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THE DEVELOPMENT OF THE PARAPHYSIS AND THE PINEAL REGION IN NECTURUS MACULATUS.

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WITH 23 TEXT FIGURES.

The presence of the paraphysis in Necturus was noted by Prof. C. S. Minot in his article "On the Morphology of the Pineal Region, based on its Development in Acanthias" (28), and a brief description of certain stages given. C. L. Herrick (15, Pl. VIII, Fig. 1, 3, 4) gives a brief account of the adult paraphysis, and shows it in the above figures, where it is named "Preparaphysis." Osborn (31, Pl. IV) shows the paraphysis in an adult brain in comparison with the brains of other amphibia. Kingsbury (21) describes briefly the adult paraphysis as well as a few of the earlier stages, and also gives an account of the epiphysis and the plexuses. I have found, however, no detailed account of all the stages in the development of the paraphysis and the pineal region. This term is used here in the same sense as in Minot's article, quoted above.

The greater part of the specimens studied for this article were taken from the Embryological Collection of the Harvard Medical School, and the numbers of each section used are given. Other specimens were prepared specially for this purpose. In some cases where the plane of section was uneven, two or more sections were used in drawing the figures in order to show all the structures, which should appear in the median line.

Fig. 1 is a median sagittal section through the brain of an embryo of 8-9 mm. I am indebted to a colleague for the drawing of this section, as this stage is wanting in the collection. In the roof of the fore brain three arches are seen. From before backward these are the paraphysal arch, *P. A.*, the post-velar arch, *P. V. A.*, and the epiphysal arch, *Ep. A.* The first two are separated by the velum transversum, *V*, which marks the limit between the two subdivisions of the fore brain. Hence the paraphysal arch belongs to the telencephalon, the other two to the

diencephalon. The epiphysal arch is bounded by two angles, which represent the position of the future supra and posterior commissures. The velum transversum is a simple infolding of the brain roof, and consists of two distinct layers, one caudad and one cephalad, the space between them being filled by a loose mesenchymal tissue, which later contains numerous blood vessels. This figure is practically identical with Minot's figure of acanthias of the same stage (28, Fig. 1), and is, therefore, of great importance in showing the homologies of these parts in elasmobranchs and amphibians. It is probable, as Minot states, that these arches occur in most of the vertebrate series.

The term post-velar arch, introduced by Minot (28), is much better for purposes of description than the terms "zirbelpolster" of German writers, and the "dorsal sack" or "postparaphysis" of American authors.

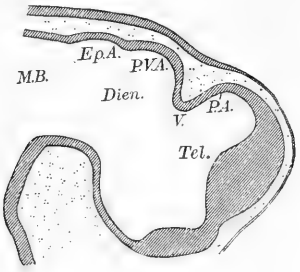

Fig. 1.

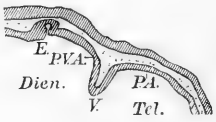

Fig. 2.

Fig. 1. Embryo of 8-9 mm. Sagittal section, × 63 diams.

Fig. 2. Embryo of 10 mm. Harvard Embryological Collection, Sagittal Series, No. 269, Section 39, × 63 diams.

Fig. 2 represents the roof of the diencephalon and telencephalon of an embryo of 10 mm. The two layers of the velum are nearer together and in the region of the epiphysal arch are seen the first signs of the epiphysis, *E*. This structure is a small rounded diverticulum, which arises from the cephalic end of the arch. It is hollow and opens into the cavity of the fore brain.

Fig. 3 is a similar section of an embryo of 12 mm. The velum is a trifle longer and the epiphysis a little larger than in the preceding figure. Immediately cephalad to the velum a very small evagination in the paraphysal arch can be seen, *P*. This is the first sign of the paraphysis, and it appears distinctly later than the epiphysis. The latter overlaps its short stalk both caudad and cephalad, and at this stage the stalk is still hollow, though its cavity was obliterated in this section.

Fig. 4 is a section of an embryo of 13 mm. The velum is again a little longer and its caudal layer is now distinctly thinner than its cephalic layer. The paraphysis is now a well-marked narrow diverticulum extending dorsad from the paraphysal arch parallel to the velum. The paraphysal arch just cephalad to the opening of the paraphysis has been

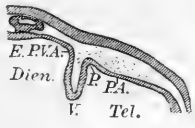

Fig. 3.

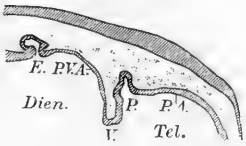

Fig. 4.

Fig. 3. Embryo of 12 mm. Harvard Embryological Collection, Sagittal Series, No. 49, Section 58, × 63 diams.

Fig. 4. Embryo of 13 mm. Harvard Embryological Collection, Sagittal Series, No. 598, Sections 71 and 75, × 63 diams.

forced downward to a slight degree, as there is relatively more space between it and the ectoderm than in the previous figures. The epiphysis is about the same size as in Fig. 3, and its opening into the brain is clearly seen.

Fig. 5 is a section of an embryo of 12.4 mm., which is, however, further advanced than that of Fig. 4. The velum, the post-velar arch, and the epiphysis are about the same, but the paraphysis is distinctly longer, and

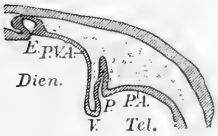

Fig. 5.

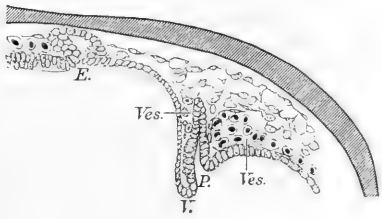

Fig. 6.

Fig. 5. Embryo of 12.4 mm. Harvard Embryological Collection, Sagittal Series, No. 675, Section 57, × 63 diams.

Fig. 6. Same as Fig. 5, × about 120 diams.

has become a narrow tube. The brain roof cephalad to it has descended still more into the cavity of the telencephalon and the opening of the paraphysis is much nearer the tip of the velum. Fig. 6 is the same section as Fig. 5, only drawn on a higher scale to show the histological details. The walls of the paraphysis and velum consist of a single layer of cells, with large oval nuclei and without very distinct cell boundaries.

These cells are, of course, continuous with those which form the brain wall in this region. The same is true of the epiphysis, but the walls seem thicker, as the organ has been cut somewhat obliquely. Close to the paraphysis two vessels can be seen, a larger one cephalad and a much smaller one caudad, *Ves.* The vessels lie in intimate relation to this structure, and it is important to note their relation at this early stage, because as development progresses the relation between paraphysis and blood vessels becomes more and more intimate.

Fig. 7 is a section of an embryo of 15 mm. The most striking feature here is the increase in size of the paraphysis, which has become a long tube with a lumen extending its entire length, and at its distal end a lateral diverticulum has appeared. The roof of the fore brain has now descended to such a degree that the opening of the paraphysis is on a level with the tip of the velum. The velum itself has lost its cephalic layer, and consists of one layer only, which, however, is much longer than the velum in Fig. 5. If Figs 4, 5, and 7 are compared it will be seen that

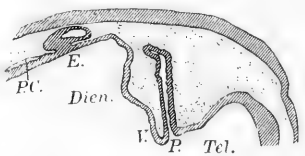

Fig. 7. Embryo of 15 mm. Harvard Embryological Collection, Sagittal Series, No. 79, Sections 85 and 89, × 63 diams.

the distal end of the paraphysis is practically at the same distance from the ectoderm in each case. As the paraphysis has developed during those stages into a long tube, its growth must have occurred by a downward extension of the neighboring parts into the cavity of the fore brain. This is practically the same process described by Minot in Acanthias. It is also shown by the great increase in distance between the roof of the telencephalon and the ectoderm from Fig. 4 to Fig. 7. The opening of the paraphysis in Fig. 3 is nearly on a level with the base of the velum, and as the down growth of the parts takes place the opening of the paraphysis and the paraphysal arch descend, apparently pushing the cephalic layer of the velum ahead of them. Therefore the single layer of the velum in Fig. 7 really corresponds to the original caudal layer, plus the cephalic layer, which has been forced down ahead of the opening of the paraphysis.

In studying Fig. 7 it might seem as if the posterior wall of the paraphysis corresponded to the cephalic layer of the velum. This, however, is

not the case, as can be seen in a wax reconstruction of the parts, Fig. 8. This is a reconstruction of the brain of an embryo of 14.5 mm. The tops of the hemispheres, *H*, have been removed to give a clear view of the paraphysis, *P*, which otherwise would be more or less covered in by them. The paraphysis appears as a straight tube in the median line and caudad to it is seen a broad partition, *V*, extending the whole width of the diencephalon. This is the velum, consisting of one layer only, which represents the two originally distinct cephalic and caudal layers. The down growth of the parts in order to provide room for the development of the paraphysis has formed a deep angle in the roof of the fore brain. This

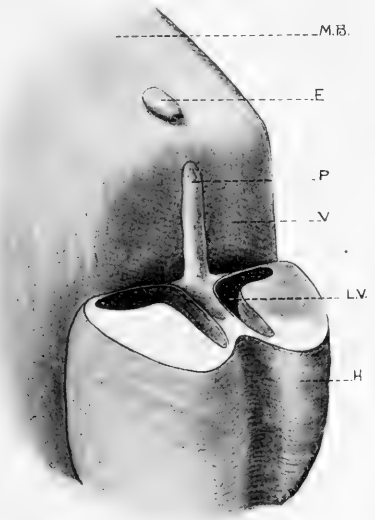

Fig. 8. Wax model of brain of embryo of 14.5 mm. Harvard Embryological Collection, Sagittal Series, × 120 times.

angle is bounded caudad by the velum and cephalad or ventrad by the narrow roof of the telencephalon (paraphysal arch) immediately cephalad to the paraphysis. As the hemispheres develop, they grow at first in a dorsal direction and occupy the space left by the formation of this angle, so that the paraphysis is practically buried between the hemispheres in front and the velum behind, Fig. 10. The growth of the paraphysis must, therefore, be regarded as having an important effect on the development of the fore brain at this stage.

Up to this stage the development of the velum has been in a ventral direction towards the floor of the fore brain, but now it begins to grow in quite a different direction. In Fig. 7 a distinct bulging of the velum is

seen, which is extending caudad at nearly a right angle to its previous line of growth. If the roof of the telencephalon be closely examined a slight bulging will be seen just cephalad to the opening of the paraphysis. These two outgrowths into the fore brain mark the beginning of the choroid plexuses, which, therefore, have in their origin a very intimate and definite relation to the opening of the paraphysis, one arising caudad and the other cephalad to it. The epiphysis at this stage has increased considerably in size, and the cavity in its stalk is now permanently obliterated. The body of the organ overlaps the stalk a little behind, and is beginning to grow well forward of it. The posterior commissure, *P. C.*, appears here for the first time, a distinct interval in the roof of the brain lying between it and the stalk of the epiphysis.

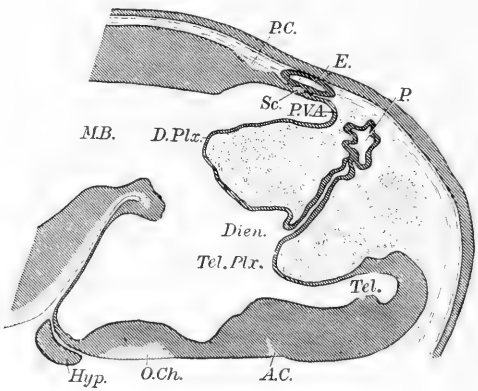

FIG. 9. Embryo of 17.5 mm. Harvard Embryological Collection, Sagittal Series, No. 540, Sections 113-115, × 63 diams.

Fig. 9 is a section through the brain of an embryo of 17.5 mm. The paraphysis has increased in length, and from its distal end, which is somewhat enlarged, small tubules are given off. The whole tube is tipped somewhat forward. The choroid plexus is now well developed, and consists of two distinct parts, one dorsal and one ventral. The dorsal part corresponds to the velum, which has grown caudad as far as the mid brain and has absorbed a large part of the post-velar arch. The ventral part is developed from the original paraphysal arch, and is growing towards the floor of the fore brain. Burckhardt (3) refers to these plexuses as "plexus medius" and "plexus inferioris," respectively, and Mrs. Gage (13), who studied them in Diemyctylus, where the anatomical conditions closely resemble those of Necturus, names them the "diaplexus" and "prosoplexus." Prof. Minot has suggested the terms diencephalic

plexus for the dorsal part, and telencephalic plexus for the ventral part, and I shall use these terms, as they express more clearly the exact origin of each plexus.

The diencephalic plexus, *D. Plx.*, appears as a large wedge-shaped mass covered by a thin layer of cells, and consisting of a loose connective tissue in the interstices of which numerous blood corpuscles can be seen. The telencephalic plexus, *Tel. Plx.*, has the same general characteristics as the diencephalic. The epiphysis has become flattened and more elongated, and is attached by a narrow stalk to the brain wall. The supra commissure, *S. C.*, is seen just cephalad to the stalk of the epiphysis, which is prolonged forward above it. I was unable to obtain any sagittal series between 15 and 17.5 mm., but in a transverse series of 16.5 mm. the first traces of this commissure can just be made out, and therefore it

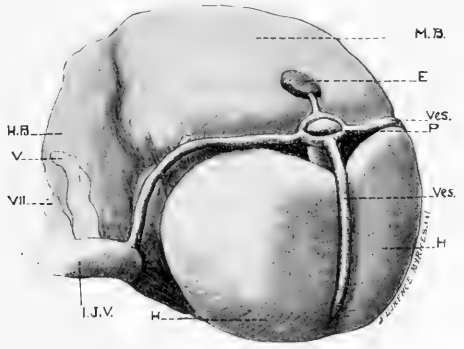

Fig. 10. Wax model of brain of embryo of 18 mm. Harvard Embryological Collection, Frontal Series, No. 850, × about 75 diams.

probably appears between 16 mm. and 17 mm. as a rule, but at these early stages there is a good deal of variation in the development of all these parts. The posterior commissure is rather larger than in the previous stage.

Fig. 10 is the drawing of the model of the brain of an embryo of 18 mm. This model is intended to show the circulation of the paraphysis at this stage. The distal end of the paraphysis, *P*, is surrounded by a venous circle, from either side of which veins, *Ves.*, run outward and backward just caudad to the hemispheres, *H*, to terminate in the internal jugular vein, *I. J. V.* This vein is passing backward external to the fifth, *V*, and seventh, *VII*, cranial nerves. Fig. 6 showed the intimate relation of the paraphysis to these vessels at 12.4 mm., and when the sections of this series were followed out it was found that here the vessels surrounded the tip of the paraphysis. It seems that as the paraphysis devel-

ops it forces its way into the veins lying over this part of the fore brain, and the tubules, as they are given off at later stages, force their way into these veins, Fig. 15, forming the sinusoidal type of circulation described by Minot (29) and Lewis (25). From the venous circle shown in Fig. 10 smaller vessels run down along the sides of the paraphysis and anastomose with the vessels of the choroid plexuses. A vessel also runs back to the epiphysis, and a larger one forward between the hemispheres. The circulation of this region appears at this stage to be mostly venous, as I could trace the arteries only to their point of entrance in the anlage of the skull, and the return circulation probably occurs by means of a minute capillary network over the surface of the brain.

Fig. 11 is a section through the brain of an embryo of 26 mm. The

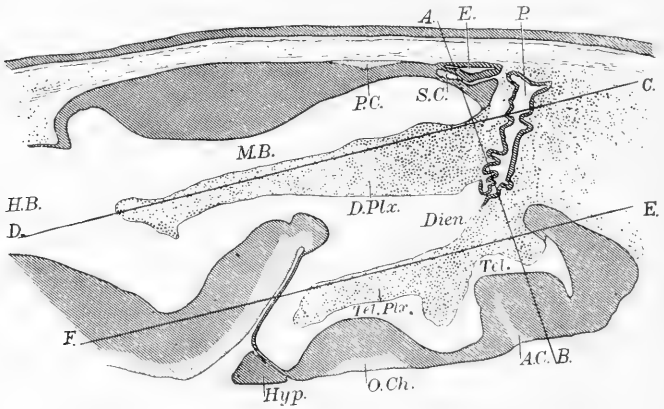

FIG. 11. Embryo of 26 mm. Harvard Embryological Collection, Sagittal Series, No. 377, Sections 125 and 126, × 63 diams.

paraphysis here is much more developed. It inclines somewhat forward, and from its wide central lumen a number of tubules are given off in every direction. The epiphysis and the commissures show but little change. The striking feature of this figure is the great development of the plexuses. The diencephalic plexus, *D. Plx.*, has grown through the mid-brain nearly to the hind-brain, and the telencephalic plexus, *Tel. Plx.*, has grown downwards into the depths of the cavity of the fore brain towards the infundibular recess.

Fig. 12 is a transverse section of an embryo of 26 mm., corresponding approximately to the line *A-B*, Fig. 11. The section passes through the epiphysis, *E*, and the supra commissure, *S. C.*, just beneath it. Then through the diencephalic plexus, *D. Plx.*, and that part of the cavity of the diencephalon between this plexus and the roof, *Dien.* The section

then passes through the paraphysis at a point where two small tubules are given off, then through the telencephalic plexus, *Tel. Plx.*, the telen-

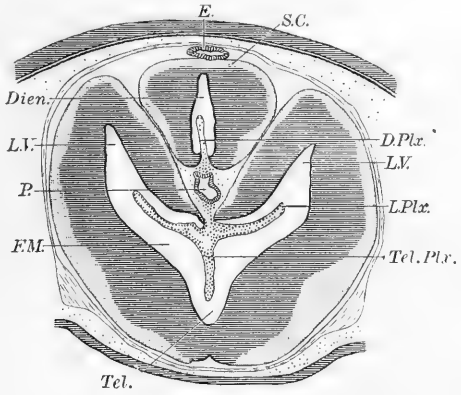

FIG. 12. Embryo of 26 mm. Harvard Embryological Collection, Transverse Series, No. 376, Section 89, × 63 diams. (See line *A-B*, Fig. 11.)

cephalon, *Tel.*, the lateral ventricles, *L. V.*, and the foramina of Munro, *F. M.* In this section the plexuses of the hemispheres, *L. Plx.*, are seen. They arise on either side of the origin of the telencephalic plexus, and

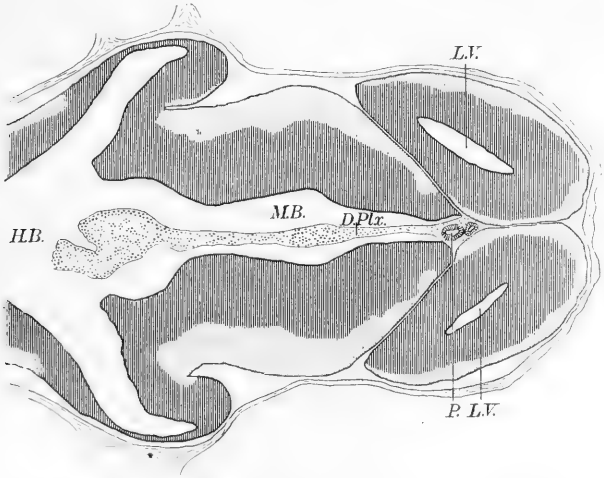

FIG. 13. Embryo of 26 mm. Harvard Embryological Collection, Frontal Series, No. 378, Section 138, × 63 diams. (See line *C-D*, Fig. 11.)

pass outward at right angles to it through the foramina of Munro into the lateral ventricles.

Fig. 13 is a frontal section through an embryo of 26 mm., corresponding

closely to the line *C-D*, Fig. 11. The section passes through the paraphyses, *P*, and a large lateral tubule, and then through the entire length

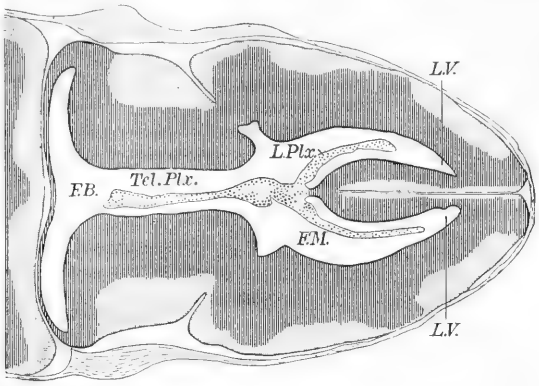

FIG. 14. Same Series as Fig. 13. Section 108. (See line *E-F*, Fig. 11.)

of the diencephalic plexus, *D. Plx.*, the distal end of which is here enlarged and has reached to the hind brain, *H. B.* Fig. 14 is of the same

FIG. 15. Same as Fig. 11. × about 150 diams.

series as Fig. 13, and corresponds approximately to the line *E-F*, Fig. 11. It passes through the telencephalic plexus, *Tel. Plx.*, the plexus of the

hemispheres, *L. Plx.*, and the lateral ventricles, *L. V.* It shows clearly how the plexuses of the hemispheres arise from the telencephalic plexus and pass at first outward and then forward through the foramina of Munro towards the cephalic extremity of the lateral ventricles.

Fig. 15 is a high power drawing of Fig. 11, magnified 150 diams. The wall of the paraphysis consists of a single layer of cells with large oval nuclei, and these cells are continuous with the cells covering the choroid plexuses, but the latter are flatter and form a thinner layer. On either side of the paraphysis two large vessels are seen, *ves.*, the epithelial cells of which lie directly against the wall of the paraphysis. The little tubules seem to be forcing their way into these vessels, which are branches of the vessels seen in Fig. 6 and Fig. 10. The vessels also pass down into the choroid plexuses. Fig. 16 is a section of an embryo of 31.4 mm.

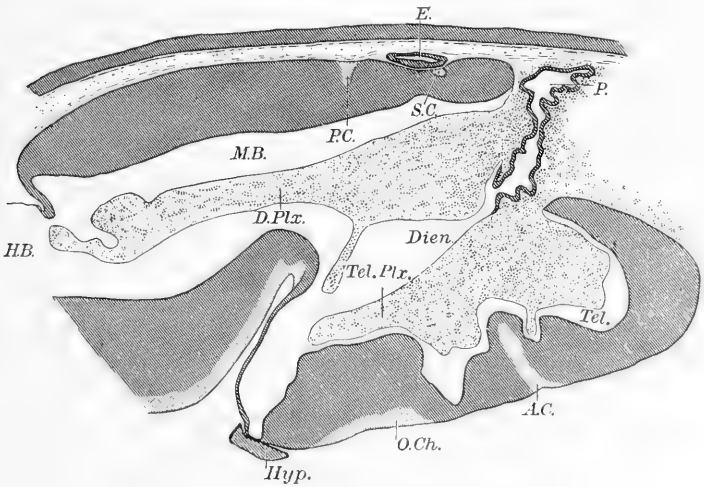

FIG. 16. Embryo of 31.4 mm. Harvard Embryological Collection, Sagittal Series, No. 537, Sections 119-122, × 63 diams.

The general arrangement is practically the same as in Fig. 11, except that all the parts have progressed somewhat in their development. The distal end of the paraphysis has begun to grow distinctly more cephalad, and the whole structure is much larger than at 26 mm. The choroid plexuses are more extensive, and from the diencephalic plexus a prolongation is extending downwards towards the telencephalic plexus. This latter has pretty well filled up the depths of the third ventricle, and from it prolongations dip down into the recesses in the floor of the fore brain. The two commissures are practically the same as they were at 26 mm., and

though the epiphysis is a little larger it has been displaced considerably caudad, as this part of these sections was unluckily somewhat injured.

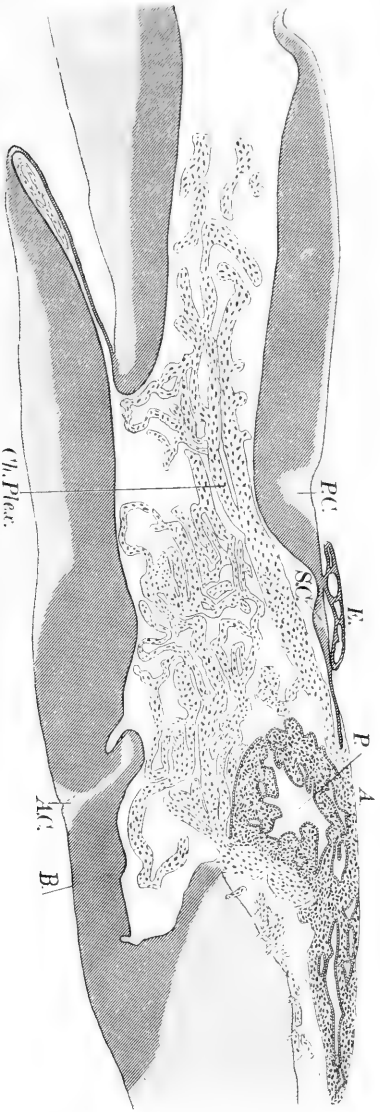

FIG. 17. Brain of adult necturus. Sagittal Section, × 38 diams.

Fig. 17 is a section through the brain of an adult necturus. This drawing is magnified 38 diams. only, as it was too large to draw on the same scale as the preceding figures. The paraphysis, *P*, forms a very complex structure extending far forward above and between the hemispheres. It consists in a general way of a proximal and a distal part. The former is broad and thick, and extends forward and upward. It then turns forward at quite a marked angle to form the distal part, which is narrow and tapering. The central canal in the proximal part is very large and irregular, but in the distal portion much narrower. From all parts of this canal a large number of tubules are given off, which extend in every direction, and between which lies a confused mass of blood-vessels. One sees here a large vessel ventrad to the organ, and a smaller dorsad to it, the same relations as appear at 12.4 mm., Fig. 6. From these vessels branches pass into the choroid plexuses.

Fig. 18 is a transverse section of an adult brain corresponding approximately to the line *A-B*, Fig. 17. This is drawn on the same scale as most of the preceding figures, 63 diams. The paraphysis, *P*, is seen in the median line between the hemispheres. It shows a distinct central cavity, with many tubules running out in every direction, between which is a mass of blood-vessels of all sizes. Below a portion of the telencephalic plexus and the plexus of the hemispheres are seen.

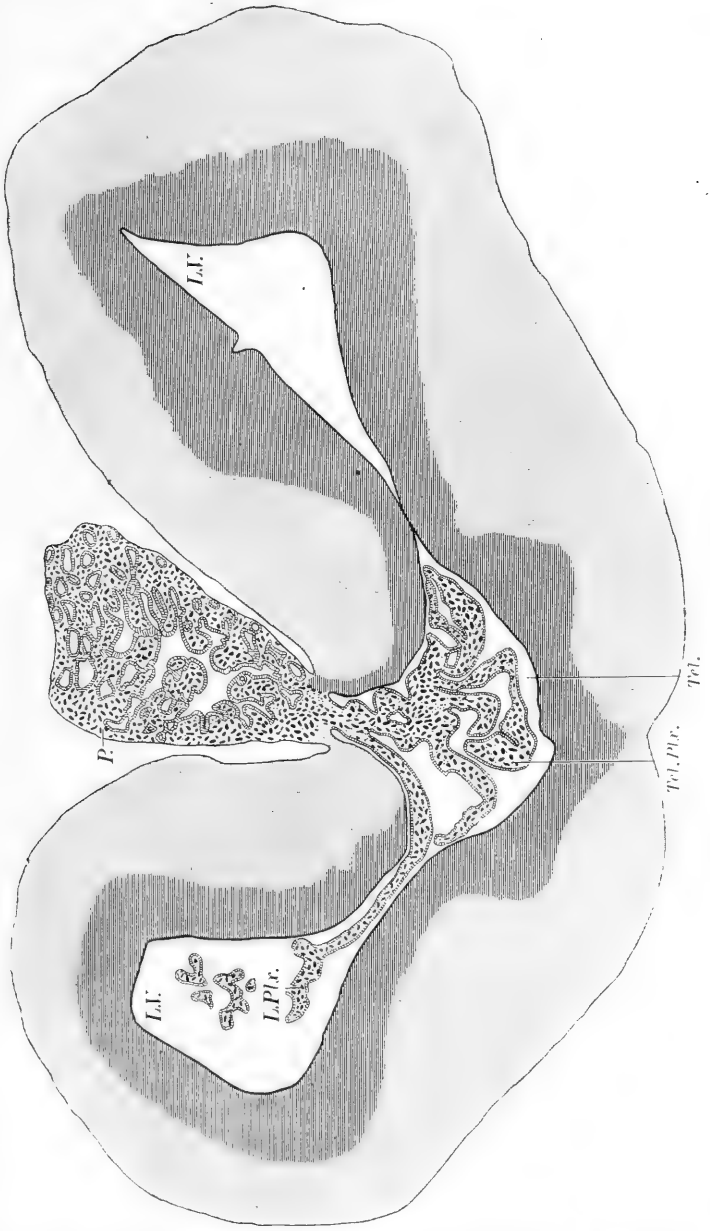

FIG. 18. Brain of adult necturus. Transverse section. × 63 diams. (See line *A-B*, Fig. 17.)

Fig. 19 is from a wax reconstruction of the adult paraphysis on a scale of 120 diams., made from the same series as Fig. 18. The angle between the proximal and distal parts is quite striking, and is much more marked in Ichthyophis (Burckhardt, 4, Fig. 1), but of course this division into proximal and distal parts is really a purely arbitrary one. This model gives a good idea of the complex structure of the organ. The tubules are of all shapes and sizes, often convoluted and anastomosing with each other. The spaces between them, which the vessels occupy, are quite large and striking.

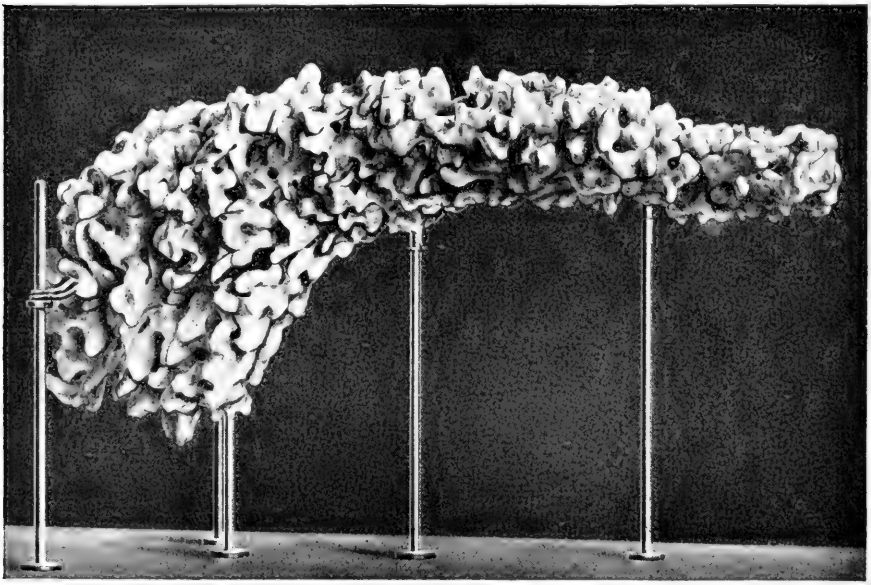

Fig. 19. Wax model of paraphysis of adult necturus, same series as Fig. 18, × about 120 diams.

Fig. 20 represents a small portion of the paraphysis of Fig. 17, magnified 560 diams., and shows clearly the relation of the tubules to the vessels. In the centre of the figure is a tubule, T, dividing into two branches, T^1, T^2. Surrounding these tubules on every side are sinusoids, *si.*, whose flat endothelial cells are seen lying directly against the epithelial wall of the tubules with no connective tissue between them. We find here in order first a sinusoid, then a tubule, then another sinusoid and another tubule, and finally a sinusoid. The wall of the tubules consists of a single layer of cells with large oval nuclei and very indistinct cell boundaries. The nuclei contain masses of granules arranged very irregu-

larly. There can be no question about the glandular nature of the paraphysis, and its circulation is evidently sinusoidal.

The choroid plexus, *Ch. Plx.*, Fig. 17, appears as a confused mass of

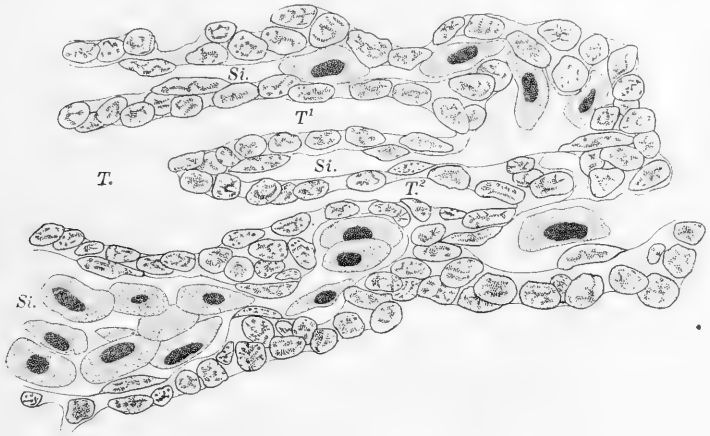

FIG. 20. Small portion of adult paraphysis, same section as Fig. 17, × 560 diams.

vessels covered by a thin layer of cells. This mass completely fills up the cavity of the fore and mid brains, and may in some cases appear in the hind brain, Fig. 23, though there seems to be considerable variation

FIG. 21. Wax model of epiphysis of adult necturus. × 280 diams.

in the caudad development of this part of the plexus. The two parts of the plexus overlap each other, and are also closely interlaced.

The epiphysis, *E.* is still attached to the brain by a very narrow stalk. The body overlaps the stalk somewhat behind, and then is prolonged forward as an oval flattened body above the roof of the diencephalon, and

its cavity seems to be divided more or less into compartments. Fig. 21 represents a wax reconstruction of an adult epiphysis seen from above. It is irregularly triangular in shape with a broad base and a blunt apex. Its surface is grooved more or less by vessels which lie against its walls.

Fig. 22 is the same model with the top removed. The interior is more or less subdivided by incomplete septa. At its apex there is a small cavity

Fig. 22. Same as Fig. 21, with top of epiphysis removed.

bounded behind by a partial septum, then comes a large chamber, which divides into two passages running back towards the angles at the base. Between these two passages appears a comparatively solid area, interrupted, however, to some extent by small spaces, which communicate with each other and the larger chambers. This solid area lies over the stalk of the organ. The supra commissure appears to be comparatively small,

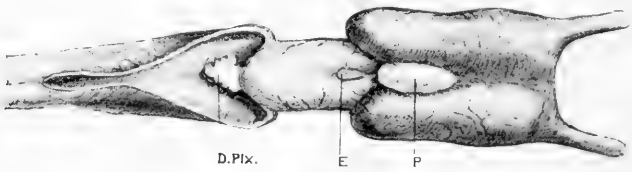

Fig. 23. Brain of adult necturus. Viewed from above. × 7 diams.

while the posterior is large and forms a deep groove in the roof of the brain. Fig. 23 is a view of the brain of an adult necturus showing the relative positions of paraphysis and epiphysis. The tufted extremity of the diencephalic plexus can be seen in the fourth ventricle.

If Fig. 1, the embryo of 8-9 mm., is compared with Fig. 17, the adult, one sees that the paraphysal arch has been wholly taken up in the formation of the telencephalic plexus, the plexus of the hemispheres, and

the paraphysis. The velum and the greater part of the post-velar arch have been absorbed in the formation of the diencephalic plexus. A portion of this arch, however, persists and forms that part of the roof of the diencephalon between the diencephalic plexus and the supra commissure. The epiphysal arch has formed the epiphysis.

The paraphysis is a structure common to all vertebrates either in the adult or embryonic condition (Selenka, 34, Francotte, 11), but previous observations on mammals leave much to be desired. It always arises from the telencephalon cephalad to the velum transversum, and its opening is placed between and dorsad to the foramina of Munro as emphasized by Dexter (5).

In the cyclostomes, Ammocoetes (Kupffer, 24), and Petromyzon (Burckhardt, 3), the paraphysis appears as a small sac-like diverticulum lying ventrad and close to the enlarged distal end of the epiphysis. In elasmobranchs, Minot (28) and Locy (27) found that the paraphysis in Acanthias appears at quite a late stage as a small outgrowth from the paraphysal arch and, owing to the small size of the post-velar arch and the compression of the velum, it comes to lie immediately cephalad to the epiphysis. In ganoids, Kupffer found in Accipenser that the paraphysis appears first as a small outgrowth which later becomes a somewhat sacculated vesicle (23, Fig. 19). Hill (18) and Eycleshymer and Davis (9) studied the paraphysis in Amia. Here it begins as a simple vesicle, which increases rapidly in size and gives off diverticuli from its central cavity. In teleosts (Burckhardt, 3) the paraphysis appears late and remains in a rudimentary condition. In the dipnoi, Burckhardt (3) describes the paraphysis in Protopterus as a wide outgrowth giving off small diverticuli.

In amphibia the organ becomes highly differentiated and its appearance in the adult brain is very striking. It appears as an elongated body lying above and between the hemispheres, and extending cephalad for a varying distance in various forms, Fig. 23. Osborn (31, Pl. 4) shows a view of the brains of Siredon, Necturus, Proteus, and Siren. The paraphysis has the same general form in each of these, but it is somewhat larger in Necturus. The paraphysis of Triton and Ichthyophis (Burckhardt, 4) has the same characteristics. In the latter the paraphysis appears in sagittal section as a hammer-shaped organ extending forward above the hemispheres. (4, Fig. 1). In Rana the paraphysis has the same position as in Necturus, but is smaller. On removing the top of the skull in Necturus the paraphysis is seen lying beneath the pia surrounded by the blood-vessels which cover this part of the brain. It appears to the naked eye so vascular and also in sections so intimately related to the

choroid plexus that it is not astonishing that it was at first regarded as a portion of this plexus. According to Minot (28) the paraphysis of Rana is characterized by the character of its epithelium, its tubular structure and its apparently sinusoidal circulation. This is practically similar to the conditions found in Necturus.

In Menopoma Sorensen (36) describes the paraphysis as a solid vascular mass, and in Ichthyophis Burckhardt (3) describes it as an elaborately folded structure of a glandular character. In Amblystoma, Eycleshymer (8) shows that the organ gives off tubules and has a digitated appearance. In Diemyctylus (Mrs. Gage, 13), in an embryo of 10 mm. the paraphysis closely resembles that of Necturus at 18 mm. (Minot, 28, Fig. 13), and in the adult it is a long tube giving off many tubules in close relation to vessels.

Herrick (15) calls the paraphysis of an adult Necturus the "preparaphysis" and says it consists of an irregular central chamber with complicated diverticuli in close relation to vessels. This description corresponds closely with Fig. 17. The model of the adult paraphysis, Fig. 19, shows the complicated arrangement of the tubules, many of which anastamose with each other, and the spaces between the tubules are filled by blood-vessels.

Fig. 6 shows how the paraphysis at 12.5 mm. is beginning to invade a large vessel lying over it on the surface of the brain. This vessel at this point is much enlarged. Fig. 10 shows this relation much clearer and also that these vessels in relation to the paraphysis are tributaries of the internal jugular vein. Fig. 15 shows the relation of the paraphysis to these vessels at 26 mm. and that the vessels pass into the choroid plexuses both dorsad and ventrad to the organ. The little tubules can be seen growing out into the vessels. Fig. 17 shows how these relations between vessels and tubules in the adult become much more intimate, and the vessels corresponding to those in Fig. 15 are seen passing into the choroid plexuses dorsad as well as ventrad to the paraphysis. Schöbel (33), who studied the circulation in the brain of certain amphibia, of which Necturus was one, shows that in the adult two large vessels pass outward just caudad to the hemispheres to empty eventually in the internal jugular vein. These vessels surround the paraphysis and anastomose with two or three large vessels running forward between the hemispheres. This is practically the same arrangement shown in the model in Fig. 10. He does not mention the paraphysis but refers to it as a large venous plexus. Rex (32) also has studied the veins in the amphibian brain, and his preparations are practically the same as Schöbel's. He refers to the paraphysis as the "nodus chorioideus" and says that it is a sort

of meeting point for the veins of the fore and mid brains. He shows beautifully in his injections how veins pass both dorsad and ventrad to the paraphysis to enter the plexuses, and how closely these vessels are related to the tubules of this structure. Mrs. Gage shows practically the same arrangement in Diemyctylus

As regards the arteries Schöbel shows that they are much smaller than the veins, and describes a small vessel passing caudad to the hemispheres to pass eventually into the plexuses. The intercrescence of the tubules of the adult paraphysis and the veins is shown clearly in Fig. 20, each vessel and tubule lying back to back with no connective tissue between them. In view of all these facts it seems evident that the circulation of the paraphysis is sinusoidal. According to the above descriptions, the development of the paraphysis into a complicated, glandular organ, which is also very vascular, seems to be a striking characteristic of the amphibia.

In lacertilia the paraphysis of Anguis fragilis has been studied by Francotte and of Lacerta vivipara by Francotte (10, 11, 12) and Burckhardt (3). The latter shows the paraphysis in an embryo of 13 mm. as a narrow tube with a slightly expanded distal extremity, much as that of Necturus of 15 mm. Francotte describes the paraphysis of Lacerta vivipara as a long tube giving off a mass of tubules which lies under the parietal eye, and resembles the epiphysis of birds (11, Fig. 14; 12, Fig. 24). In Anguis (10, Figs. 15 and 19) the paraphysis forms a long narrow sack, with somewhat convoluted walls, which curves back over the post-velar arch to end in close relation to the parietal eye.

The conditions in the lizard are essentially the same (10, Fig. 31). In Phrynosoma coronata Sorensen (35, Fig. 2) describes the paraphysis as a long, narrow tube, immediately cephalad to the epiphysis.

In the ophidia Leydig (26, Fig. 6) shows the paraphysis of an embryo of Vivipara urcini near birth as a large, wide tube with no convolutions or diverticuli and practically the same conditons in a young "Ringelnatter" and Tropidonatus natrix (26, Figs. 5 and 2).

Among the chelonia Voelzkow (39) has described the paraphysis in Chelone imbricata as at first a wide tube much convoluted, which later decreases somewhat in size. (Figs. 21 and 22.) Its distal end inclines caudad close to the epiphysis. In Chelone mydas Humphrey (20, Fig. 7, Pl. II) shows the paraphysis as a long tubular structure, giving off small tubules, and in an embryo of Chelydra it appears as a large, wide sack, from which tubules arise. It is in closer relation to the epiphysis than in Chelone mydas or imbricata.

In Cistudo Herrick shows a model of Sorensen (15, Fig. 5, Pl. VI) of the paraphysis, which is a wide tube with convoluted walls and tubules.

In the crocodilia Voelzkow (39) has described the paraphysis of Crocodilus madagascarensis grand and Caiman niger spix. In the former the paraphysis is at first a wide tube which becomes convoluted and much longer and narrower. In the latter the paraphysis forms a larger tube and the convolutions and tubules are more complicated. In both cases the organ reaches its greatest development in embryonic life and retrogrades later, though more so in the crocodile. He was unable, however, to follow the development in the caiman as far as in the crocodile. Owing to the thickenings in the brain wall the organ is crowded somewhat caudad against the post-velar arch.

In birds the paraphysis is relatively rudimentary. Burckhardt (3) shows the paraphysis in an embryo of the crow as a small diverticulum not unlike that of Petromyzon. Dexter (5) worked out in detail the development of the organ in the common fowl, and showed that it appeared at first as a small diverticulum. The walls become much thickened and in a chicken of 10 days it is a small, oval structure, about 150 μ in its greatest diameter, with very thick walls (5, Fig. 5). Selenka (34) has described the paraphysis in the oppossum, but as far as I am aware little is known of the development of the paraphysis in mammals, though Francotte (11) has observed it in human embryo of twelve weeks.

From the cyclostomes to the amphibia the paraphysis shows a steadily progressive development, and the various forms through which it passes, from the simple diverticulum of Petromyzon to the elaborate gland of the urodela, are illustrated in a general way by the stages of its development in Necturus. In the vertebrates above the amphibia the paraphysis retrogrades and practically retraces its steps through the reptilia and birds to mammals, reaching in the chick essentially the same form in which it started in Petromyzon. Its development, therefore, may be indicated by a curve, which ascends steadily from the cyclostomes, reaches its height in urodela, and descends through the reptilia and birds to mammals.

The epiphysis is present in nearly all vertebrates. It is stated to be absent in the alligator (Sorensen, 36 and 37), and in the caiman and crocodile (Voelzkow, 39) and in Torpedo (d'Erchia, 7).

The epiphysis of Necturus as compared with the paraphysis is relatively poorly developed and in this respect resembles the epiphysis of other urodela (Mrs. Gage, 13). In Diemyctylus Mrs. Gage found that the epiphysis was entirely cut off from the brain and that its cavity was nearly obliterated. In Ichthyophis (Burckhardt, 1 and 4) the epiphysis is a small, pear-shaped organ attached to the brain by a narrow solid stalk. Herrick (15) in Menopoma, describes the epiphysis as an irreg-

ular number of vesicles attached to the brain by a narrow opening. According to Kingsbury (21) the structure in Necturus consists of an aggregation of closed vesicles, forming an oval, flattened body, and there is no connection with the brain. The cavity of the epiphysis communicates through its stalk with the cavity of the diencephalon up to 15 mm., when the cavity in the stalk becomes obliterated. The stalk persists and was present in all the adult brains which I examined, but in some cases it was so small that it could easily be overlooked. The reconstruction of an adult epiphysis, Fig. 22, shows that the cavity of the organ forms a large chamber subdivided to a certain extent by incomplete septa. A much more solid area is seen towards the caudal extremity, which is placed just over the stalk. The same characteristics I have observed in another model made from a different brain. One gets the idea that the epiphysis consists of a series of vesicles in studying sagittal sections a little to one side of the median line, as for instance in Fig. 17, where the epiphysis was displaced a little to one side.

. There has been such a vast amount written on the origin of the epiphysis and the pineal or parietal eye and their homologies that it seems superfluous to add anything more here. In a very general way, however, there seems to be some sort of proportion in the relative development of the paraphysis, epiphysis, and the parietal eye. In urodela where there is no parietal eye and a small epiphysis, the paraphysis reaches its highest degree of development. In those forms where the paraphysis is rudimentary or relatively slightly developed the parietal eye is present or else the epiphysis is relatively highly developed. Compare, for example, the figures of Burckhardt (3) of Petromyzon, Minot (28) of Acanthias, Burckhardt (3) of Trout, Leydig (26) and Voelzkow (38) of reptilia, and Dexter (5) of the fowl. Rana, however, seems to be a marked exception to this statement, as there the paraphysis, epiphysis, and pineal eye are all present and well developed, and the same may be said for Lacerta (Francotte, 10 and 11) and Sphenodon (Dendy, 6). As the paraphysis and epiphysis are glandular structures they have probably some sort of compensatory function and where one is highly developed the other is relatively rudimentary or even absent. Compare in this respect also Torpedo with Acanthias and the crocodile and alligator with the chick.

As a rule the stalk of the epiphysis is placed immediately caudad to the supra commissure, in all cases I believe, except in the toad, where there is a distinct interval between it and this commissure (Sorenson, 36). In Necturus there is an interval in the roof of the brain between the stalk and the posterior commissure. This portion of the roof of the brain was

described by Kupffer (23) as the "schaltstück," and according to him it is best developed in amphibia. Burckhardt (3) maintains that it occurs in all vertebrates from Petromyzon up, but according to Kupffer it is absent in Accipenser (23, Fig. 19), and it is also wanting in Acanthias (Minot, 28, Fig. 10) and in the fowl (Dexter, 5, Fig. 9).

The velum transversum is probably characteristic of all vertebrates. Minot (28). In Petromyzon the velum appears as a small transverse fold, and the post-velar arch is well marked. The plexus development is, however, very slight. In elasmobranchs the velum of Acanthias forms a long, narrow, transverse fold, and the post-velar arch is so small that the origin of the velum seems to be close to the supra commissure. The caudal layer of the velum is distinctly thinner than the cephalic (Minot, 28, Fig. 6). This is also seen in Torpedo (d'Erchia, 7, Fig. 12), and in Necturus, Fig. 6. The velum later on has the character of a choroid plexus, but the plexus of the hemispheres is very rudimentary (Minot, 28). In Notidanus Burckhardt (3) shows a long, narrow velum, a short post-velar arch, and a small telencephalic plexus. The plexus of the hemispheres, however, is absent.

In Accipenser (Kupffer, 23, Fig. 19) the velum is long, well developed and folded to a certain extent, and the post-velar arch is quite extensive. In ganoids (Studnicka, 38) the membranous roof of the brain serves as the tela choroidea of higher types. In this class of vertebrates according to Burckhardt (3) the plexus of the hemispheres is lacking, but the telencephalic plexus is well developed, and in teleosts the former is also wanting, but the latter present in a reduced form.

In amphibia all the plexuses are highly developed, and in Necturus they are of marked extent (Kingsbury, 21). The velum in Necturus appears at first as a transverse fold in the roof of the brain separating the diencephalon from the telencephalon. This fold develops at first ventrad and then caudad through the mid brain as far as the hind brain. This great growth of the velum forms the diencephalic plexus. The post-velar arch, which at first is wide and well marked, is practically absorbed by the overgrowth of the velum, and a small portion only persists in the roof of the diencephalon between the origin of the diencephalic plexus and the supra commissure, Fig. 17. The telencephalic plexus develops from the paraphysal arch immediately cephalad to the paraphysis, the opening of which therefore is surrounded by these two plexuses. They fill up the cavity of the third ventricle and mid brain, and the diencephalic plexus may appear in the hind brain (Osborn, 29). This seems to vary in different cases, and in the majority of brains which I was able to examine the extremity of this plexus did not actually extend

into the hind brain. In Fig. 23, however, this extremity appears as a marked tuft in the fourth ventricle. The plexuses of the hemispheres arise on either side from the origin of the telencephalic plexus and pass into the lateral ventricles, extending nearly to their cephalic extremities.

In Lacerta vivipara (Francotte, 12, Fig. 24) the post-velar arch has been much compressed from before backward so as to form a deep narrow angle. At the apex of the angle the folds of the diencephalic plexus are seen. The velum is smooth and apparently is not included in the formation of the plexus. In Anguis fragilis (Francotte, 10, Figs. 19 and 15), the post-velar arch does not seem to be so much compressed, and the plexus formation somewhat greater. As he says, however, the development of those parts in Lacerta is practically the same as in Anguis. According to Burckhardt the telencephalic plexus is much reduced in size, consisting merely of small folds, but the plexus of the hemispheres is well developed (Burckhardt, 3).

In the turtles Humphrey (20) found that the velum of Chelydra is but slightly developed, and no diencephalic plexus is formed. All the other plexuses are telencephalic in origin.

Herrick (15, Fig. 5, Pl. VI), shows in Cistudo a well-developed telencephalic plexus and a diencephalic plexus represented by many folds in the caudal layer of the velum and the post-velar arch. In Chelone imbricata Voelzkow (39, Figs. 19 and 22) shows at first a well marked velum and a wide post-velar arch. In later stages the velum and practically all the arch are thrown into folds to form the diencephalic plexus. The telencephalic plexus is also well developed. In the serpents much the same arrangement can be seen. The velum (Leydig, 26, Figs. 2, 5, and 6) forms a prominent fold, and it and the post-velar arch form a very vascular plexus. In the crocodilia (Voelzkow, 39, Figs. 7, 11, 13, 15), the velum and the post-velar arch are at first well marked, but the parts later become so compressed from before backward that the arch forms a deep acute angle in the depths of which plexus foldings are seen. The caudal layers of the velum, however, takes no part in the plexus formation. In birds, Dexter (5) found that the velum of the fowl is small and the post-velar arch broad at first. This becomes compressed so as to form an acute angle much as in the crocodilia. The cephalic limb of this angle and all the velum is converted into the choroid plexus. In birds the plexus of the hemispheres is very well developed, but the telencephalic plexus is practically absent.

In fishes the plexus development is quite simple, in many cases being merely the thin membranous roof of the third ventricle; in others, however, this is much folded and vascular (Sorensen, 35). In certain forms

there is a telencephalic plexus, but the plexus of the hemispheres is absent or rudimentary (Burckhardt, 3). In amphibia there is a great overgrowth of all the plexuses, especially of the diencephalic plexus, which here reaches its highest development. In reptilia the plexus of the hemispheres is well developed, but the telencephalic plexus is reduced in size (Burckhardt, 3), and the diencephalic much more so. In birds the plexus of the hemispheres is highly developed, the telencephalic plexus practically absent, and the diencephalic plexus, while very similar to that of reptilia, approaches nearer to the tela choroidea of higher mammalia.

Osborn first named the supra-commissure and worked out its homologies. According to him (30) the urodela are distinguished from the anura by the frequent extensive development of this commissure, which is large in Amphiuma, smaller in Necturus, and much reduced in Rana. It appears in Necturus a little later than the posterior commissure, as is usual in most cases, as far as I am aware, except in Ammocoetes, where it appears shortly before the posterior commissure (Kupffer, 24, Fig. 5). It is found in all the chief types of vertebrates, and is usually smaller than the posterior (Minot, 28). It is developed from the diencephalon, while the posterior belongs to the cephalic limit of the mid brain.

Conclusions.

1. The paraphysis appears first in an embryo of 12 mm. It is developed from the telencephalon immediately cephalad to the velum transversum as a small diverticulum, which becomes eventually a complicated gland with anastomosing tubules. The gland is very vascular, and has a sinusoidal circulation.

2. The epiphysis appears first in an embryo of 9-10 mm., and is developed from the diencephalon. It is always attached to the brain by a small solid stalk, and the cavity is partially subdivided by incomplete septa.

3. The velum transversum grows at first ventrad and then caudad as far as the hind brain, forming in this way the diencephalic portion of the choroid plexus. The post-velar arch, which is at first quite extensive, is almost entirely absorbed in this extensive growth of the velum.

4. The telencephalic plexus arises from the roof of the telencephalon, and fills up the depths of the cavity of the third ventricle. The opening of the paraphysis is surrounded by these two plexuses.

5. The plexus of the hemispheres arises at a right angle from the telencephalic plexus just cephalad and ventrad to the opening of the paraphysis.

6. The supra-commissure appears first at 16-17 mm. It lies immediately cephalad to the stalk of the epiphysis and is comparatively small.

7. The posterior commissure appears first at 15 mm., and there is a marked interval in the roof of the diencephalon between it and the epiphysis.

I wish in conclusion to express my acknowledgments to Prof. Minot for his kind advise and interest in the preparation of this article.

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ABBREVIATIONS.

A. C.—Anterior commissure.
Ch. Plx.—Choroid plexus.
Dien.—Diencephalon.
D. Plex.—Diencephalic plexus.
E.—Epiphysis.
Ep. A.—Epiphysal arch.
F. B.—Fore-brain.
F. M.—Foramen of Munro.
H.—Hemisphere.
H. B.—Hind brain.
Hyp.—Hypophysis.
I. J. V.—Internal jugular vein.
L. Plx.—Choroid plexus of lateral ventricle.
L. V.—Lateral ventricle.
M. B.—Mid-brain.
O. C.—Optic commissure.
Tel. Plx.—Telencephalic plexus.
Tel.—Telencephalon.
P. C.—Posterior commissure.
P. V. A.—Post-velar arch.
P. A.—Paraphysal arch.
P.—Paraphysis.
S. C.—Superior commissure.
Si.—Sinusoid.
T.—Tubule.
Ves.—Vessel.
V.—Velum transversum.

THE DEVELOPMENT OF THE THYMUS.

BY

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WITH 3 PLATES AND 5 TEXT FIGURES.

This paper is intended mainly as a contribution to our knowledge of the histogenesis of the thymus in mammals. Special attention is given to the origin and development of the corpuscles of Hassall, since their mode of formation has never been satisfactorily described in mammals and their significance in all forms is in dispute. An attempt is also made to show in detail the changes that occur during the transformation of the thymus from the epithelial to the lymphoid condition.

This work was begun at the suggestion of Dr. D. D. Lewis at the University of Chicago. The greater part of it has been done at the University of Missouri. Special acknowledgments are due Dr. C. M. Jackson for valuable criticism and suggestions. I wish also to thank Mr. Charles H. Miller of the University of Chicago for his kindness in sending me material.

MATERIAL AND METHODS.

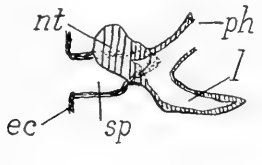
As material for the greater part of my work, I have used pig embryos from 8 mm. to full term (26 cm. to 30 cm.). These are especially suitable for such work since they may be procured in abundance from the large packing houses at almost any stage of development. For special purposes I have studied a few specimens from human fœtuses, and from the cat, rat, and guinea pig. The smaller pigs used (8 mm. to 27 mm.[1]) belong to the collection in the anatomical laboratory at the University of Missouri. These were stained in bulk with alum-cochineal and mounted in serial sections. In the later stages, which were prepared specially for this work, the ventral half of the cervical and anterior thoracic regions was usually cut out and embedded from pigs from 3 cm. to 8 cm. On specimens from 8 cm. to 30 cm., I dissected out the thymus and used such parts as were desired.

[1] The crown-rump measurement is used in all cases.

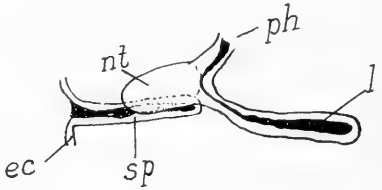
All pig material was fixed in Zenker's fluid, embedded in paraffin, and mounted in serial sections from 3 μ to 10 μ thick. Except those of the young stages (8 mm. to 27 mm.) the sections were stained on the slide. Most of them were stained with hæmatoxylin or iron-hæmatoxylin and counterstained with Congo red. For special purposes many other stains were used.

To demonstrate the delicate protoplasmic threads of the syncytium during the later stages of the lymphoid transformation, I stained by the iron-hæmatoxylin method but omitted the final decolorization in iron-alum. Protoplasm is stained deep black; nuclear structure is poorly shown, but the finest cytoplasmic processes may be seen.

For the demonstration of connective tissue fibrillæ in the syncytium, I found the method recommended by Jackson (13, S. 39) most satisfactory.



Text Fig. 1.



Text Fig. 2.

Text Figure 1. Cranial view of third gill pouch (thymic anlage); × 33; pig embryo, 11 mm.; *ec*, ectoderm; *l*, lumen; *n t*, nodulus thymicus; *ph*, connection to pharynx; *s p*, sinus præcervicalis.

Text Figure 2. Ventral view of thymic anlage; × 33; pig embryo, 15 mm.; *ec*, ectoderm; *l*, lumen; *n t*, nodulus thymicus; *ph*, connection to pharynx; *s p*, sinus præcervicalis.

To determine the relation of the blood-vessels to the corpuscles of Hassall, I put a young kitten under deep anæsthesia and injected a large quantity of a strong aqueous solution of Prussian blue into the aorta through the common carotid artery. The heart continues to beat even after an amount of fluid twice as great as the total volume of the blood has been injected. An injection made in this way is under a slightly increased blood pressure and easily reaches the finest capillaries. There is therefore a thorough injection with little danger of rupturing delicate blood-vessels.

Organogenesis.

My observations on this phase of development are not sufficiently complete to warrant a full discussion. A brief survey may however prepare the way for a better understanding of the histogenesis. The text figures show the shape in outline of the third gill pouch and thymic anlage

from 11 mm. to 27 mm. They are graphic reconstructions. Since this pouch is nearly all converted into thymus it may be regarded as the thymic anlage from a very early stage.

At 11 mm. (Text Figure 1), the pouch is a hollow epithelial tube directed from without ventrally and mesially. The lumen (*l*) is large and communicates freely with the pharynx. On the dorso-lateral aspect of the pouch is a solid epithelial mass (*n t*) distinctly different in structure from the rest of the pouch. This is the nodulus thymicus (Kastschenko, 14) and will be referred to by that term. This structure has been described by Stieda (26), Prenant[2] (22), and others as the anlage of the carotid gland.

It was evidently mistaken by Minot[3] in a 12-mm. pig for the anlage of the entire thymus. It may be seen as early as the 8 mm. stage budding off from the cranio-lateral aspect of the pouch. Immediately behind the nodulus thymicus, but not connected to it at this stage is the inner blind extremity of the sinus præcervicalis (*s p*). These become fused at 12 mm. or 13 mm.

At 15 mm. (Text Figure 2) the thymic anlage is more elongated. It now projects ventrally and medianwards, its free end lying immediately caudad to the median thyroid anlage and just craniad to the pericardium. Its lumen (*l*) is still in communication with the pharynx. The sinus præcervicalis (*s p*) is drawn out, its lumen being smaller and longer. It is now fused to the outer two-thirds of the posterior aspect of the nodulus thymicus (*n t*).

At 18 mm. (Text Figure 3) the anlage is growing rapidly in a caudal direction and just entering the thoracic cavity. It is connected to the pharynx by a delicate epithelial cord. There is still a lumen in its caudal part. The sinus præcervicalis has lost its connection to the nodulus thymicus.[4] The outer part of its lumen has disappeared and it seems about to lose its connection with the ectoderm.

At 20 mm. (Text Figure 4) the thymus extends well into the thoracic cavity. Its thoracic segment (*t h*) has increased considerably in size and is united to the gland of the opposite side.

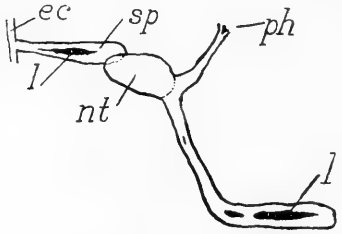
The nodulus thymicus still forms the greater part of the head. The anlage has no connection with the pharynx or the epidermis. There is

[2] Prenant is said to have since abandoned this idea and accepted Kastschenko's view. (v. Ebner in Kölliker's Gewebelehre des Menschen. Aufl. 6, Bd. 3, 1, S. 340.)

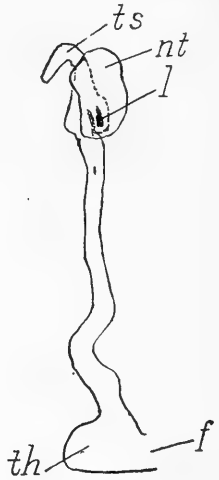
[3] Laboratory Text of Embryology, p. 191; also p. 209 and Fig. 124.

[4] On the opposite side in this specimen, these structures were fused over a very small area.

now an elongated mass of thymic tissue extending upwards behind the nodulus thymicus, and fused with it. Its upper pointed extremity curves outwards around the hypoglossal nerve. This is the "thymus superficialis" (*t s*) of Kastschenko and is regarded by him as being formed from the sinus præcervicalis. Kastschenko describes this elongated portion as being always separate from the rest of the head, being connected only by connective tissue. In my preparations it is clearly continuous with the rest of the anlage, and seems to have formed by growing out from it. The presence of a lumen in its lower end favors Kast-



Text Fig. 3.



Text Fig. 4.

Text Figure 3. Ventral view of thymic anlage; × 33; pig embryo, 18 mm.; *ec*, ectoderm; *l*, lumen; *n t*, nodulus thymicus; *ph*, connection to pharynx; *s p*, sinus præcervicalis.

Text Figure 4. Ventral view of thymic anlage; × 33; pig embryo, 20 mm.; *f*, area fused with gland of opposite side; *l*, lumen; *n t*, nodulus thymicus; *th*, thoracic segment; *t s*, thymus superficialis.

schenko's view, for there is no lumen in the head at 18 mm. in my preparations. On the other hand the separation from the head at the 18 mm. stage favors the idea that the sinus præcervicalis degenerates. I have not studied a sufficient number of specimens at this transition stage to enable me to decide this point, though I believe the ectoderm takes no part in the formation of the thymic anlage. Kastschenko's results are opposed by nearly all other students of this problem, but his work should not be discarded before the development of the thymus superficialis in the pig has been accurately determined.

At 27 mm. (Text Figure 5) the thymus is much longer and extends

well down into the thoracic cavity in relation to the base of the heart where it has fused with the gland of the opposite side. Buds are now beginning to form through the greater part of its extent. Traces of the original lumen (*l*) may still be seen in several places. The " thymus superficialis " is bent around the twelfth nerve. A delicate cord of thymic

TEXT FIG. 5.

TEXT FIGURE 5. Ventral view of thymic anlage; × 33; pig embryo, 27 mm.; *f*, area fused with gland of opposite side; *l*, lumen; *n t*, nodulus thymicus; *th*, thoracic segment; *t s*, thymus superficialis.

tissue fused with the back of the nodulus thymicus connects the thymus superficialis to the rest of the head.

From about 3 cm. until toward the end of fœtal life the thymus shows the two constrictions, described by Prenant (22) (for the sheep) as the intermediary and the cervico-thoracic cords. These cords connect three

enlargements which we may call the head, the mid-cervical segment, and the thoracic segment. I will consider each part separately. The thoracic segment develops rapidly, spreading out above and in front of the heart. The glands of the two sides fuse completely in this region. The lymphoid transformation is noticeable at 3.5 cm. and well advanced at 4.5 cm. The medulla begins to form at 8 cm. The cervico-thoracic cord is at first very narrow but soon thickens and joins the cord of the opposite side. At full term they form a sharp constriction, 3 mm. to 4 mm. wide and 5 mm. to 6 mm. long, situated at the superior aperture of the thorax, and connecting the mid-cervical and thoracic segments. The histological changes take place here later than in the enlargements.

The mid-cervical segment develops like the thoracic segment but somewhat more slowly. Budding, lymphoid transformation, and formation of the medulla all begin here a little later than in the head and thoracic segment. Its caudal end is slightly in advance of its cranial end. The intermediary cord is well marked at 4 cm. It soon becomes very attenuated, having at 6 cm. in many places a diameter of only 15 μ. Prenant suggests that this drawing out of the gland is caused by the rapid growth of the neck. Later it increases in size and at full term is noticeable only as a very slight constriction between the head and the mid-cervical segment. The histological changes are much later here than in other parts of the gland.

The greater part of the head in the early stages is formed by the nodulus thymicus. This body grows slowly, attaining a diameter in cross-section of .5 mm. at 8 cm. Its cross-sectional area at 8 cm. is about one-third that of the rest of the head of the thymus. At this stage the nodulus thymicus is a rounded body lying on the inner aspect of the head in relation to the carotid artery. A small area of its outer surface is fused with the lymphoid tissue of the thymus. Its histological structure has been fully described by Prenant (22). From the earliest stages, it consists of cords of epithelial cells separated by blood capillaries. At 8 cm. the thymus superficialis (Kastschenko) is a large body lying craniad and dorsal to the rest of the head but connected to it at its caudal extremity.

I have no observations on the head of the thymus between 8 cm. and full term, having overlooked it in collecting my material. In a full term pig (30 cm.), the cervical part of the thymus is in two distinct parts. The postero-ventral part, representing part of the head, the intermediary cord, and the mid-cervical segment, is about 3 cm. long and 1 cm. wide. It extends from the upper border of the thyroid cartilage to the thorax, and with the corresponding part of the opposite gland encloses the

trachea, thyroid, and lower part of the larynx. A slight narrowing at the junction of the upper and middle thirds indicates the position of the intermediary cord. The antero-dorsal part, the thymus superficialis, is rounded in cross-section, tapering to a point behind. Its anterior end is about 7 mm. in diameter and loops around the twelfth nerve as in the earliest stages. A lobule hangs over ventral to the nerve, a thin cord being dorsal to it. The posterior end of the thymus superficialis extends to the cricoid cartilage dorsal to the postero-ventral part of the gland. It is united to this part of the gland by a very delicate band of thymic tissue. I did not find the nodulus thymicus at full term. It has either moved away from the head or degenerated.

The duct of the thymus is the lumen of the third gill pouch. A glance at the Text Figures will show its development. It is broken up into segments and finally obliterated. I could find no traces of it at 3.7 cm. or later. On the theory of the exclusively endodermal origin of the thymus, I cannot explain the absence of a lumen in the head at 18 mm. and its presence at 20 mm. and 27 mm. unless it be due to individual variation in different specimens.

It appears from the foregoing that in the pig the exclusively endodermal origin of the thymus from the third gill pouch is probable, but a slight participation by the ectoderm has not been satisfactorily excluded. Kastschenko's conclusions, however, as to the ectodermal origin of the thymus are unwarranted by his recorded observations. Prenant, after his careful work (on the sheep), was not sure that a small mass of ectoderm did not enter into the formation of the head. Practically all other investigators of this problem maintain that the ectoderm takes no part in the formation of the thymus. The epithelial body (nodulus thymicus) developing in connection with the head of the thymus from the third gill pouch does not form the carotid gland. Kastschenko's description of the origin of the carotid gland in mammals from the adventitia of the internal carotid is now accepted by the majority of anatomists, and it therefore has nothing to do with the thymus in origin.

The Histology of the Fully-Formed Thymus.

Before taking up the histogenesis, I shall briefly consider the histology of the gland as shown in a 24-cm. embryo. At this stage the gland may be regarded as fully formed. As is well known, the thymic lobule consists of a cortical and a medullary portion,—the medulla of all the lobules being united by the medullary cord. The cortex consists of a delicate reticulum with its spaces well filled by cells, usually lymphocytes. The reticulum may be regarded as composed of small branched anastom-

osing cells, though of course no cell boundaries are distinguishable. The nuclei are poor in chromatin, rounded, and usually 4.5 μ to 5.5 μ in diameter. The amount of cytoplasm around the nuclei and connecting them is usually very small. At some nodes there is a greater amount of cytoplasm giving the appearance of a large reticulum cell. In connection with the blood-vessels, which are numerous in the cortex, are often found branched cells with pale nuclei and cytoplasm that stains more intensely than that of the rest of the reticulum. By a modification of Mallory's method used by Jackson (13, S. 39), I have been able to demonstrate numerous fibrillæ in the reticulum. In some cortical areas at this stage there are a great many erythroblasts. Masses of free erythrocytes are often found, usually near a comparatively large vessel, but such cells occur singly anywhere in the cortex.

In the medulla, the syncytial character of the stroma is much more pronounced. The cytoplasm is much more abundant than in the cortex, and the spaces are smaller and not so numerous. There is not so much room for lymphocytes as in the cortex, hence the lighter color of the medulla in stained preparations. The nuclei of the syncytium are either pale or dark, both types showing wide variations in size. By Jackson's method (13, S. 39), fibrillæ may be readily demonstrated in the syncytium. In Plate I, Fig. 6, is shown the arrangement of these fibrillæ (*s f*) in the medulla at 24 cm. They often may be traced into the areas which are forming the concentric corpuscles. In some parts of the medulla the fibrillæ are very numerous; in a few places, entirely absent. In both cortex and medulla eosinophile cells are often found. These occur in groups in the interlobular tissue around the blood-vessels, around some of the corpuscles of Hassall, and singly in the reticulum. These have been described by Schaffer (24). Free erythrocytes are rarely found in the medulla. In the medulla are also found the corpuscles of Hassall. Since the structure of these bodies depends largely upon their age, it may be better understood from the consideration of their development.

The Lymphoid Transformation.[5]

Kölliker, in 79, first advanced the idea that the leucocytes are formed directly from the epithelial cells of the thymic anlage. According to Minot (in human embryology, p. 878), "he records for the rabbit that between the twentieth and twenty-third days the cells of the thymus become smaller and their outlines disappear, so that the organ appears to

[5] This term will be used to include those changes that occur in the thymus during its passage from an epithelial to a characteristic lymphoid structure.

be an accumulation of small round nuclei. At about the same period blood-vessels and connective tissue grow into the epithelial anlage."

His (12 b), **80,** and Stieda (26), **81,** claimed that the corpuscles of Hassall are the only remnants of the epithelial anlage, that the lymphocytes, reticulum, etc., are of mesenchymal origin.

Maurer (17 a), **86,** described the leucocytes as arising directly from the cells of the epithelial anlage in the thymus of teleosts. In the amphibian thymus (17 b), **88,** he thinks that the leucocytes are probably of mesenchymal origin. He was unwilling to conclude that they arose from the epithelium because he could not find transition forms. In lizards (17 c), **99,** he records that even before the separation of the epithelial anlage of the thymus from the pharynx, changes begin. The peripheral cells are closely crowded together and show many mitoses. There arises between the central cells, or is formed in vacuoles in their protoplasm, a fluid which accumulates until the nuclei surrounded by a thin zone of protoplasm are connected only by protoplasmic threads. A loose medulla is thus formed which looks like a cellular reticulum. The cortex is still solid. The lymphocytes are formed from the epithelial cells; none come from without. Later, blood-vessels and connective tissue grow in. He believes that the reticulum is of mesenchymal origin in all forms. Maurer (17 d), **02,** still holds that in amphibians the lymphocytes are probably of mesenchymal origin.

Hermann et Tourneux (11), **87,** find that in man and other mammals the epithelial anlage of the thymus is gradually converted into leucocytes and reticulum cells. Vacuoles appear during the transformation which seem to be formed by the absorption of large cells. In a sheep embryo of 130 mm., the clear epithelial cells have all disappeared, giving rise to small round cells and reticulum cells. Prolongations of connective tissue, each containing a blood-vessel, grow into the anlage during the transformation. They are not sure that all the thymic elements are epithelial in origin, being especially in doubt about the origin of some of the reticulum cells.

Gulland (8), **91,** describes the development of the tonsil in the rabbit. Leucocytes first appear in the connective tissue around the thymus. Later they appear in the connective tissue around the tonsil. They infiltrate the tonsillar epithelium. No leucocytes are of epithelial origin. After studying the tonsil he examined the thymus in a few specimens and concluded that the same process of leucocyte infiltration occurred there. He does not give the details of their infiltration, and did not see any of the transition forms of nuclei in the thymus at that period.

Prenant (22), **94,** made a careful study of the development of the

thymus in sheep embryos. His results are as follows. At 25 mm. the gland is composed of distinct polyhedral cells with nuclei of only one kind. A few mitoses and an occasional direct division are to be seen. At 26 mm. mitoses are numerous (one nucleus in fifty). Nuclei are regularly rounded or elliptical and some small nuclei occur juxtaposed to large nuclei. At 28 mm. many mitoses are present and irregular spaces have appeared. These spaces are not blood-vessels nor parts of the thymic duct but vacuoles. Some nuclei, noticeably small and darkly colored, lie close to the large, clear nuclei and seem to be budded off from them. Some nuclei (rare) are broken into three or four chromatic bodies. Embryos of 40 mm. have undergone in great part the lymphoid transformation. All transitions are found between the large, pale elliptical nuclei of clear reticular structure and the small, deeply colored rounded nuclei whose sap is strongly stained. These last are certainly lymphocytes and constitute an immense majority of the cellular elements. Large, clear nuclei are found joined to small dark ones—nuclear couples.

At 85 mm. the medulla appears; the cortex corresponds to the entire thymic mass of preceding stages. The cortex contains a great many lymphocytes separated by islands and rows of pale nuclei. There are about thirty lymphocytes to one pale nucleus. Mitoses are numerous in the cortex. In the medulla at this stage, the large clear, and small dark, nuclei are about equal in number, and mitoses are rarer than in the cortex. In later embryonic stages a clear peripheral zone is present where cell proliferation takes place. Mitoses are now more numerous in the medulla than in the cortex. It is probable that a certain number of the epithelial cells persist as reticulum cells in the fully-formed organ.

J. Beard (3 a), **94**, (3 b), **99**, thinks that the function of the thymus is to form the first leucocytes. He finds that in the skate the epithelial cells are converted early into lymphocytes which emigrate into the blood. There are many breaks in the gland where the lymphocytes escape in masses. The thymus is the only source of leucocytes until the other lymphoid organs are formed.

Ver Eecke (28), **99**, finds that in the frog the epithelial thymus is invaded by lymphocytes and connective tissue. The epithelial cells are not destroyed but merely dispersed by the mesenchymal elements. He calls the resulting tissue lympho-epithelial. This idea of the commingling of the two tissues had already been advanced by Retterer.

Nusbaum and Prymak (20), **01**, on teleosts, agree with Maurer that the lymphocytes are of epithelial origin but disagree on the details of their formation. They find that the epithelial anlage is at first composed of cells with distinct boundaries. It is not different from the epithelium

of the pharynx. Before any blood-vessels or connective tissue have invaded the organ, changes begin in the central part. These changes consist in the breaking up of the cytoplasm so that the cells become branched and connected by delicate processes. These processes finally break apart leaving a nucleus surrounded by a thin layer of protoplasm—a lymphocyte. The peripheral epithelial layer multiplies rapidly, forming nuclei somewhat smaller and darker than their own. These nuclei become gradually changed into the nuclei of lymphocytes and break away from the other cells. All transitions are present between the large, clear epithelial nuclei and the lymphocytes. Blood-vessels and connective tissue grow in from the outside.

It appears from a survey of the literature that, of those who have studied the origin of lymphocytes in the mamalian thymus, His, Stieda, and Gulland have advocated the idea that they invade the gland from without, and that the original epithelial anlage persists only as remnants, the corpuscles of Hassall. They also consider the stroma of mesenchymal origin. On the other hand, Kölliker, Hermann and Tourneux, and Prenant, have described the lymphocytes as derived directly from the epithelial cells of the anlage. Hermann and Tourneux and Prenant ascribed a similar origin to part of the reticulum.

Neither His, Stieda, nor Gulland made a detailed histological study of the changes that take place in the thymus during the transformation. They did not see the vacuolization of the cytoplasm, the changes in the epithelial nuclei, etc.—processes which undoubtedly occur. Gulland made nearly all his observations on the tonsil and then from a superficial examination of the thymus concluded that the process is the same there. The conclusions of these men are therefore not to be compared on this point with those obtained by the thorough and careful work of Prenant.

On amphibians, Maurer hesitatingly agrees with His, and Ver Eecke accepts the mesenchymal origin of the leucocytes; while on fishes Maurer, Beard, and Nusbaum and Prymak believe in the epithelial origin of lymphocytes. Maurer's work on reptiles is in agreement with his work on teleosts.

As to the origin of leucocytes in the lymphoid organs of the alimentary canal, opinion is divided. Retterer, v. Davidoff, Rudinger, Klaatsch, and others have described the leucocytes as arising from epithelium and being invaded by mesenchymal elements forming adenoid tissue. Stöhr, Gulland, Tomarkin, and others describe them as penetrating the epithelium from without.

I shall now discuss my own observations on the lymphoid transformation in the thymus of the pig. From a very early stage (11 mm.), the

epithelium of the third gill pouch is a syncytium. No cell boundaries exist. The nuclei, large and irregular in shape, are embedded in a common mass of cytoplasm. In the thymus at 20 mm. I find a syncytium of dense cytoplasm embedded in which are large nuclei of irregular shape and size. No distinct types of nuclei are present yet; all stain with medium intensity. A few mitoses are to be seen.

In a section of the mid-cervical segment at 3.7 cm. (Plate I, Fig. 1), I find evidence that the lymphoid transformation has begun. The syncytium is composed of coarsely reticulated cytoplasm much looser in texture than that of the preceding stage. It contains a few irregular spaces (*s s*) which are evidently of the nature of vacuoles. These may be formed, as Maurer suggests, by liquefaction of the cytoplasm. There is no reason to suppose that cells degenerate and form them as Hermann and Tourneux believed. Three types of nuclei may be distinguished; large pale nuclei (*l p n*) large dark nuclei (*l d n*), and small dark nuclei (lymphoblasts) (*lb*). Transition forms occur between these types. The large dark nuclei are intermediate forms between the pale nuclei and the lymphoblasts. A few mitoses (*m*) occur. No blood-vessels are present inside the anlage but they may be seen between the buds just outside. At this stage, the head and the thoracic segment have areas that are somewhat farther advanced than this. The intermediary and cervico-thoracic cords show no changes.

At a later stage than the above (Plate I, Fig. 2), in the thoracic segment of a 4.5-cm. pig, the spaces of the syncytium (*s s*) have increased greatly in number and size. The anlage is now a cellular reticulum. The large pale nuclei are somewhat less numerous than the dark nuclei and many have become angular, adapting themselves to the nodes of the syncytium. They contain less chromatin than in the preceding stage. Large dark nuclei and lymphoblasts are present; the lymphoblasts are much more numerous than in the preceding stage. A very few small dark nuclei are completely separated from the syncytium. These are lymphocytes. There are no lymphocytes in the connective tissue around the thymus or in the blood at this stage. I did not examine the tonsil or spleen at any stage. A few small blood-vessels are to be seen; their walls consist of endothelium only. There are more mitoses than at the preceding stage, but none happened to be present in the area shown in the figure. During mitosis, at all stages of development, except the early epithelial condition, the chromosomes are so closely packed that it is very difficult to distinguish them individually.

In a section through the mid-cervical segment of a 7-cm. pig (Plate I, Fig. 5), we see a stage somewhat later than the one shown in

Fig. 2. In various parts of the section lymphocytes (*l*) are completely formed. The great majority of the small round nuclei are in the lymphoblast (*l b*) condition, i. e., they are not yet completely separated from the syncytium. There are a few lymphocytes outside the thymus in the interlobular tissue in this region; around the head and the thoracic segment at 7 cm. they are numerous, these parts being in a later stage of transformation. I have never seen lymphocytes outside the thymus, where there were none inside it; but they appear outside shortly after they are formed here. Those formed next the interlobular septa seem to pass out very early. Of course the lymphoblasts, which are distinguishable from the lymphocytes only by being imbedded in the syncytium, are to be seen in the thymus long before any appear outside.

At the stage shown in Fig. 5, a great many nuclei are in mitosis. I have not seen at any stage, the amitoses and nuclear couples described by Prenant for the sheep. In some parts of the section comparatively large solid epithelial areas occur. These are found as often in the central as in the peripheral part. Many of the pale nuclei are smaller than those shown in Fig. 2. The blood-vessels are somewhat larger and more numerous than those at 4.5 cm.

It is to be noted that the epithelial anlage does not at any stage become converted entirely into small round cells as many observers have stated. Distinctly pale angular reticular nuclei can always be seen.

In the mid-cervical segment at 8.5 cm. (Plate I, Fig. 4), a great many lymphocytes (*l*) are formed. These lie between the persisting epithelial cells which are now arranged in irregular cords and islands. In these epithelial masses, lymphoblasts may still be seen indicating that the formation of lymphocytes is still in progress. Many of the pale nuclei are now small. The heavy hæmatoxylin stain in this case makes the nuclei darker than they would appear with an ordinary stain. A few nuclei are in mitosis.

This figure shows also the first appearance of the medulla (*md*). The medulla is formed directly, as shown in the figure, from persisting parts of the epithelial syncytium. Certain centrally situated masses of this syncytium undergo changes of such a nature that they stain readily with cytoplasmic stains such as Congo red. In sections stained with hæmatoxylin and Congo red, the medulla is first recognized as a brightly colored area situated usually about the center of the lobule. These epithelial masses that give rise to the medulla seem to increase in size about the time of the change in staining capacity. The first differentiation of the medulla is chemical rather than morphological, for there are other persisting epithelial masses even larger than it in the same section

that do not react in the same way with the cytoplasmic stains. The medulla appears in the head and the thoracic segment at 7.5 cm. to 8 cm. All the gland except this small central area forms the cortex. Blood-vessels now reach all parts of the gland, but are still few in number. I cannot distinguish any wall except the endothelium on those actually inside the gland.

In a 9.5-cm. pig, the medulla is larger. It contains pale nuclei of various sizes, large dark nuclei, and lymphoblasts. Its spaces are smaller than those of the cortex. The early stages in the formation of the corpuscles of Hassall appear as soon as the medulla begins to form. The epithelial cords in the cortex have become less conspicuous, but are still forming lymphocytes. A few nuclei are in mitosis. Many blood capillaries may now be seen penetrating the gland from the periphery. These vessels run in the epithelial masses and have a wall of large endothelial cells which gives them the appearance of radiating cords. When these vessels first appear, as at this stage, they have only an endothelial wall. The blood-vessels grow in as small capillaries which, after their entrance, increase in size and branch; they do not break in as large vessels surrounded by mesenchymal tissue. I am fairly sure that aside from the endothelial cells few or no mesenchymal cells come into the thymus. Around the greater part of the periphery of the gland is a solid zone of syncytium two or three nuclei deep which is in transformation like the epithelial cords inside. This zone, described by Prenant as a zone of proliferation, grows rapidly, as the frequent mitoses indicate. Its inner boundary is forming lymphocytes and reticulum cells.

In a 14-cm. pig, the lymphoid transformation is practically at an end except in the medulla. The peripheral zone of proliferation has disappeared. The cortex has about the same structure as at 24 cm., as previously described. In the medulla, lymphoblasts, large pale nuclei, and the large dark intermediate types are still present. There are a few mitoses here. It is very probable therefore that the formation of lymphocytes is still in progress in the medulla. The medulla at 24 cm. shows a similar structure except that there are fewer lymphoblasts. These facts persuade me to regard the medulla as a center for lymphocyte formation at least as late as birth. Connective tissue fibrillæ begin to appear in the gland along the large blood-vessels and the interlobular septa as early as 10.5 cm. They are only a little farther in at 16 cm.; but near full term they are present in nearly all parts of the stroma. (See Plate I, Fig. 6.)

The above account may be summarized as follows: In the pig the epithelial syncytium of the thymic anlage becomes loosened up by the

formation of vacuoles in it. These vacuoles increase in number and size, converting the anlage into a cellular reticulum. While this vacuolization is in progress, the nuclei, which at first are of one kind with a medium amount of chromatin, differentiate into large clear, large dark, and small dark (lymphoblast) forms. The large dark nuclei probably divide by mitosis and form the lymphoblasts. The lymphoblasts gradually break loose from the syncytium, passing into its spaces and becoming lymphocytes. Shortly after lymphocytes begin to be formed, some of them pass out of the gland into the surrounding connective tissue. The lymphoid transformation begins in embryos of 2.5 cm. to 3 cm. and continues in the cortex until 12 cm. or 13 cm. In the medulla it is not complete at birth. Since the thymus increases greatly in size during this period the epithelial syncytium must grow rapidly. Lymphocytes are constantly being formed at the expense of the growing syncytium. A peripheral zone of proliferation is present from about 8 cm. to 12 cm. The medulla is formed as a chemical differentiation of certain centrally situated areas of the epithelial syncytium. The histological changes occur earlier in the head and thoracic segment than in the mid-cervical segment and very much earlier than in the cords. The reticulum of both cortex and medulla is practically all of epithelial origin. Some branched cells around the blood-vessels in the cortex may be of mesenchymal origin.

My reasons for regarding the lymphocytes as of epithelial origin are as follows:

A. The lymphoblasts are true epithelial nuclei, because (1) there are numerous transition forms between them and the large dark nuclei which later cannot be regarded as invading lymphocytes; (2) they are closely embedded in the syncytium and show no evidence of having eaten their way through the protoplasm; (3) they are present from a very early stage and increase in number as development proceeds; (4) they are present before blood-vessels invade the gland and have no constant relation to blood-vessels or to the surface of the gland that indicates an invasion from either of these directions; (5) they are present before lymphocytes appear in the connective-tissue around the thymus.

B. Some observers admit that the small dark nuclei (lymphoblasts) are of epithelial origin but do not admit that they form lymphocytes. The considerations that lead me to believe that the lymphoblasts do form the lymphocytes are: (1) the small dark nuclei (lymphoblasts) show every possible relation to the syncytium from being completely embedded in it to lying free in the syncytial spaces. A comparison with later stages shows that this appearance is not due to poor fixation or to the

adherence of the nuclei to the reticulum; (2) the first free nuclei often appear in the center of the gland when there are no other free nuclei in the periphery at that level; (3) there is good evidence that lymphocytes emigrate from the thymus in large numbers. If we examine the thymus of a 7-cm. pig in serial sections we find that the lymphoid transformation is less advanced in the mid-cervical segment than in the head. In the mid-cervical segment there are a few lymphocytes in the interlobular tissue. In the lower end of the head where there are more lymphocytes inside the gland, lymphocytes pack the interlobular tissue and form a thin zone around the periphery of the gland. In the middle of the head where the transformation is far advanced, lymphocytes pack the interlobular tissue and form a thick zone around the entire gland. Indeed, in some sections, there are more lymphocytes in the zone outside than are present inside the gland. If this zone of lymphocytes be passing into the gland, it is not easy to understand why it is formed from within outwards, and why it is thickest where the greatest number of lymphocytes are already present inside. No satisfactory suggestion has yet been made as to why lymphocytes should thus suddenly pour into the thymus at a time when if present at all elsewhere they are rare. They do not come to break up the thymic epithelium, for that is already a reticulum before free cells are present (Fig. 2, Plate I). Where lymphocytes invade intestinal epithelium as in the tonsil they eat paths through it leaving spaces. The epithelial reticulum of the thymus is not formed in that way. On the other hand it is not difficult to believe that this zone of lymphocytes is formed by cells passing out the periphery of the thymus and that the gland thus contributes a great number of lymphocytes to the organism; (4) I have not been able to find lymphocytes in the connective-tissue around the thymus before they are present inside. An invasion by way of the blood-vessels may be excluded, since the thick zone of lymphocytes formed around the gland shows that these cells either enter or leave it through the preiphery.

The Corpuscles of Hassall.

These bodies were first mentioned by Hassall (10) in **46**. He speaks of them as being composed of mother cells which enclose the newly-formed daughter cells and nuclei. He thought the central mass was formed by the outer enclosing layers. He found bodies which he regarded of the same nature in fibrous coagulations in the heart.

Virchow (29), **51**, in a discussion of endogenous cell formation, compares Hassall's corpuscles to carcinoma pearls. He had about the same

conception of the nature of the corpuscles as Hassall. This oft-quoted comparison was therefore not based upon a deep insight into their nature.

Günzburg (9), 57, did not advance beyond Hassall's conception that the central mass is formed by the peripheral layers.

Paulitzky (21), 63, described the center of the corpuscles as homogeneous or granular. They sometimes contain an elliptical nucleus, sometimes fat droplets. The larger ones have in the center several nuclei or cell-like forms. The central part is formed from masses of epithelial cells. Connective tissue cells grow around them and are transformed into epithelial cells forming the peripheral part of the corpuscle.

The term "concentric corpuscles" was introduced by Ecker (6), who described them as arising directly from gland cells by fatty metamorphosis. He distinguished (1) simple corpuscles, round vesicles with thick concentric hulls, containing inside a fatty opalescent mass, and (2) compound corpuscles, which consist of several vesicles with a common hull. The peripheral layers of a corpuscle consist of flattened cells.

His (12 a, 12 b), 60, 80, described the corpuscles as consisting of an outer striated shell, probably composed of nucleated cells, and containing lymphocyte-like cells inside. He supposed them to be the original cells of the epithelial anlage which become entangled in the reticulum in some way. Their rapid growth in their narrow confines causes the concentric form.

Cornil et Ranvier (5), 69, considered the corpuscles as arising from the endothelium of blood-vessels and compared them to the spheres of their "Sarcôme angiolithique."

This suggestion of a vascular origin, made by Cornil et Ranvier, was elaborated by Afanassiew (1a), 77.

Afanassiew held that the corpuscles of Hassall arise from the endothelium of the smaller veins and capillaries. The endothelial cells increase in size, become cubical, and later fill the lumen of the vessel. During the proliferation of the endothelium, the blood-vessels break up into segments which are now nearly solid cords. These cords are at first connected to each other and to blood-vessels, but they soon break apart. The surest proof that the corpuscles are of vascular origin is that erythrocytes may be found inside them. Vascular injections, however, do not go into a corpuscle except in a very early stage, since the lumen is soon obliterated by the endothelial plugs. The corpuscles are formed entirely by the endothelial cells. Afanassiew worked on embryos of man, the rabbit, and the calf.

Stieda (26), 81, in sheep embryos, describes the epithelial mass of the

thymic anlage as being broken up by ingrowing adenoid tissue. From 50 mm. to 60 mm., there are no large epithelial cells; but later at 100 mm. he finds in the adenoid tissue large cells 9 μ to 15 μ in diameter, isolated or united in groups, whose protoplasm colors light-red with carmine. These large cells have a concentric structure. Some of them are enclosed by large cells whose cytoplasm does not color with carmine, giving rise to a yellowish mass of irregular form and stratified appearance. In older embryos (250 mm.), the cellular masses are numerous but the large colored cells are rare. The yellowish masses are groups of the large cells which have undergone a transformation like that of the stratum corneum of the epidermis. Stieda considers the large colored cells which form the corpuscles as remnants of the epithelial anlage, although he admits that for a long period during development he found no trace of them. He explains the formation of the corpuscles in accordance with Cohnheim's hypothesis that most tumors arise from unused tissue remnants.

Ammann (2) **82,** made most of his observations on human fœtuses. He describes the corpuscles as arising from connective tissue. The corpuscles are cellular in structure and are formed of one, two, or three central cells around which a variable number of cells, increasing with age, are arranged like the coats of an onion. The corpuscles are formed from reticulum cells and leucocytes. Growth consists in the apposition of cells from without. The life of a corpuscle consists usually of four stages: (1) Stadium der Transparenz; (2) Stadium der colloiden Entartung; (3) Stadium der Verkalkung; (4) Stadium des Zerfalls. The nucleus of a reticulum cell or leucocyte increases in size at the expense of the cell body. Its increase in size establishes the concentric form. The corpuscle undergoes colloid and usually calcareous degeneration. Fat droplets, cholesterin crystals, and colloid granules are found together in the degenerating corpuscles. Breaking up in this way makes absorption possible. No epithelial remnants are to be observed. No erythrocytes are found in the corpuscles.

In four cases of atrophic thymus gland which yet contained lymphoid tissue Ammann found corpuscles in all stages of development. He also found that the corpuscles are formed most rapidly when the thymus is at the height of its development. From these facts he concluded that they are not connected with the involution of the thymus as Afanassiew thought. He thought that their formation is due to a physiological decrease in the intensity of growth of the medulla, due to the rapid growth of the cortex.

Watney (31), **83,** agreed with Ammann that the corpuscles arise from connective tissue cells.

Monguidi (18), **85,** distinguished true and false concentric corpuscles—the latter being only sections of blood-vessels.

Hermann et Tourneux (11), **87,** gave a description of the structure and formation of the concentric corpuscles about like that given by Ammann except that they regard the reticulum cells from which the corpuscles develop as of epithelial origin.

Gulland (8), **91,** regarded the corpuscles as epithelial remnants and compared them to the epithelial pearls of the tonsil.

Maurer (17 c), **99,** described the corpuscles as epithelial in origin. His description of their formation is however different from that of His. All the cells of the epithelial anlage at first assume a lymphoid character. Later, some of these cells reassume their epithelial nature and then form the corpuscles. His conclusions for teleosts and amphibians are similar to the above results which he obtained from the lizard.

Ver Eecke (28), **99,** for the frog, describes the leucocytes and connective tissue cells as invading the thymic anlage and separating the epithelial cells. The epithelial cells, separated by the mesenchymal elements, lie at first in groups or singly. They go through a cycle of two phases, a stage of growth, and a stage of involution. In the former stage, they increase to three or four times their original size and their cytoplasm differentiates into circular layers like the coats of an onion. The majority are monocellular. Some cells grow together making a more complex multicellular type. There are some intermediate forms, cells with a dense dark protoplasmic body, indistinct striations, and a nucleus partly or completely hidden in a precocious degeneration. In the stage of involution, which sets in early, the cytoplasm degenerates by the formation of vacuoles containing a hyaline liquid. The liquefaction may be in the form of a diffuse vacuolization, a large central vacuole, or a peripheral vacuole circular in section. The nucleus loses its affinity for stains, becomes deformed, breaks up, and finally disappears. The corpuscles are finally absorbed. They never contain erythrocytes. The cells do not degenerate to form a corpuscle. The liquefaction forms an internal secretion which is forced out by the muscle tissue in the reticulum.

Entirely different results on amphibians are reported by Nusbaum and Machowski (19), **02.** These investigators revive the old idea of Afanassiew, accepting his results except that they think the adventitia as well as the endothelium of the blood-vessels takes part in the formation of the corpuscles. They find erythrocytes in the corpuscles. These erythrocytes either gradually shrivel and disappear, or they are absorbed by leucocytes or endothelial cells. The leucocytes after digesting the hæmoglobin of the erythrocytes become eosinophile cells which are numerous in the thymus.

Wallisch (30), 03, measured the volume of the human thymus and of the corpuscles of Hassall at various stages. He finds that the total volume of the corpuscles of a 7-mo. embryo is 4.6 mm.,[3] and of those of a 6-mo. child, 174.6 mm.[3] The total volume of the thymus of a 78-mm. embryo, when it has already been partly transformed into adenoid tissue is only 6.8 mm.[3] Since there is no evidence that the cells of the corpuscles multiply, he concludes that they cannot be regarded merely as remnants of the original epithelial anlage.

Disregarding the crude observations of the earliest investigators, there remain three distinct theories of the formation of the corpuscles of Hassall.

1. The epithelial anlage of the thymus is broken up by the invading mesenchymal elements. The separated masses of epithelial cells undergo further changes mainly of a degenerative nature to form the corpuscles. This was the belief of His and Kölliker. According to this interpretation, the corpuscles are to be regarded as remnants that have nothing further to do with the gland. Stieda, Maurer, and Ver Eecke held this view in a modified form. Stieda regarded the cells forming the corpuscles as epithelial remnants but admitted that they go through a stage in which, for a time, they lose their epithelial character. This is substantially the same as Maurer's view. He thinks that the cells of the epithelial anlage all become lymphoid, and that some of them afterwards reassume their epithelial nature and form the corpuscles. Ver Eecke regards the corpuscles as epithelial remnants but thinks that they are glandular in nature, not mere useless remains.

2. The corpuscles are formed from the proliferating walls of blood-vessels. This idea was suggested by Cornil and Ranvier and elaborated by Afanassiew. Nusbaum and Machowski accept Afanassiew's view except that they believe the adventitia of the blood-vessels as well as their endothelium takes part in the formation of a corpuscle. These investigators thought that the formation of the corpuscles is connected with the involution of the thymus.

3. The corpuscles are formed from reticulum cells of the medulla and grow by apposition of the surrounding cells. This view was advanced by Ammann. Ammann thought that the reticulum is of connective tissue origin. He also believed that leucocytes formed the central part at least of some corpuscles. Hermann and Tourneux accepted Ammann's results, except that they ascribed an epithelial origin to the reticulum. (I do not know whether they accepted the origin from leucocytes described by Ammann.) Ammann thought that the corpuscles formed because of a physiological decrease in the rate of growth in the medulla.

My own observations on the development of the corpuscles of Hassall in pig embryos, will now be considered. The medulla, as previously described, begins to form from the epithelial syncytium usually near the center of the lobule. It is first recognized by its more marked reaction with cytoplasmic stains such as Congo red. Shortly after the medulla begins to form, the earliest stages of the corpuscles may be observed. A few corpuscles have appeared at 9.5 cm. I did not find them earlier. They are all formed from the epithelial syncytium of the medulla.

Before beginning this discussion I will explain the use of my terms. By a corpuscle of Hassall, I mean a modified area of the epithelial syncytium of the medulla, containing at some period of its development, one or more nuclei, and whose cytoplasm has been in part or entirely transformed into colloid. The term colloid is applied to various substances probably of widely different chemical nature, but is fairly adapted to our imperfect knowledge. I shall use the term here in the restricted sense employed by Ziegler,[6] i. e., hyaline substances of epithelial origin, that do not give the reactions of mucin.

Colloid in the corpuscles of Hassall does not usually appear as solid masses in its early formation, but as fibers, granules, or sheets which are separated by more or less cytoplasm that is not yet changed. This stage I have called, "colloid in formation" (*c f*). It later assumes a more solid homogeneous appearance which I call solid colloid (*c s*). Often the solid colloid stains intensely with cytoplasmic stains. I call this kind solid deeply-staining colloid (*c s d*). In later stages, the colloid often loses its affinity for cytoplasmic stains, staining a very pale color or not staining at all. I call this variety old colloid (*o c*).

According to their mode of development, the corpuscles of Hassall may be classified as follows:

A. Concentric Corpuscles.
   a. Simple.
      1. Ordinary type.
      2. Epithelioid type.
      3. Cystic type.
   b. Compound.

B. Irregular Corpuscles.
   a. Compact type.
   b. Reticular type.

[6] Gen. Pathology, 10th ed., Warthin's translation, p. 205.

A. The *concentric corpuscles* include those that from their earliest appearance are concentric in structure. Adopting Ecker's classification, I distinguish simple concentric corpuscles and compound concentric corpuscles.

(a) Three types of simple concentric corpuscles are to be considered. (1) The *ordinary type* is far more numerous than any other. The earliest recognizable stage is shown in Plate II, Fig. 11. A nucleus (*n*) of the syncytium of the medulla has enlarged to perhaps twice its ordinary volume and has lost the ability to stain in the characteristic way with hæmatoxylin. Its sap is clear and a few reddish stained granules represent its chromatin. Around it in the cytoplasm is an indistinct uneven layer of colloid (*c f*). The colloid is not yet solid and is being formed in concentric fibers or sheets. A slightly later stage is shown in Plate II, Fig. 14 and Fig. 15 (left side of figure). Some of the colloid (*c s*) next to the nucleus is now solid. The next stage is shown in Plate II, Fig. 15 (right side of figure). These corpuscles show a thick layer of colloid (*c s d*) that stains intensely with Congo red. Just outside the deeply staining colloid, colloid in formation may be seen. The nuclei are clear, and have become smaller and irregular in outline. The colloid seems to be pressing upon them and obliterating them. The colloid transformation gradually involves the adjacent cytoplasm of the syncytium until other nuclei are involved. The corpuscle has now reached the condition shown in Plate II, Fig. 12. The central area (*o c*) is solid, the nucleus having disappeared entirely. Another (*n'*) is nearly obliterated by the colloid. Part of the central area (*o c*) no longer stains intensely, and it is breaking loose by the formation of a concentric space. Several nuclei are surrounded by colloid in formation. Their long axes are nearly in a tangential direction. These nuclei are clear but only moderately swollen.

In the further development of the corpuscle (Plate III, Fig. 17 and Plate II, Fig. 7), the central area (*c s d*) increases in size. The nuclei involved in this area become obliterated probably by the pressure of the colloid and are no longer distinguishable. This central area usually splits off and may break up into many smaller masses. The peripheral part of the corpuscle increases by extension of the colloid formation into the adjacent part of the syncytium. This extension takes place in the early stages by direct progressive involvement of the immediately adjacent cytoplasm; in later stages (Fig. 7), by the formation of concentric lamellæ which cut off unchanged areas of cytoplasm. The lamellæ increase in size and number, the cytoplasm included between them is changed into colloid. They finally become closely packed, giving the characteristic and

well-known onion-like structure found in the fully-formed corpuscle. The nuclei that are enclosed between the lamellæ gradually lose their chromatin and become flattened out. They do not swell and are not obliterated. It seems that swelling occurs only in nuclei that are surrounded by deeply staining colloid, and that this change is preparatory to their obliteration by or transformation into colloid. The amount of the corpuscle that breaks up to form the softer center is very variable. The size of the center usually seems to increase with the age of the corpuscle.

Plate III, Fig. 21, shows a variation from the ordinary concentric type. The central nucleus (*n*) stains reddish but is not enlarged. Most of the other nuclei are unchanged. All the colloid (*c f*) is in the early fibrous and granular stage.

From 20 cm. to full term many corpuscles show masses of calcareous material in or near the center. This material rarely appears in younger corpuscles (Plate III, Fig. 17, *c l*). It stains a violet blue with Delafield's hæmatoxylin.

The majority of the corpuscles of Hassall belong to the ordinary type of simple concentric corpuscles described above. It is very clear that they have nothing to do with blood-vessels. They never contain erythrocytes nor anything resembling them. Rarely a lymphoblast or leucocyte is found inside the corpuscle. These seem to be usually involved in the corpuscle like ordinary stroma nuclei during the formation of the lamellæ. (Their occurrence in other types will be discussed later.) It is also clear that these corpuscles arise from the syncytium of the medulla. They are epithelial in origin, since the entire stroma of the gland is derived from epithelium, but they are certainly not remnants of the original epithelial anlage. Neither are they formed from lymphoid-like elements that reassume their epithelial nature as Maurer described for the lizard.

Some of Ammann's observations are in accord with my results. The swelling of the nucleus was noted by Ammann as the first step toward the formation of the corpuscle. It should be noted, however, that rarely a corpuscle begins to form as a mass of colloid out in the cytoplasm and involves nuclei secondarily. I cannot distinguish his "Stadium der Transparenz" for I cannot be sure that a corpuscle is beginning to form until some colloid is present. The formation of the colloid is associated with the swelling of the nucleus. His other three stages, "Stadium der colloiden Entartung," "Stadium der Verkalkung," and "Stadium des Zerfalls" are easily seen. I have never seen corpuscles begin in leucocytes as Ammann described. His statement that the corpuscle grows by apposition of reticulum cells is true in a modified sense. He thought that

the outer part of a corpuscle is formed of reticulum cells that have moved up and flattened themselves out around it. The description just given shows that the corpuscles are never composed of distinct cells, and that the increase in size is due to an extension outward of the colloid formation and not to a moving in of the adjacent tissue.

The concentric form of this type of corpuscle is due at first to its being formed around a spherical or ellipsoidal nucleus. The swelling of this nucleus creates a centrifugal pressure in the adjacent cytoplasm. Before or during its transformation into colloid, the cytoplasm also increases in quantity. That the cytoplasm increases in quantity is shown by the fact that the nuclei are fewer in the corpuscle than in any adjacent area of the syncytium of equal size. This centrifugal pressure presses the newly formed colloid into concentric lamellæ. It at first turns the long axes of the nuclei tangentially, and later flattens them and makes them concave toward the center.

2. The *epithelioid type* of corpuscle is characterized by large areas of cytoplasm so marked off by colloid lamellæ as to give the appearance of a mass of large epithelial cells. They may contain only one nucleus embedded in a well-defined area of cytoplasm (Plate III, Figs. 18 and 20). These correspond to the monocellular corpuscles that have been described for lizards and amphibians. They are rare in the pig. I have not been able to trace these very far, as they soon become indistinguishable from other forms. The only difference I have noted is that the outer colloid lamellæ begin to form early, causing the peculiar appearance of a large epithelial cell. Again the epithelioid type may present an appearance such as shown in Plate I, Fig. 3. These do not seem to be formed around any special nucleus. The outer colloid lamellæ form before any center has been established, marking off large cytoplasmic areas that may look like large cells. The centrifugal pressure of expansion caused by the great increase of cytoplasm in this area determines the concentric form in these corpuscles. Pure epithelioid corpuscles are very rare, but epithelioid areas in other corpuscles are not uncommon. The occurrence of epithelioid areas in corpuscles of the ordinary type shows that it is due to variations in a fundamentally similar process.

3. In the cystic type of corpuscle, the central part, instead of becoming transformed into colloid, undergoes early liquefaction, forming vacuoles. The central nucleus does not increase in size as in the ordinary type, but shrivels up and disappears. The corpuscle begins by the formation of outer colloid lamellæ—the central mass is not changed into colloid. In Plate II, Fig. 10, the central area (p m) is undergoing a diffuse liquefaction. The nucleus (n) is colorless and shrunken. In Plate II,

Fig. 8 (right side of figure), the central area has formed two large vacuoles (v). On the left side of the same figure, a concentric vacuole (v) has formed, separating off a central spherical nucleated mass of protoplasm. The nucleus of this mass of protoplasm is shrunken and the cytoplasm shows many small vacuoles. The corpuscle shown in Plate II, Fig. 9, is probably a later stage of the form just described. The central protoplasmic mass has become converted into an ellipsoidal pale body ($p\ m$). The small circular body in this shriveled mass is probably the nucleus. Some corpuscles like the one shown in Fig. 9 are found in which the central mass has entirely disappeared. The further growth of corpuscles of this type seems to be by formation of colloid lamellæ as in the ordinary type. They soon become indistinguishable from other forms.

The cystic type of corpuscle is rare in the pig. This evidently corresponds to the form in amphibia that misled Nusbaum and Machowski into reviving Afanassiew's theory. The central masses, in Figs. 8 and 9, might readily be mistaken for red corpuscles in animals in which these cells are nucleated. But the red cells of the blood of the pig are not nucleated at this stage. I have traced a number of these corpuscles (as well as those of other types) in serial sections and have never seen any indications of a connection to blood-vessels. Nusbaum and Machowski (19), (Fig. 1, c, S. 116) show a corpuscle which is similar to my Fig. 9. It will be noted that the central space in neither of these figures is lined by endothelium. The early form of corpuscle shown by Nusbaum and Machowski (Fig. 1, d, S. 116) is very probably a normal blood-vessel with cubical endothelium. I have often found such vessels with cubical endothelium in the interlobular tissue of the pig's thymus at 10 cm. to 12 cm. They probably may be found at other stages also. In the thymus of a kitten, injected by the intra-vitam Prussian blue method previously described, the majority of the corpuscles were found to be in early stages. The injection did not penetrate any corpuscle. I had a somewhat better opportunity to study the relations of the corpuscles to the blood-vessels in a 14 cm. human embryo. Here the vessels of the thymus were all very much distended with blood and the corpuscles were in early stages. No blood cells were found in the corpuscles.

(b) *Compound concentric corpuscles* are formed whenever two or more simple concentric corpuscles begin to form so close together that they come in contact during their later growth. An early stage of such a corpuscle is shown in Plate II, Fig. 15. The colloid lamellæ are formed around each center until they come in contact; they are then formed around both centers. In Plate III, Fig. 22, a compound concentric cor-

puscle is shown. There are three simple concentric corpuscles in it—one of them (the lowest in the figure) in a very early stage. Several lamellæ are common to the older corpuscles, and one is common to all three. This arrangement of the lamellæ is a mechanical effect of the tension in the cytoplasm, due to the centrifugal pressure from the two centers. The size of the separate centers in a compound corpuscle depends upon the stage they have reached when they come in contact. If a compound corpuscle be formed by the union of two simple corpuscles in an early stage, as in Plate III, Fig. 19, all indications of its compound nature are soon lost. A corpuscle originally compound may, then, in later stages, become indistinguishable from simple corpuscles. The simple corpuscles uniting to form a compound concentric corpuscle may be of any of the types previously described.

B. Irregular Corpuscles.

This group includes those corpuscles which are not at first concentric. Concentric areas may appear later. According to the classification previously given, I distinguish a compact type and a reticular type.

(a) The compact type (Plate III, Fig. 16) first appears as a compact area of syncytium of irregular shape. It is recognizable by the colloid it contains. The nuclei are not noticeably increased in size and have no regular arrangement. Their chromatin still stains dark with nuclear stains. The colloid (*c f*) is not yet solid. The corpuscle has no distinct center. These corpuscles grow by direct colloid transformation of the adjacent syncytium. No distinct lamellæ are formed. The colloid may remain in the fibrous condition shown in the figure (*c f*) or it may become solid, but it never reaches the deeply staining condition unless a concentric area be established.

A later stage of this type is shown in Plate III, Fig. 23. The corpuscle is sharply marked off from the syncytium. Some of its colloid is solid. A concentric area (*c s*) is beginning to form. The nuclei are not markedly different from those of the adjacent syncytium. These corpuscles may become large and branched. Often one or more concentric areas are developed after the corpuscle has attained considerable size. By the growth of these concentric areas, irregular corpuscles may become converted into concentric corpuscles.

(b) The reticular type is produced by colloid formation in the ordinary reticulum of the medulla. In the types previously described, the spaces of the reticulum are usually obliterated as the colloid formation advances; but in this form the spaces persist as a part of the corpuscle. Pure reticular corpuscles vary greatly in size, sometimes involving only

one node of the syncytium. They are never concentric, and never form lamellæ. Reticular areas often occur in other forms of corpuscles. In this way leucocytes are often involved in the corpuscle, since they lie in the spaces of the reticulum. Lymphocytes often get into a corpuscle in the lymphoblast condition, being cut off by the formation of lamellæ outside them (Plate III, Fig. 22). The leucocytes shut in the corpuscle in this way during development may not degenerate. They probably persist and help to remove the corpuscle in its final stages of degeneration.

The amount of expansion of the cytoplasm before or during the colloid transformation is probably small in the irregular reticular corpuscles, since it does not obliterate the spaces of the syncytium. In the compact type, the spaces of the syncytium are obliterated and there is evidence of some expansive force (note the arrangement of the nuclei in the upper part of Fig. 23, Plate III). In the figure referred to, the number of nuclei in any part of the corpuscle is less than in an equal area of the adjacent reticulum. These facts indicate that there is an expansion of the cytoplasm. That this expansive force does not produce a concentric form is due primarily to the fact that there is no expansion of a nucleus and distinct center of formation as is present in concentric corpuscles of the ordinary type. The absence of the onion-like structure in irregular corpuscles is due to the fact that the colloid is not laid down in lamellæ.

Significance of the corpuscles of Hassall. It has been shown in the preceding pages that the corpuscles of Hassall in the pig are not epithelial remnants, and also that they are not formed from blood-vessels. There is no evidence connecting their development with the involution of the thymus, for they begin to form before the lymphoid transformation is complete and are most numerous when the thymus is at the height of its development. I have not been able to see the decrease in the rate of growth of the medulla described by Ammann, and even if such did occur it is difficult to understand how it could cause the formation of a corpuscle.

The above theories are, therefore, inconsistent with the facts of development in the pig. It seems to me that the formation of a corpuscle is not to be regarded as a process of degeneration. The fact that the formation of colloid is an essential feature in the development of every corpuscle is a strong argument that it is a form of secretion such as occurs in its neighboring branchial derivative, the thyroid. The fact that the corpuscles differentiate in an apparently uniform syncytium is further evidence against a theory of degeneration. Since the lymphocyte-forming function of the thymus is probably secondary, it is not

unreasonable to suppose that its primitive function was the formation of a colloid secretion such as occurs in the thyroid, and that the corpuscles are abortive expressions of this primitive function.[7]

Giant Cells.

Polykaryocytes may often be seen in the medulla. These bodies develop from the syncytium of the medulla. They are first noticeable as groups of small spherical nuclei in a solid area of the syncytium. These nuclei stain with medium intensity and are all very similar in size and color. The area containing this group of nuclei becomes a well-defined node of the reticulum and persists as such. A polykaryocyte is, therefore, a large node of the reticulum containing a number of small nuclei very similar in appearance. These cells often occur in groups. They are entirely distinct from the corpuscles. They are evidently similar to the polykaryocytes found in bone marrow and other lymphoid tissues.

Summary.

The following is a resume of the development of the thymus in the pig:

The thymus of the pig is probably developed entirely from the endoderm of the third gill pouch.

By a gradual process of vacuolization and liquefaction of the cytoplasm, the epithelial syncytium of the thymic anlage is converted into a cellular reticulum.

From the first appearance of vacuolization, three types of nuclei are present: large pale nuclei; small dark nuclei (lymphoblasts), and large dark intermediate forms.

The lymphoblasts gradually break loose from the cellular reticulum, moving into its spaces and forming lymphocytes. Mitoses are most numerous at the period of the most rapid formation of lymphocytes. The medulla continues to form lymphocytes at least as late as birth.

Lymphocytes appear in the connective tissue around the thymus shortly after they are formed; and lymphoblasts, which are distinguishable from lymphocytes only by being embedded in the syncytium, are present in the thymus a long period before lymphocytes are found anywhere in the neighborhood of the thymus.

The cellular reticulum of the earlier stages persists in a modified form as the reticulum of both cortex and medulla. It retains more cytoplasm

[7] Ver Eecke (28) believes that the corpuscles in amphibians are of a glandular nature.

in the medulla. Practically all the reticulum of both cortex and medulla, as well as the lymphocytes, are, therefore, of epithelial origin.

The corpuscles of Hassall develop from the syncytium and are, therefore, epithelial in origin. They are, however, not to be considered as remnants of the original epithelial anlage.

In development various types of corpuscles are distinguished. The ordinary type of concentric corpuscles first appears as an enlarged clear nucleus around which colloid is being formed. Before or during the formation of colloid, the cytoplasm increases in quantity, filling the spaces of the reticulum and producing a centrifugal pressure which shapes the newly-formed colloid into concentric lamellæ and flattens the neighboring nuclei, making them concave toward the center. The central nuclei usually become obliterated.

The epithelioid type is distinguished by its resemblance to large epithelial cells, this appearance being due to the formation of colloid lamellæ around large areas of clear cytoplasm. The central part of the corpuscle usually remains unchanged until after some of the colloid lamellæ are formed.

The cystic type differs from the ordinary type only in that the central part undergoes vacuolization instead of colloid transformation. Those with concentric vacuoles may simulate blood-vessels containing nucleated red cells. Corpuscles never contain erythrocytes; neither can they be injected at any stage of development. Serial sections also show that there is no connection to blood-vessels at any stage.

Compound concentric corpuscles are formed by the union of two or more simple concentric corpuscles during development.

Irregular corpuscles are not concentric in arrangement, and are formed in the syncytium in an irregular manner. In the compact type of irregular corpuscles, concentric areas may form.

The formation of colloid is an essential feature in the development of every corpuscle, and is not to be considered as a process of degeneration.

Since the conclusion of my work and after my manuscript was given to the publishers, two articles dealing with the thymus have appeared.

Ph. Stöhr (Ueber die Thymus, Sitzungsberichte der phys.-med. Gesellschaft zu Würzburg, June 8, 1905) believes that the thymus first epithelial in nature becomes converted entirely into small cells of lymphoid appearance. Later the large reticulum cells are formed from these by enlargement. The corpuscles of Hassall are formed by the massing together and enlargement of these lymphoid-like cells. The small round cells of the gland are epithelial in origin but are to be regarded not as lymphocytes but as epithelial cells. The thymus is not a source of lymphocytes.

The author apparently believes that none of the small round cells leave the gland though he admits that lymphocytes enter. But as mentioned above the zone of connective tissue immediately around the head at 7 cm. may contain even more lymphocytes than are present inside the gland at that time If these are all entering the gland then it is probable that most of the small round cells are really lymphocytes. This conception then does not simplify the problem but is only a theoretical compromise between the two views as to the origin of the lymphocytes.

J. Aug. Hammar (Zur Histogenese und Involution der Thymusdrüse, Anat. Anz. Bd. XXVII, June 17, 1905) regards the reticulum as formed from the epithelial anlage but thinks the evidence at hand insufficient to decide the question as to the origin of the lymphocytes. He finds lymphocytes outside the thymus in many animals (man, cat, chick, frog) before any are present inside the gland. The corpuscles of Hassall develop from the epithelial reticulum and undergo hyaline (colloid?) degeneration.

My description of the formation of the corpuscles of Hassall differs essentially from Hammar's, in that I believe the formation of the corpuscle consists in the expansion of the cytoplasm of the syncytium and its conversion into colloid. Hammar did not recognize "colloid in formation," though he speaks of the coarse fibrillar structure of the protoplasm. He did not describe such corpuscles as are shown in Fig. 7, Plate II.

The considerations presented above in favor of the epithelial origin of the lymphocytes seem to me much stronger than those given by Hammar. His statements as to the presence of lymphocytes around the thymus before they are present inside are to be taken with some reservation inasmuch as he mentions small round cells separate from the syncytium earlier, but regards them as degenerating epithelial cells (S. 65). His figure from the human fœtus (Fig. 18, S. 66) does not seem to be strong support for his statement. Certainly many lymphocytes are present in the pig thymus when the reticulum is broken up as much as shown in the figure referred to. It is also to be borne in mind that the different parts of the thymus undergo the lymphoid transformation at different times and that a single section may therefore be misleading.

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EXPLANATION OF PLATES.

All the figures were drawn with Leitz obj. 1/12, oc. 4, and camera lucida. The magnification after the reduction of the plates is about 1060 diameters. All drawings were made from transverse sections of the mid-cervical segment of the thymus unless they are otherwise indicated.

The following abbreviations designate the structures indicated in all the figures:

c f—colloid in formation.
cl—calcareous deposit.
c s—solid colloid.
c s d—solid colloid that stains deeply.
e—erythrocyte.
end—endothelial nucleus.
l—lymphocyte.
lb—lymphoblast.
l d n—large dark nucleus.
l p n—large pale nucleus.
m—nucleus in mitosis.
md—beginning of medulla.
n—nucleus.
o c—old colloid.
p m—protoplasmic mass.
sf—fibril in syncytium.
ss—space in syncytium.
v—vacuole.

Plate I.

Fig. 1. From a 3.7-cm. pig embryo. Stained with iron-hæmatoxylin and Congo red. Vacuolization of the cytoplasm and differentiation of the nuclei have begun.

Fig. 2. From the thoracic segment of a 4.5-cm. pig embryo. Stained with iron-hæmatoxylin and Congo red. A cellular reticulum is now formed. Large pale nuclei, lymphoblasts, and the large dark intermediate forms are present.

Fig. 3. Epithelioid type of concentric corpuscle. From a 16-cm. pig embryo. Stained with hæmatoxylin and Congo red. Colloid lamellæ (*c s d*)

separate large areas of clear cytoplasm, causing the appearance of large epithelial cells. Colloid is being formed between the lamellæ and around several nuclei.

Fig. 4. From a 8.5-cm. pig embryo. Stained with iron-hæmatoxylin (not decolorized). The medulla has appeared. Lymphocytes are present between the epithelial cords.

Fig. 5. From a 7-cm. pig embryo. Stained with iron-hæmatoxylin and Congo red. A few lymphocytes have been formed. In the cellular reticulum are large pale nuclei, lymphoblasts, and large dark intermediate nuclei. The nuclei in mitosis are very compact.

Fig. 6. From the medulla of a 24-cm. pig embryo. Stained with Jackson's modification of Mallory's method (ref. in text). Many fibrillæ are seen in the syncytium.

Plate II.

Fig. 7. Ordinary type of simple concentric corpuscle. From a 16.5-cm. pig embryo. Stained with hæmatoxylin and Congo red. The corpuscle is well advanced in development. Concentric lamellæ of colloid have been formed. The cytoplasm between the lamellæ is in an early stage of colloid transformation. Colloid fibers cut transversely appear as dots. The nuclei are becoming flattened by the pressure of expansion. The central mass stains irregularly and all traces of the nuclei in that region are gone.

Fig. 8. Two cystic concentric corpuscles. From a 16-cm. pig embryo. Stained with iron-hæmatoxylin and Congo red. On the left, a nucleated mass of protoplasm has been separated off by the formation of a vacuole annular in section. This might be mistaken for a blood-vessel containing a nucleated red cell. In this central protoplasmic mass the nucleus is shrunken and the cytoplasm vacuolated. In the small corpuscle on the right, two large vacuoles have formed.

Fig. 9. Cystic concentric corpuscle. From a 14-cm. pig embryo. Stained with hæmatoxylin and Congo red. The central protoplasmic mass is pale and shrunken. The small circular body in it probably is the remains of the nucleus. Colloid lamellæ are forming. Colloid fibers cut transversely appear as dots.

Fig. 10. Cystic concentric corpuscle. From a 10.5-cm. pig embryo. Stained with hæmatoxylin and Congo red. The center contains no colloid and seems to be softening. The nucleus is shrunken.

Fig. 11. Ordinary concentric corpuscle in a very early stage. From a 10.5-cm. pig embryo. Stained with iron-hæmatoxylin and Congo red. The nucleus is enlarged and colloid is forming around it. A few colloid fibers may be seen in the cytoplasm for some distance from the central nucleus.

Fig. 12. Ordinary concentric corpuscle. Several nuclei are involved. From a 10.5-cm. pig embryo. Stained with hæmatoxylin and Congo red. The deeply-staining colloid has completely obliterated the central nucleus (in the region *o c*), and nearly obliterated another (*n'*). Some of the colloid now stains pale (*o c*).

Fig. 13. Ordinary concentric corpuscle. From a 10.5-cm. pig. Stained with hæmatoxylin and Congo red. The central nucleus is being obliterated

by the deeply-staining colloid. The neighboring nuclei are beginning to show the effect of the centrifugal pressure.

Fig. 14. Ordinary concentric corpuscle in an early stage. From a 10.5-cm. pig. Stained with hæmatoxylin and Congo red. A band of deeply-staining colloid has been formed. Just outside this is colloid in formation.

Fig. 15. Two simple concentric corpuscles which would have formed a compound concentric corpuscle. From a 10.5-cm. pig. Stained with hæmatoxylin and Congo red. The left corpuscle is a little more advanced than Fig. 11. The right corpuscle shows a large area of deeply-staining colloid which has pressed the nucleus into a small irregular shape.

Plate III.

Fig. 16. Compact irregular corpuscle in an early stage. From a 14-cm. pig embryo. Stained with hæmatoxylin and Congo red. The colloid is not yet solid. The nuclei are not essentially different from those of the adjacent syncytium.

Fig. 17. Ordinary concentric corpuscle. From a 12-cm. pig embryo. Stained with hæmatoxylin and Congo red. There is a large, central, deeply-staining colloid mass in which calcareous deposits (*cl*) have been made. The neighboring nuclei show the effects of the centrifugal pressure.

Fig. 18. Epithelioid concentric corpuscle in an early stage. From a 10.5-cm. pig embryo. Stained with hæmatoxylin and Congo red. The outer colloid lamella marks off a nucleated mass of cytoplasm resembling a large cell. The nucleus is undergoing the same changes as occur in the central nucleus of an ordinary concentric corpuscle.

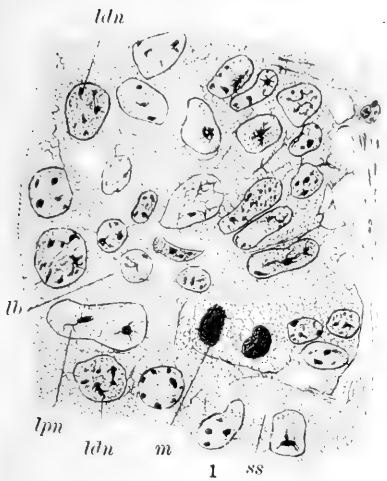
Fig. 19. Compound concentric corpuscle. From a 10.5-cm. pig embryo. Stained with hæmatoxylin and Congo red. This would have soon lost all evidence of its compound nature.

Fig. 20. Epithelioid concentric corpuscle. From a 10.5-cm. pig embryo. Stained with hæmatoxylin and Congo red. Some colloid is forming outside the circular area. Solid deeply-staining colloid is forming.

Fig. 21. Ordinary concentric corpuscle, showing a variation from the usual type. From a 16.5-cm. pig embryo. Stained with iron-hæmatoxylin and Congo red. The central nucleus is reddish but not enlarged. No solid colloid has been formed.

Fig. 22. Compound concentric corpuscle. From a 16.5-cm. pig embryo. Three centers are present. The pale colloid in the upper part is probably older than the deeply-staining variety. In the lower part of the figure, a young corpuscle is shown.

Fig. 23. Compact irregular corpuscle. From a 16-cm. pig embryo. Stained with hæmatoxylin and Congo red. Some of the colloid is solid. No definite center is present but one is beginning to form (*c s*). The nuclei are not markedly different from those of the adjacent syncytium.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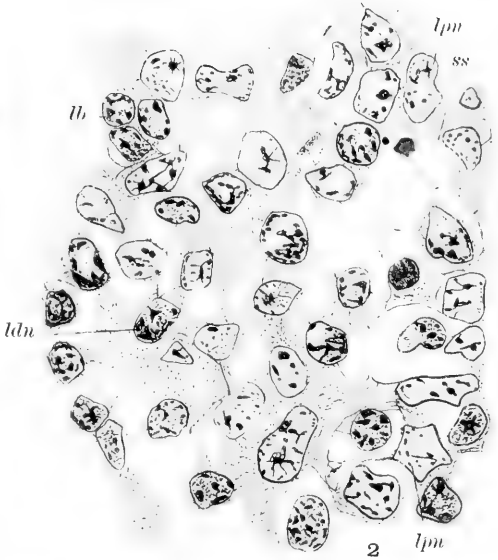
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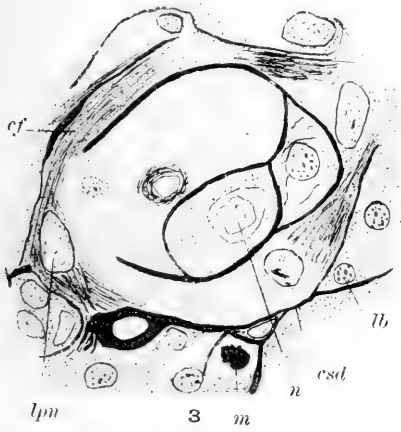
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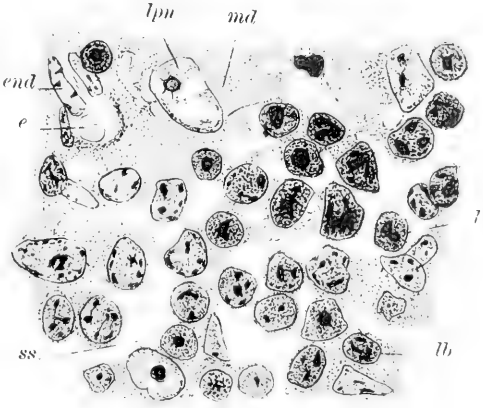
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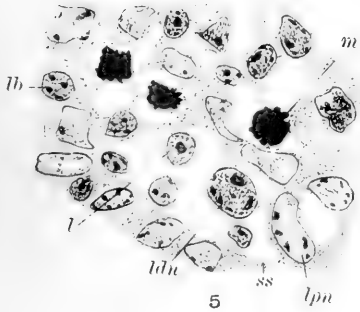
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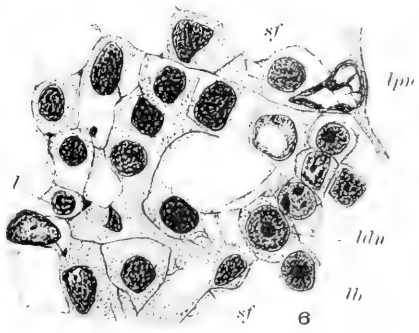
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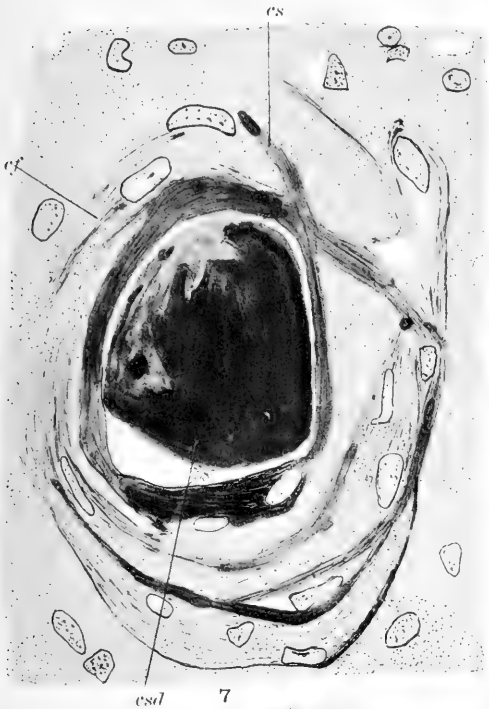

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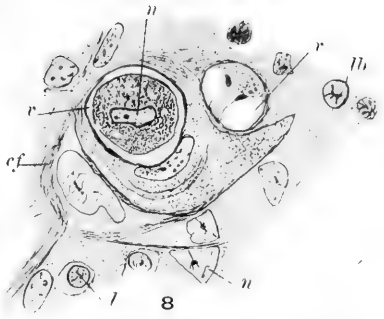

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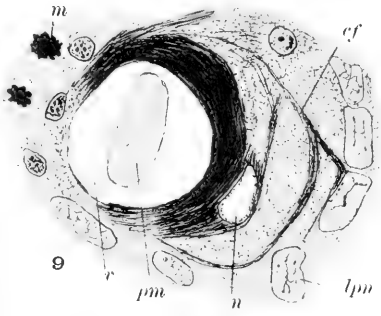

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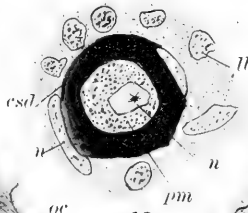

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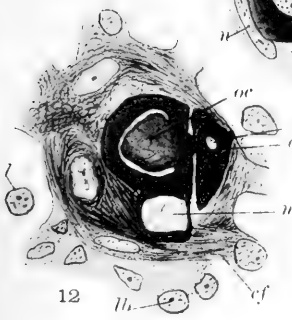

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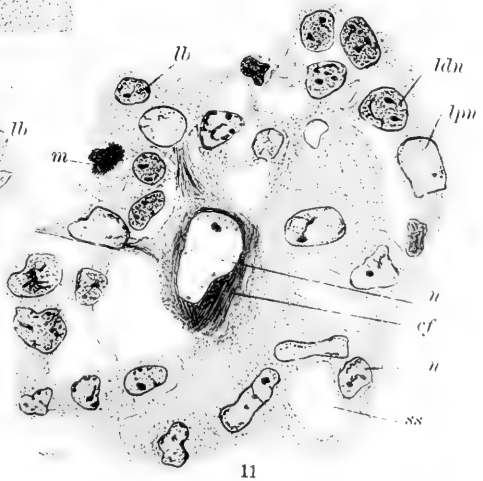

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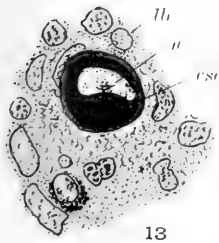

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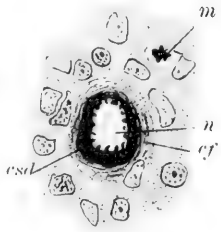

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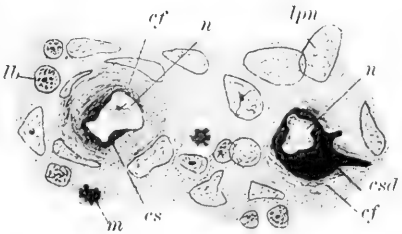

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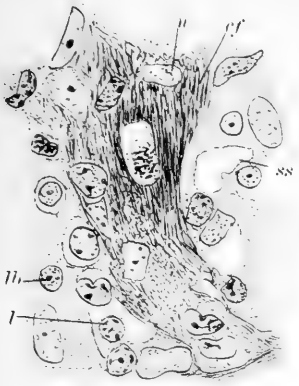

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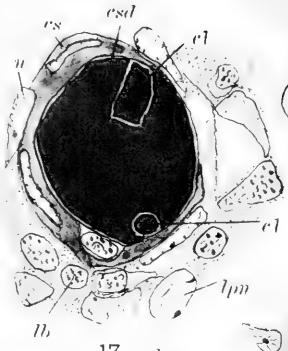

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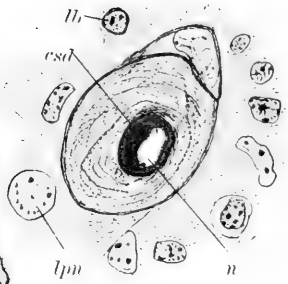

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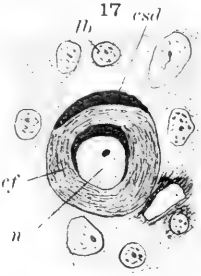

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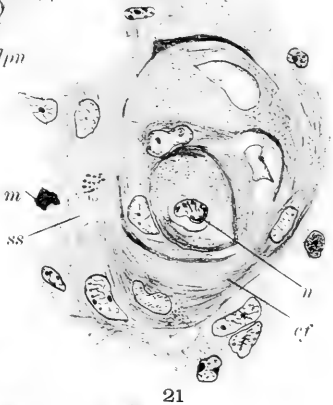

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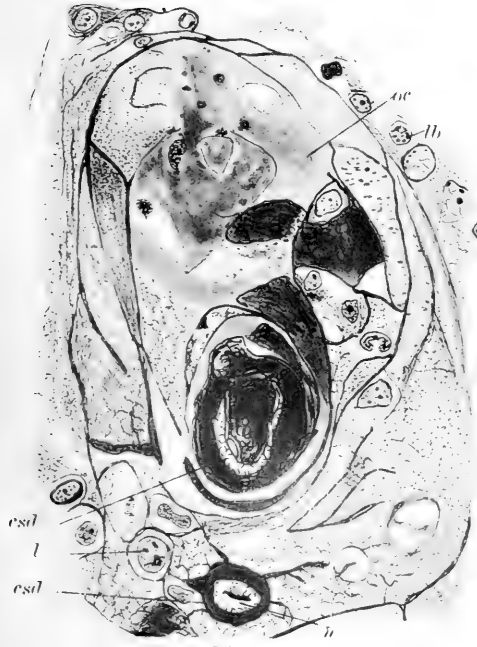

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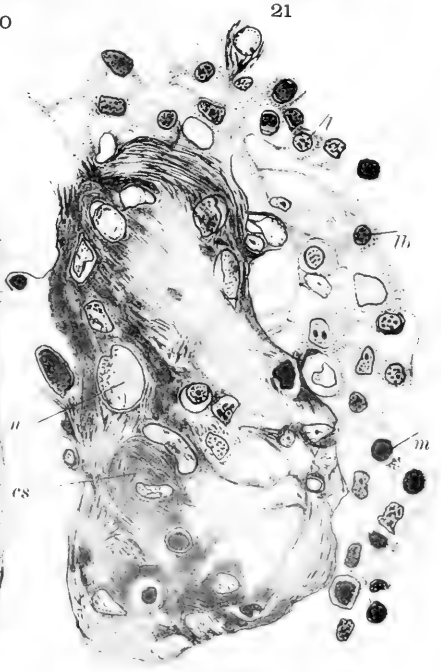

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THE VEINS OF THE ADRENAL.

BY

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WITH 3 TEXT FIGURES.

Within the past decade our knowledge of the functions of the adrenal glands, and of their relations to the rest of the economy, has been greatly enhanced by many careful chemical and physiological researches. The recent studies of Aichel (1), Wiesel (2, 3), Soulie (4), and others have placed the early development of the organ upon a fairly certain basis. These advances in the physiology and embryology of the organ have not as yet been accompanied by corresponding advances in our appreciation of its minute anatomy. Hence this branch of the subject is, at the present time, one of unusual interest.

The intimate relation of the parenchyma of the adrenal to its blood-vessels, as shown by the general tendency to regard the organ as a true gland whose secretion enters its blood-vessels and leaves the organ through its efferent veins, makes it specially important that these vessels should be carefully studied and their structure and distribution accurately recorded.

The exhaustive study of Flint (5), on the course of the adrenal vessels, based as it was upon carefully prepared reconstructions, leaves little to be desired along this line. The writer is, however, unable to find in the literature any reference to the minute structure of the veins of the adrenal, with the notable exception of Minot's (6) article on sinusoids.

To be sure Pfaundler (7) mentioned the occurrence, in the medulla of the adrenal, of venous vessels whose only wall consisted of endothelium. Gottschau (8) also, though omitting their description, has figured similar vessels in his Plate XVIII, Fig. 1. But as to the structure of the larger blood-vessels of the adrenal glands the literature seems to be entirely barren.

The architecture of the arterial walls does not appear to offer any distinctive peculiarities, the tissues of which they consist being arranged in a manner precisely similar to that which characterizes the arteries of

similar size occurring in other organs. The veins, however, present distinct and remarkable peculiarities which it is the purpose of the present paper to describe.

Methods and Material.—The tissue used for this study has included specimens of the adrenal from twenty-one human adults, together with the casual examination of fetal adrenals of the pig and of man. The adrenals of other mammals, e. g., monkey, dog, cat, rabbit, and guinea-pig, have also been more or less carefully studied.

These tissues have been fixed and hardened with many reagents, among which are Zenker's solution, formol, Müller-formol, alcohol, corrosive acetic mixture, Tellyesniczky's fluid, and Flemming's solution. The stains used were hematein by various methods, acid hematein, iron hematein, etc., and for counter stains eosin, orange, Van Gieson's picro-fuchsin, Weigert's elastic tissue stain, Mann's methyl blue-eosin mixture, Congo-red, and Ehrlich's triacid mixture. A combination of Mann's hematein, Weigert's elastic tissue stain and Van Gieson's picro-fuchsin, gave the best results for the differentiation of the muscular and connective tissues. This method was applied as follows, and may be used after any of the above fixatives.

1. Stain 10-12 minutes in Mann's hematein or in Böhmer's hematoxylin, until somewhat overstained. 2. Wash well in water. 3. Stain 10-20 minutes in recently prepared resorcin-fuchsin solution after the method of Weigert (9). 4. Wash in water. 5. Stain 1-3 minutes in the freshly prepared picric acid-acid fuchsin solution of Van Gieson (10). 6. Wash and dehydrate in 95 per cent, or in absolute alcohol. 7. Clear, and mount.

Types of Adrenal Veins.—The efferent veins of the adrenals arise in the medulla of the organ by the union of the broad capillaries of the medulla and the adjacent zone of the cortex. These capillaries form broad thin-walled vessels which have been described by Minot (6) as *sinusoids.* They converge toward the middle of the medulla, where they pass into somewhat larger vessels, which, for convenience, may be termed *small central veins.* These veins tend toward the hilum, are relatively short, and by union with one another soon form thicker-walled vessels which may be described as *large central veins.* These large veins pass toward the hilum, near which they unite to form a large efferent vessel, the *suprarenal vein.* This last vein makes its exit from the hilum of the organ and enters either the vena cava inferior, as is the rule on the right side, or the renal vein, as frequently occurs on the left.

From this brief review of the course of these vessels it will be seen that four distinct venous types have been enumerated, and it is the purpose

of the writer to show that these types exhibit well-defined structural peculiarities.

Observations.—The *sinusoids,* after the careful description by Minot (6), will require but brief mention. These vessels possess the wall of a capillary and the lumen of a venule. A number of such vessels may be seen in Fig. 1, in the central portion of the medulla, on either side of the group of central veins. Their wall consists of nucleated endothelial plates which rest directly upon the parenchymal cells. Their lumen is several times the diameter of the medullary capillaries. They are dis-

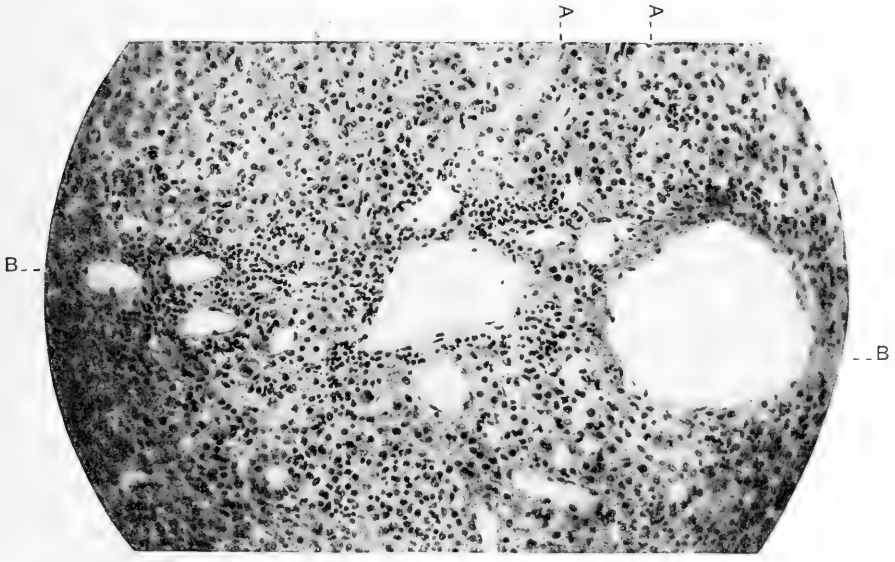

Fig. 1. A group of vessels from the central portion of the medulla of the human suprarenal gland. *a*, sinusoids; *b*, small central veins. Fixation, 5 per cent formalin; stain, Mayer's hematein; thickness, 8μ; photomicrograph, $\times$ 100.

tinguished from the small central veins by the absence of connective tissue from the wall of the sinusoids.

The *small central veins* are of the type shown in Fig. 1. The wall of these vessels consists of two coats, endothelial and connective tissue. The latter is always relatively thin, though the vessels possess a very considerable lumen. Venules of this type of structure, Fig. 1, collect the blood from the sinusoids of the medulla. Frequently, however, the sinusoids open directly into the small central veins and venules, the connective tissue of the venous wall being occasionally continued for a very short distance upon the endothelium of the sinusoid.

The connective tissue of the small central veins is richly supplied with elastic fibers, which are disposed in oblique and circular directions,

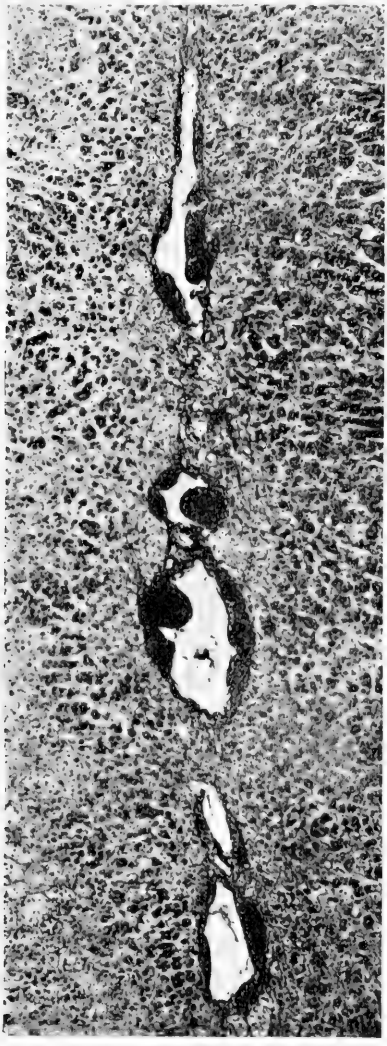

FIG. 2. The medulla of a suprarenal gland of man, showing a group of large central veins. The middle and lowermost veins are in transection, the uppermost vessel in longitudinal section. The series of sections shows that this last vessel is a branch of that in the middle of the figure. Fixation, Zenker's fluid; stain, hematein and methyl-blue, Mann's method; thickness, 10 μ; photomicrograph, $\times$ 60.

occasional elastic fibers are also longitudinal. The typical small central veins contain no muscle. As they approach their termination in the

large central veins a few smooth muscle fibers are found, but these are always disposed in a longitudinal direction. As soon as longitudinal muscle fibers appear in appreciable numbers the venous wall acquires the type of the succeeding variety, the large central vein.

In the *large central veins,* as in the small, but two coats can be readily distinguished. The inner coat, or intima, in these vessels consists of a lining endothelium, which rests upon a very thin membrane of delicate connective tissue, containing many elastic fibers.

The outer coat, or adventitia, is also a very thin membrane of fibro-elastic tissue, but its fibrous bundles are coarser than those of the intima, and its elastic fibers form a very close network. The outer portion of this coat contains a few longitudinal smooth muscle fibers. The great majority of these fibers, however, are arranged in the form of longitudinal ridges which project into the adjacent medullary tissue. From one to five of these muscular ridges occur in the circumference of the vein (Fig. 2 and 3). Except at those points at which the muscle occurs, the venous wall is extremely thin (Fig. 3). The muscular ridges are frequently so large as to materially obstruct the lumen of the vessel (e. g., the middle vessel in Fig. 2, also the uppermost vessel, which is cut in very nearly longitudinal section), and they form so noticeable a peculiarity that their presence may be considered characteristic of this type of vessel. The writer has never failed to find these peculiar muscular ridges more or less highly developed in each of the human adrenals which he has examined: he believes them to be constantly present. They are less highly developed in the suprarenal vessels of the lower mammals, but even there they may frequently be demonstrated.

The muscle fibers of the larger ridges are arranged in bundles which are enveloped in fibro-elastic septa of connective tissue. All of the muscle fibers in these bundles are longitudinally disposed. This arrangement is well shown in Fig. 3, in which a large central vein is seen in transection at a point near the entrance of a large branch. Examination of sections somewhat higher in the series shows the union of these two vessels.

In the section photographed, the branch has been longitudinally cut. The fine dark lines shown in the figure are bands of elastic fibers which are enveloped in delicate white fibrous tissue inclosing the cut ends of the bundles of smooth muscle. The tendency to form longitudinal ridges is shown in this figure by the irregular distribution of the muscle, one side of the vessel, in both the parent stem and the branch, being almost devoid of muscle fibers. The muscular character of these ridges is beyond doubt.

FIG. 3. Large central veins from the medulla of the human suprarenal gland. The figure shows the distribution of the elastic tissue and the bundles of smooth muscle which are seen in transection in the larger vein and in longitudinal section in the smaller ones below. The series shows these latter vessels to be branches of the former, the section being selected to show a plane near the point of division. The smaller vessels are very obliquely cut and the muscle is distinctly longitudinal. Fixation, Zanker's fluid; stain, Mann's hematein, Weigert's elastic tissue, and Van Gieson's picro-fuchsin; thickness, 10 μ; photomicrograph, $\times$ 37.

The writer has observed that the formation of such heavy ridges as those shown in Fig. 3, nearly always occurs at those points where the vessel branches. It is possible that, as in the case of the somewhat similar ridges in the veins of the erectile tissues (see Kölliker's Handbuch der Gewebelehre, 6te Aufl., 1902, pages 486 and 487), these muscular protuberances may to some extent serve the purpose of valves.

As the large central veins approach the hilum of the organ they form still larger vessels which partake of the structure of the suprarenal vein. The point of transition from the one type to the other is variable, occasionally the type of the large central veins is continued to the exit of the suprarenal vein at the hilum of the organ. More frequently the primary branches of the suprarenal vein may be traced for a considerable distance into the medulla of the organ, still retaining the type of structure found in the larger vessel.

The *suprarenal vein* presents three coats, intima, media, and adventitia. The tunica intima, in addition to its endothelial lining, possesses a thin membrane of very delicate connective tissue in which occasional branched connective tissue cells may be distinguished; such cells are, however, very scanty. This coat also contains a delicate network of elastic fibers.

The tunica media of the suprarenal vein is extremely thin, rarely ever does it exceed in thickness the tunica intima. It consists chiefly of fibro-elastic tissue, the elastic fibers forming quite a dense network. Few muscle fibers occur in this coat, nowhere are they found in sufficient numbers to form a definite layer, as in veins of similar size in other organs. Some of the muscle fibers are circularly disposed, but many of them are longitudinal.

The tunica adventitia is by far the thickest of the three coats and forms two-thirds to five-sixths of the entire vascular wall. It consists chiefly of smooth muscle fibers, all of which are longitudinally disposed. These smooth muscle fibers form characteristic coarse bundles which are distributed around the entire circumference of the vessel. The largest of these bundles may occasionally form projecting ridges as in the smaller veins, but as a rule the muscular tissue is more evenly distributed than in the central veins. Each of the muscle bundles is enveloped in a perimysial sheath of connective tissue, which blends with that of the tunica media. These adventitial sheaths possess a dense network of elastic fibers, in fact the greater part of the elastic tissue in the vascular wall is frequently found in the adventitia. On its outer surface the tunica adventitia is continuous with the capsule of the adrenal or with the adjacent connective tissue.

This peculiar type of vessel is not strictly confined to the suprarenal

gland, but occurs, more or less typically developed, in many of the large abdominal veins, notably in the renal veins and vena cava, into which the suprarenal veins empty. But nowhere is this peculiar venous type more strikingly developed, nowhere is the adventitia relatively so much thicker than the media, nowhere is a greater proportion of the smooth muscle of the venous wall longitudinally disposed, nowhere is there relatively less circular muscle, than in the suprarenal vein. Realizing the intimate relation of the parenchyma of the organ to its blood-vessels, and adopting, if we may, the accepted physiological function of the adrenal—the formation of an internal secretion, a powerful vaso-constrictor which is poured into the blood within the capillaries and veins of the organ—the peculiar longitudinal arrangement of the muscular tissue, the valve-like protuberances at the junctions of the venous vessels, the absence of circular muscle from the walls of the veins of all sizes, and the general appearance of these vessels which are so remarkably different from the veins of most other organs, become, to say the least, extremely significant of a close structural relation, physiologically speaking, to the presence of an astringent secretion in the outflowing blood current.

In this connection, one further observation is of importance. In the periadrenal connective tissue are numbers of small veins which return the abundant blood supply of the tissues of this region, most of them emptying into the phrenic veins. Many of these veins do not differ from the similar veins of other parts, but in many others the writer has observed that the muscle tissue is almost entirely disposed in a longitudinal direction, a condition which is quite the reverse of that found in the adipose and areolar tissues of other portions of the body.

The writer also finds that many of the small veins of the adrenal, instead of opening into the central veins as is usually the case, pursue a less frequent course, penetrating the cortex and capsule of the organ, and emptying into the small veins of the surrounding connective tissue. The frequency with which this condition was associated with the occurrence of longitudinal muscle fibers in the periadrenal veins, suggests a more than casual relationship between the two conditions.

Summary.

In conclusion, the above facts may be summarized as follows:

1. The efferent blood-vessels of the adrenals form four successive vascular types, the sinusoids, the small central vein, the large central vein, and the suprarenal vein.

2. Each of these types presents distinctive characteristics.

3. In all four types circular muscle is either absent or noticably deficient.

4. In the large central veins prominent and characteristic muscular ridges are constantly present, and are frequently in relation with those points at which the branches of these vessels enter.

5. These peculiarities of structure may possibly bear a close physiological relation to the function of the adrenal as a gland that forms an internal secretion which has been shown to be a powerful vaso-constrictor and stimulant of smooth or involuntary muscle.

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THE BLOOD VESSELS OF THE PROSTATE GLAND.

BY

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WITH 2 COLORED PLATES.

As the structures of the body are being more and more carefully investigated it is found that organs are composed of like structural units, which when repeated a number of times form the whole organ. In general these units are formed by the glandular structures, the blood-vessels, or by both, as is the case in the liver.

Some eight years ago, at the suggestion of Dr. Mall, I undertook the study of the prostate gland, with the hope of finding structural units in it. In this search I was successful. Since then my work has been continued in the laboratories of Professor Born[1] of Breslau and Professor Spalteholz[2] of Leipzig, and although this communication is several years late in appearing, it should in reality have preceded those that were published in 1899.

In the present study for the most part the prostate glands of dogs were used. Several cadavers were injected and the gross blood supply was studied in part from these. After the animals had been killed by chloroform, the aorta was exposed just above the bifurcation and injected with various substances. A preliminary washing out of the blood-vessels with salt solution was practised in a few of the first injections, but this was soon discarded as it did not seem to enhance the value of the method.

Carmine gelatine, followed by ultramarine-blue gelatine, as an injecting mass, gave the most satisfactory results. About 250 cc. of the carmine gelatine were injected first, the injection being stopped as soon as all of the tissues had acquired a maximum carmine hue. This was followed by the injection of ultramarine-blue gelatine, which was kept up until no more of the material would pass in. The carmine gelatine

[1] Walker, George: Ueber die Lymphgefässe der Prostata beim Hunde. Arch. für Anatomie, 1899.

[2] Walker, George: Beitrag zur Kenntniss der Anatomie und Physiologie der Prostata, etc. *Ibid.*, 1899.

filled the arteries, capillaries, and veins; the blue passed into the arteries and arterioles, displacing the gelatine and filling them, but was stopped at the capillaries because the ultramarine-blue granules were too large to enter them. In a specimen thus prepared the arteries appear blue, and the capillaries and veins red. This is shown in Figure 1, with colors reversed, in order to present the conventional appearance. As it was impossible to get a perfectly complete injection in one specimen, several of the best were selected and the gaps filled in, with the results as shown in Figure 1. One section, however, is remarkably beautiful and presents a picture very closely resembling that seen in this figure.

In order to map out the complete network of arteries surrounding a separate lobule, I injected them with Prussian blue, then opened the urethra, and injected carmine gelatine into a prostatic duct through a very fine blunt hypodermic needle. A specimen made in this way is shown in Figure 2 where the ducts are represented in brown. The capillaries were studied in a specimen which had been completely injected with carmine gelatine. A very thin section of this was stained with iron hæmatoxylin, and is shown in Figure 3. The basement membrane is artificially tinted with yellow so as to make it visible.

The technique of the injecting is rendered difficult by the fact that the situation of the gland in the pelvis is somewhat remote. In all, about 75 dogs were used before a complete circulation cycle could be seen. Cinnabar, lampblack, and various other substances were tried, but they did not prove as good as the combination of carmine gelatine followed by ultramarine-blue gelatine.

When the ordinary directions for preparing carmine gelatine were followed, it always proved difficult to get a perfectly transparent substance. The trouble is connected with the neutralization of the ammonia by the acetic acid. The gelatine should be rendered practically neutral, but if the reaction is carried the least bit too far, the solution becomes cloudy. Sometimes two drops of the acetic acid are sufficient to make turbid a whole litre of the prepared carmine. After a good many trials, the following method was adopted: Take 10 cc. of the ordinary laboratory ammonia and dilute with 40 cc. of distilled water, then determine by titration the exact amount of the laboratory acetic acid which will neutralize it. After this determination has been made, 10 grms. of pure carmine are rubbed up with 50 cc. of distilled water; then 25 cc. of the ordinary ammonia are measured, and a few drops at a time are poured into the carmine mixture which is kept constantly rubbed up. This process is very closely watched, and the ammonia is gradually added until the carmine is completely dissolved, and the mixture becomes

translucent and assumes a dark red color. The amount of ammonia used is determined by referring to the vessel in which the 25 cc. have been measured. The gelatine in whatever proportion it is required—according as a thin or thick solution is desired—is dissolved in the distilled water, and the carmine solution is added to it. We then calculate how much acetic acid will be required for the amount of ammonia which has been used; this is measured and added, drop by drop, to the mixture which is constantly stirred. A sufficient quantity of water is then added to bring the amount up to a litre. I found that in this way I could always obtain a beautifully clear gelatine and was never annoyed by the failures and uncertainties belonging to the other method.

Arteries.

The prostate gland derives its arterial supply from the internal iliac arteries by means of four branches; the superior vesical, the inferior vesical, a small branch from the inferior hemorrhoidal, and a small terminal branch from the internal pudic artery. These vessels will be found illustrated in my paper published in 1899. The superior vesical, a branch from the internal iliac, which is given off high up, divides before reaching the bladder, into two fair-sized branches; the lower and smaller branch extends downward and supplies the vesical third of the prostate; this branch is sometimes called the middle vesical artery. The inferior vesical, which is a large branch, is practically the main blood vessel of the prostate gland, and should be called the prostatic artery for, in the majority of instances, it does not send any branches to the bladder. The major part of the gland is supplied by this vessel; it courses along the vesicorectal fascia and meets the prostate at its lower border, where it usually divides into seven branches, four of these enveloping the anterior, and three the posterior surface. The posterior are about one-half the size of the anterior branches. These vessels are situated in the capsule of the gland and envelop it as the fingers of one's hand would do in clasping a round object. From these trunks a number of smaller ones are given off, so that a very close arterial network is formed over the surface of the gland. The branch from the inferior hemorrhoidal is not constant; in fact, it appears to be more often absent than present. When it is seen, it occurs as one or two small branches which meet the prostate in its urethral half, and extend over the surface as fine vessels which anastomose with the vesical artery. The internal pudic branch is fairly constant. It extends along the membranous urethra and plunges directly into the prostatic substance usually without giving off any branches to the surface.

A slight anastomosis is occasionally seen. The vessels supplying the two sides of the gland are distinct. The only anastomosis across the median line is by way of the venous channels around the urethra.

From the large superficial branches above described, smaller ones are given off at right angles, and pierce the gland in places corresponding to the divisions of the lobules (*Art.* Fig. 1). Here they penetrate the fibrous-tissue septa, and extend to the urethra, becoming smaller and smaller, however, as they approach it, so that in this region they are seen as very delicate terminal vessels. As they pass down, they give off branches which penetrate into the lobule and finally divide into myriads of capillaries which pass around the alveoli, and come in very close relationship with the secreting cells. From these cells they are separated simply by a delicate basement membrane composed of fine fibrils. From the superficial vessels branches are given off which enter the lobule directly, that is, they do not pass first into the fibrous-tissue septa (*Sup. Br.* Fig. 1). On the anterior surface there are usually two branches which do not give off as many smaller ones as the rest, and consequently remain larger and extend over to the middle line, where they dip into the median fissure and supply the median side of the lobules (*Med. Br.* Fig. 1).

The arrangement on the posterior surface corresponds to that seen on the anterior surface, in so far as the supply of the lobules is concerned. On the posterior surface toward the bladder one vessel penetrates the substance of the gland and runs directly to the caput gallinaceum (*Art. Col. Sem.* Fig. 1). Here it divides into a fine network and supplies the erectile tissue of the organ. Before this vessel reaches the eminence a small trunk is given off which extends to the ejaculatory duct (*Art. duct. ej.* Fig. 1). The branch supplying the caput gallinaceum is usually derived from the pudic; sometimes it comes from the inferior vesical.

The arterial supply in the connective tissue toward the urethra is much poorer than in the secreting portion. Here the vessels terminate in fine branches, relatively somewhat sparsely scattered. The arterial arrangement is shown on the red side of Figure 1.

Capillaries.

The capillaries form a very complete and elaborate network around the alveoli of the lobule. Here, as is seen in Figure 3, they surround an alveolus in a more or less circular manner, and upon these vessels the cells rest almost directly, being separated only by the very delicate connective-tissue basement membrane. From this outside capillary, a folding in is seen, which forms a definite loop (*Cap. L.* Fig. 3.) This at

first sight might appear to end blindly, but a more careful study reveals the two branches, which sometimes appear winding around each other, and presenting enlarged club-shaped ends. The cells rest on these as they do on the circular portion. Under the low power, the epithelial cells appear to be in direct contact with the capillaries, and it is only by the aid of the oil immersion that a very delicate connective-tissue basement membrane is seen. This is shown artificially colored as *B. M.* Fig. 3. This membrane contains a few elastic fibers.

Veins.

On the surface of the gland are veins corresponding to the arteries which lie in the capsule. As a rule they merge into two main trunks corresponding to the vesical arteries; occasionally several small branches pass off into the middle hemorrhoidal vein.

The superficial veins do not drain the blood from the whole gland, but only from the outer fourth, as is shown in Fig. 1. From the inner three-fourths of the gland the blood passes towards the centre, and into the large venous sinuses which are a continuation of the corpora spongiosa. (*Co. Sp.* Fig. 1). These immediately surround the urethra. The large venous trunks which collect the blood from the gland do not lie on the same plane as the arteries, but are situated in the fibrous septa some little distance removed from them. These run, as do the arteries, on the outside of the lobule, and are interlobular, not intralobular. For the venous return from the caput gallinaceum there is no distinct vessel corresponding with the artery, but there are anastomoses with the spongy plexus.

The venous plexus around the urethra is, as before stated, a continuation of the corpora spongiosa. The blood from this region passes away into the internal pudic vein. Occasionally two or three small veins drain the tissues from this region, pass out of the prostate and run along the membranous urethra and off into the vesicorectal fascia.

There is an anastomosis of the veins in the prostate and bladder where these organs come together, and also on the outside through the superior vesical veins. There is, of course, an anastomosis of the urethral veins through the corpora spongiosa plexus.

SUMMARY.

The prostate gland is supplied with blood by branches of the internal iliac arteries, viz., the superior vesicals, inferior vesicals, inferior hemorrhoidals, and internal pudics; the main blood supply comes from the inferior vesicals.

Branches of these envelop the surface of the gland and give off smaller ones, which penetrate between the lobules in the fibrous-tissue septa.

The capillaries are separated from the epithelial cells only by a very thin basement membrane.

There are superficial veins corresponding with the arteries.

For the outer superficial fourth of the gland the return flow is towards the surface. The inner three-fourths are drained by veins which empty into the venous plexus immediately around the urethra.

The lobule is formed primarily by the individual gland ducts as shown in Figure 2. The main arteries surround this lobule which they penetrate at many points. The veins leave the lobule mainly at its peripheral and central (urethral) ends as shown in Fig. 1.

EXPLANATION OF PLATES I AND II.

FIG. 1 is from a section of a prostate gland of a dog injected with carmine gelatine and ultramarine-blue gelatine. The arteries in the section were blue, the veins and capillaries red. The section was cut free hand, about 50μ in thickness, and cleared both in glycerine and in creosote. In the figure this artery is red and this vein blue. *Art.*, Arteries; *Art. Col. Sem.*, Artery of the colliculus seminalis; *Art. duct. ej.*, Artery of the ejeculatory duct; *Col. Sem.*, Colliculus seminalis; *V. Pl.*, Venous plexus around the urethra.

FIG. 2. Lobule of prostate from a gland which had been injected with ultramarine-gelatine blue into the artery, and with carmine gelatine into the prostatic duct. *Pr. duct.*, Opening of the prostatic duct into the urethra; *Gl. Tis.*, Gland tissues distended with carmine gelatine; *Art.*, Surrounding artery. In this figure the artery is represented in red and the ducts in brown.

FIG. 3. Very thin section from the prostate gland of a dog. Capillaries in red, injected with carmine gelatine. Section stained with iron hæmatoxylin, with artificial yellow tinting of basement membrane. Oil immersion with one inch eye-piece amplification. *Cap.*, Capillaries; *B. M.*, Basement membrane; *Gl. Ep.*, Glandular epithelium; *Cap. L.*, Capillary loop.

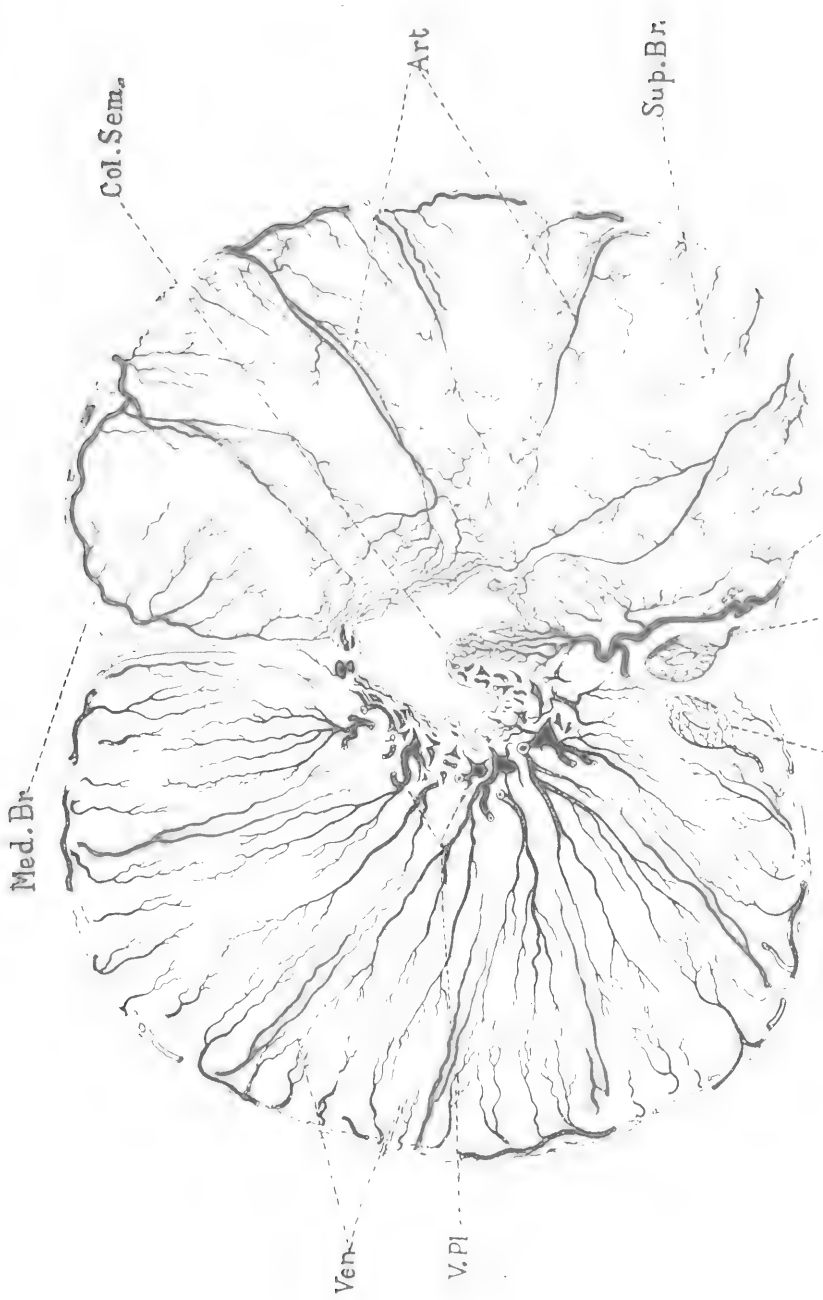

FIG. 1

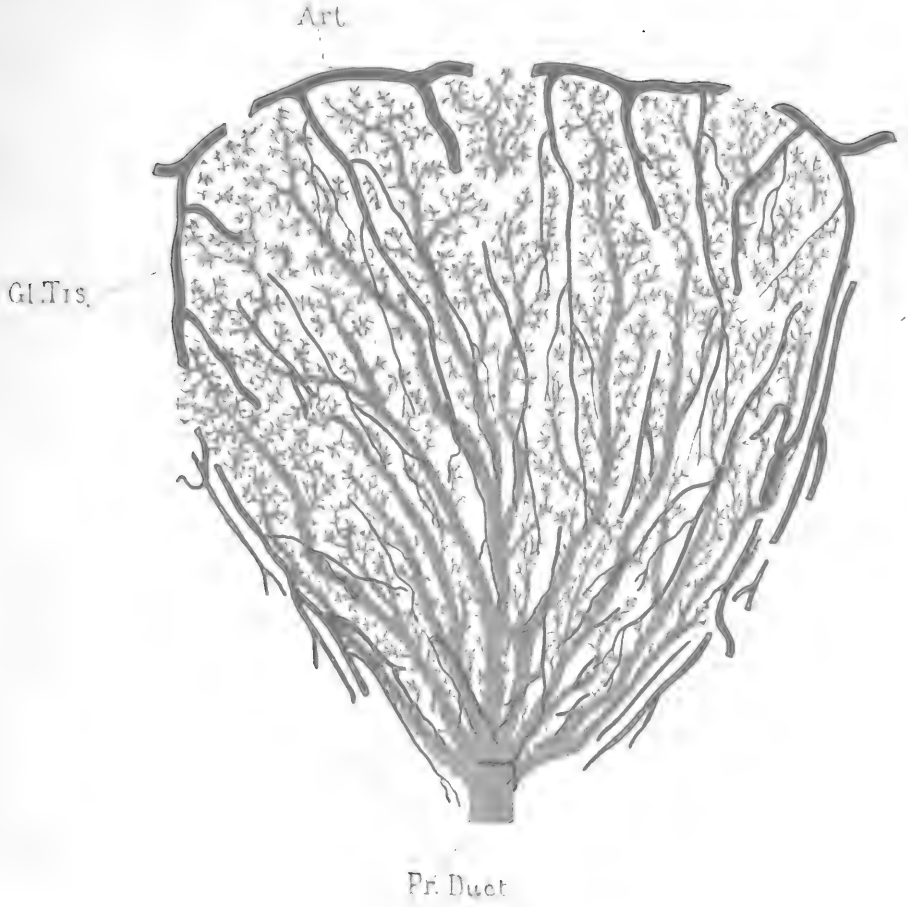

FIG. 2

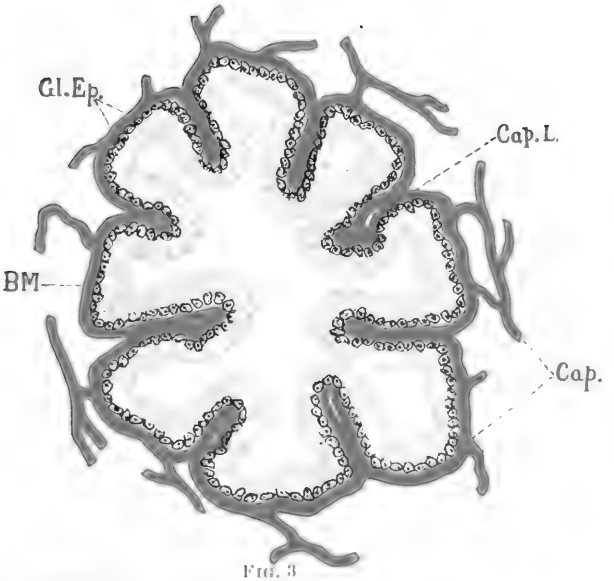

FIG. 3

THE EMBRYONIC DEVELOPMENT OF THE RETE-CORDS AND SEX-CORDS OF CHRYSEMYS.

BY

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WITH 1 DOUBLE PLATE AND 6 TEXT FIGURES.

A glance at the diagrams on the next page will at once serve to show the great difference of opinion that has prevailed in regard to the origin of the sex-cords and rete-cords of the Sauropsida. In fact, it is hard to conceive of any possible manner of origin that has not been held to be correct by some well-known embryologist.

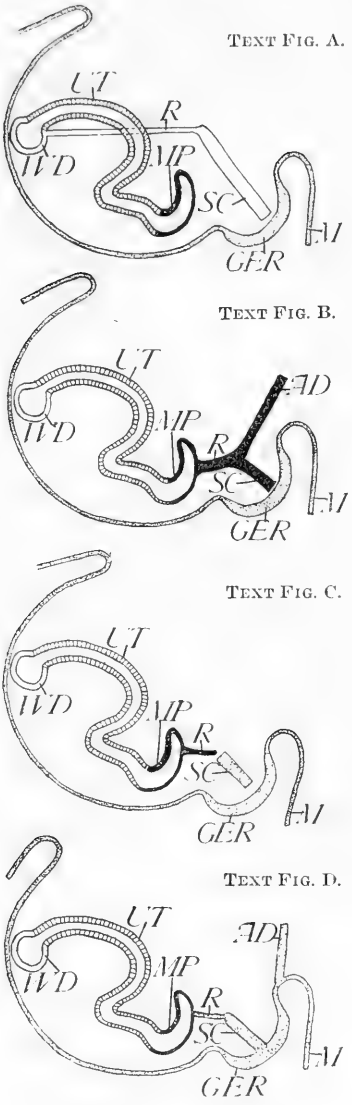
The Chelonia have remained almost untouched in the study of this problem. Only one work has appeared upon the rete-cords (Von Möller, 98), while no work has been published upon the subject of the sex-cords.

Von Möller studied two turtles, one a specimen of Emys lutaria of 2.5 cm. plastron length, and the other Clemmys leprosa of 4.9 cm. plastron length. He observed no connection between the testis and Wolffian body. This caused him to remark:

"Dieser Befund wird höchst auffällig, wenn mann bedenkt dass die Beobachtungen an Amphibien zeigen dass die Verbindungen zwischen Hoden und Wolffschen Gänge schon dann angelegt und vollendet werden, wenn die übrigen Organe sich noch in der Entwickelung befinden, und wenn das Junge in der Eischale, respective in Uterus eingeschlossen ist. Die zwei von mir untersuchten Schildkröten hatten dagegen schon seit Monaten die Eischale verloren, und doch war bei ihnen noch kein einzige Verbindung zwischen Hoden und Wolffschen Gänge vorhanden, obwohl Anlagen dieser Verbindungen sich bereits vorfanden."

It is quite unfortunate that he considered these stages to be early enough for his purpose, since my work has shown the rete-cords to be formed at a relatively early stage of development in Chrysemys.

Von Möller sums up his results as follows: "Ich finde also bei diesem Thiere zwischen Hoden und Wolffschen Körper noch keine Verbindungen, dagegen im Mesorchium und im oberflächlichen Bindegewebe der Urniere solide Zellenstränge, für welche ich genötigt bin einen Ur-



TEXT FIGS. A-D. Diagrams illustrating various views held by authors whose writings are reviewed in this article.

AD.—Fundament of the adrenal body.
GER.—Germinal epithelium.
M.—Mesentery.
M. P.—Malpighian corpuscle.
R.—Rete-cord.
S. C.—Sex-cord.
U. T.—Uriniferous tubule.
W. D.—Wolffian duct.

sprungsort anzunehmen, der weder in den Geweben der Urniere noch in denen des Hodens liegt denn ich weder im Stande bin einen Zusammenhang mit den gewundenen Kanälchen des Hodens nachzuweisen, noch einem solchen mit dem Epithel Bowmanscher Kapseln oder sonst mit Theilen der Urniere. Ich nehme daher an, dass sie vom Peritoneum stammen." No further allusion need be made to this article.

Turning to the other groups of the Sauropsida, we find a large mass of literature. To intelligently discuss this, we must use precise terms. The sex-cords are those masses or cords of cells which eventually become the seminiferous tubules of the testis or the medullary cords of the ovary. The rete-cords are those structures which eventually give rise to the canals which unite the seminiferous tubules or medullary cords of the sex-glands with the ducts of the mesonephros.

It is not necessary to enter into a lengthy review of the literature upon this subject. That has been thoroughly done by Born, 94, Mihalkovics, 85, Janosik, 85, Coert, 98, Winiwarter, 00, and others. A few diagrams will suffice to show, in a sufficiently vivid manner, the wide differences between the many views upon this subject as expressed in the papers most worthy of note.

The names associated with the different diagrams, Text Figures *A-D*, are those of the authors who have held views represented by the diagrams so indicated. After the name of each

author are placed the names of the forms which he studied in arriving at his conclusions.

A. Tubules arise from the Wolffian duct and grow into the sex-gland fundament. Their distal portions form the sex-cords while their proximal portions form the rete-tubules.

70, Waldeyer—Chick (Gallus).

B. According to this view, evaginations grow out from the capsule of Bowman. Distal branches from these stems pass down into the sex-gland fundament to form sex-cords, while the more proximal portions of the evaginations remain attached to the capsules of Bowman and serve as rete-tubules.

Braun, 77, Platydactylus, Tropidonotus.
Weldon, 85, Lacerta.
Hoffmann, 89 and 92, Lacerta, Hæmatopsis, Sterna, Gallinula.
Semon, 87, Gallus.
Peter, 04, Lacerta.

Braun, 77, considers the rete-sex-cords to be, in the strictest sense, segmental in arrangement. He expressly denies that the cells that contribute to the formation of the adrenal body are derived from branches of the evaginations from the capsules of Bowman, as asserted by Weldon, 85 and Hoffmann, 89 and 92. These two last named authors asserted that each evagination divides into a dorsal and a ventral branch, the former supplying the cells of the cortical portion of the adrenal body, and the latter forming the sex-cords. Semon, 87, was not so clear upon the question. He merely stated that the anastomosing cords arising from the capsule of Bowman pass into the adrenal and sex-gland fundaments, —the more dorsal to the former, the more ventral to the latter.

C. Large numbers of cells migrate from the germinal epithelium into the underlying stroma. From this unorganized blastema, the sex-cords are formed, suddenly crystallized as it were. The rete-cords are formed of evaginations from the capsule of Bowman.

Schmiegelow, 82, Gallus.
Mihalkovics, 85, Lacerta, Gallus.
Laulanie, 86, Gallus.

D. According to Janosik, the sex-cords arise as direct ingrowths from the germinal epithelium. Cords of cells grow from their distal ends to the capsules of Bowman, thus forming the rete-cords. Cords of cells grow in from the peritoneum between the sex-gland fundament and the mesentery to form the cortical portion of the adrenal body.

Janosik, 90, Gallus.

The following table will show the great difference of opinion held by authors working upon the same identical species. The view held is indicated in the same manner as above.

Lacerta agilis—Weldon (B); Hoffmann (B); Mihalkovics (C).
Chick (Gallus)—Waldeyer (A); Semon (B); Mihalkovics (C); Laulanie (C); Schmiegelow (C); Janosik (D); Weldon (?).

We cannot close an account of the literature upon the subject without referring to the work of Semon, **91**, upon Ichthyophis, one of the Gymnophiona, and a paper by Semper, **75**, upon the Sex-glands of the Elasmobranchs.

Semon, **91**, considers the nephrotome to be the ventral portion of the mesoblastic somite. This view, by the way, is also held by Brauer, **02**. Semon states that after the nephrotome breaks away from the myotome and sclerotome, it still remains attached to the peritoneum (unsegmented mesoderm) by means of two bridges of cells—a lateral and a medial. The major part of each nephrotome forms a Malpighian corpuscle of the mesonephros. The lateral of the two bridges connecting it with the peritoneum becomes its peritoneal funnel (nephrostome), while the medial bridge sends out a process which divides into a dorsal branch passing to the adrenal body, and irregular branches (sex-cords) non-segmental in character, that pass to the sex-glands, there to come in contact, in the case of the male, with the seminal vesicles, which are derived from the germinal epithelium. He holds a theory that the pronephros extends in rudiment, at least, along the entire length of the mesonephros, and that this pronephric rudiment develops into the adrenal body. He considers the dorsal branches spoken of above, to be these vestiges of the pronephros.

Semper, **75**, gives the most interesting account of the rete in the male of Acanthias. According to him, each of the 34 primary Malpighian corpuscles of the kidney is connected with the body cavity by a peritoneal funnel. Seven of the most anterior of these funnels lose their union with the peritoneum and take on the form of vesicles. Three or four of them now fuse together to form the "central canal," which lies at the base of the testis and parallel with it. From this central canal there arise a number of irregular anastomosing canals which extend into the testis and come in contact with the true sex-structures (Vorkeimketten) that have arisen from the germinal epithelium. This net-work of rete-cords he calls the rete-vasculosum.

In other forms there exists a somewhat modified condition of considerable interest. In comparing Acanthias and Mustelus, Semper said: "Trotzdem scheint ein grosser Untershied in Bezug auf die Entstehung

des Centralcanals des Hodens zwischen Mustelus und Acanthias zu bestehen. Bei dieser Gattung wird er seiner ganzer Länge nach gebildet durch die Verwachsung der seitlich vom Segmantalgang nach vorn sich wendenden Trichterblasen. Seitliche Ausbuchtungen der letzteren bilden den basalem Theil der rete vascolosum. Bei Mustelus dagegen ist es nur der vorderste über die Hodenfalte hinaus vorgreifende Abschnitt des Centralcanals den mann entstanden ansehen könnte, denn nur an diesen setzen sich 2 (oder 3) Segmentalgänge an. Der ganze übrige viel längere Theil des Centralcanals entsteht aus den in das Stroma der Epithelfalte eingestülpten Keimepithel Zellen."

Balfour, **78,** shows that in the forms which he studied, the anterior end of the sex-gland only, was directly united to the mesonephros by means of the rete-canals.

The condition in the lizard Platydactylus is, according to Braun, **77,** quite similar. He considers the union to be formed in adult life by two or three rete-cords joining the anterior ends of mesonephros and testis; although he states that they are connected along the entire length of the testis in early stages. Hoffmann, **89,** finds the union of rete-cords to be complete and intact along the entire length of the testis in Lacerta at the end of the first year. He did not study older specimens. Semon, **87,** claims that there is a degeneration of the rete-cords at both the anterior and posterior ends of the sex-gland of the chick; but Janosik, **90,** denies this.

Material and Technique.

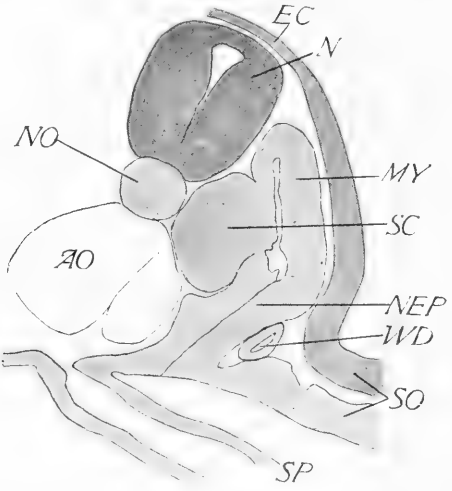
Our lakes in the vicinity of Madison abound in the little painted tortoise, Chrysemys marginata. The number of embryos to be gathered in the season is limited only by ones patience in the work of preserving them. I have prepared a large number of serial sections of the mesonephros and sex-gland, as well as of entire embryos, comprising an unbroken chain of stages from gastrulation to adult life.

As a fixative, Tellyesnitzky's Bichromate-acetic fluid was almost exclusively used, as it gave most excellent results. Haidenhein's iron-alum hæmatoxylin stain proved unsatisfactory for early stages of the embryos under 7 mm. length. For later stages than this it gave excellent results and was used almost exclusively. A counter-stain of Congo red was also employed. The sections were cut at a thickness of 7 μ.

Measurements were made of the distance between the cervical bend and the tail bend (*C-T*). In the later stages the length of carapace was also given.

To more clearly understand the origin of the sex-cords, it will be

necessary to first understand certain features in the development of the mesonephros. Reference to these features will be made only in so far as they concern the subject of this paper. In an early stage of development (*C-T.* 3.5 mm.), a section through the posterior part of the sex-gland fundament shows the mesoblastic somites to be attached to the lateral plates by the unmodified middle plate (Text Figure *E*). The cells of the latter are arranged in two rows, in such a manner as to leave a line of weakness between, which may be considered as a rudimentary lumen, connecting the body-cavity on the one hand with the cavity of the



Text Fig. E. Transverse section through the middle of the mesonephros fundament of an embryo of 3.5 mm. C-T. length.

AO.—Aorta.
EC.—Ectoderm.
MY.—Myotome.
N.—Neural canal.
NEP.—Nephrotome.
NO.—Notochord.
SO.—Somatopleure.
SP.—Splanchnopleure.
WD.—Wolffian duct.

mesoblastic somite on the other. In the region posterior to this, these relations become even more marked.

More anteriorly, just behind the interesting region which forms a transition between the pronephros and mesonephros, the middle plate is found to be wholly broken away from the mesoblastic somites, and to be divided by transverse intervals into nephrotomes which occur in the number of three to four per somite.

I found no evidence of a primary metamerism of these nephrotomes. So soon as the middle piece appeared to be broken up at all, the number of nephrotomes here recorded appeared. Special investigation along this

line, however, might show a primary metamerism, from which the above described condition was derived by further secondary splitting of the nephrogenous tissue. Each nephrotome becomes vesicular to within a short distance of the peritoneum thus forming the primary Malpighian corpuscles. The remaining portion of the nephrotome uniting it with the peritoneum becomes, in later stages, the peritoneal funnel or nephrostome, while the uriniferous tubule arises as an outgrowth from the distal end of the nephrotome. The mesonephric peritoneal funnels are vestigial structures from the time of their origin.

In later stages (*C-T.* 6 mm.), two sharply defined regions of the mesonephros may be distinguished from one another. In the anterior part of the sex-gland, only the primary Malpighian corpuscles are formed. Each is well developed, the glomerular invagination having already taken place. The 11th to 21st Malpighian corpuscles are connected with the peritoneum by peritoneal funnels (Plate I, Fig. 5), some of which are much better developed than others, there being great variation among them. In the best developed among them, the end attached to the peritoneum flares open to form an actual funnel-like mouth, yet this opening is never continuous with that of the Malpighian corpuscles. The greater part of the peritoneal funnel is merely a cord of cells. In some cases even, it has lost its continuity with the capsule of Bowman. At this stage the first ten Malpighian corpuscles are without peritoneal funnels.

Caudad of the 21st Malpighian corpuscle, each nephrotome shows two or three rudimentary vesicular enlargements. Each enlargement is destined to form a Malpighian corpuscle. The most ventral of these we shall consider as the primary Malpighian corpuscle. It is still rather broadly connected with the peritoneum. This place of union we shall consider as a rudimentary peritoneal funnel, although it has no flaring opening.

In later stages, secondary and tertiary Malpighian corpuscles appear in the anterior region described above, thus making the total number per somite approximately equal to that in the posterior region. Roughly speaking, from nine to twelve Malpighian corpuscles in all, appear in each somite.

Reference to Plate I, Fig. 1, will show certain of the points mentioned above. Furthermore, one can see an elongated mass of tissue that extends from each peritoneal funnel dorso-mediad and which lies just laterad of the V. renalis revehens (vena cava). This we shall term the *funnel-cord.* They appear in both the anterior and posterior regions of the mesonephros as described above and are co-extensive with the sex-gland

fundament, in fact they are found for a short distance anterior to it Naturally each funnel-cord lies opposite a primary Malpighian corpuscle, and likewise to the series of secondary, tertiary, etc., corpuscles formed in a vertical row above it. Each cord is made up of rather loosely arranged cells that bear a rather close resemblance to the mesenchyme cells. In fact the nuclei of these cells, the funnel cells, and the cells of the peritoneum are not to be distinguished from one another. Cytoplasmic differences alone appear and these depend upon the density of the tissue. In some cases a slight evagination of the capsule of Bowman is found at the point where it joins the peritoneal funnel. This evagination may take various forms and in many cases is wholly absent. Such an appearance may have led to the view held by some authors that these cords arise as outgrowths from the capsules of Bowman. This view would be still further justified if the peritoneal funnel were to break away from the peritoneum at a stage prior to that observed. There can be no question, however, but that the funnel-cords are outgrowths from the peritoneal funnels; in fact their bases are the funnels themselves.

The distal portions of the funnel-cords lie above the vena cava in the fundament of the adrenal body, contributing the greater part of the tissue that in later stages constitutes the cortical substance of that gland. Peritoneal ingrowths may also be seen extending dorso-laterad from the peritoneum at a point near the base of the mesentery to the adrenal fundament. These also contribute to the cortical tissue of the adrenal body. They are of less regular occurrence than the funnel cords, and in later stages lose their connection with the peritoneum, although they are easily distinguishable in the stage of 7 mm. *C-T.* length.

The sex-gland can be clearly distinguished in the embryo of 6.8 mm. *C-T.* length. It extends through six somites, although the last $\frac{1}{6}$ of it remains in a rudimentary condition. Even in this stage it consists merely of thickened peritoneum containing scattered primitive sex-cells (Ureier).

The sex-gland develops from a portion of the germinal epithelium lying between the bases of the funnel-cords and the base of the mesentery. In the embryo of 6 mm. *C-T.* length, a few primitive sex-cells were already beginning to appear in this region. At this time, the V. renalis revehens (vena cava) lies close above the germinal epithelium which has not yet begun to thicken to form the sex-cords. In an embryo of 6.8 mm. *C-T.* length the germinal epithelium has sent out masses of cells towards the V. renalis revehens, and has at the same time bent outward in such a manner as to form in transverse section, the periphery of a semi-circle, the interior of which is occupied by the sex-cords. The tips of the sex-

cords remain stationary and almost, if not quite, in contact with the wall of the V. renalis revehens, while their bases grow peripherally with the germinal epithelium. Mesenchyme cells between the sex-cords are few and far between.

At some points, the tips of the sex-cords penetrate to one side or the other of the V. renalis revehens, and penetrate to the adrenal fundament to which they contribute.

Plate I, Fig. 3 shows a wax plate reconstruction of a large part of the sex-gland of the 7 mm. *C-T.* stage. In this stage the carapace has just formed. The prominent funnel-cords afford the most striking feature of the model. Their bases are attached to the peritoneum at the lateral boundary of the sex-gland. They extend in a dorso-medial direction. It will be noticed that each is connected with a primary Malpighian corpuscle. The other Malpighian corpuscles are not shown in the model.

Mediad of the funnel-cords the peritoneum is greatly thickened, forming numerous irregular elevations and ridges between which are deep clefts and pits. These thickenings are the sex-cords. They are solid and their cells show no evidence of a radial arrangement to form a lumen. The peritoneum is far more cut up than would appear from the model. Many slight fissures separating adjacent sex-cords do not appear. In any case many of these rudimentary sex-cords are from the first, united with the funnel-cords while others anastomose freely with one another, so that all are either directly or indirectly connected with the latter.

Primitive sex-cells are frequently met with in the germinal epithelium, as well as in the distal parts of the funnel-cords. Aside from the scattered primitive sex-cells, these tissues are composed of ordinary peritoneal cells. The cells of the germinal epithelium are so crowded as to make it stain very deeply. The sex-cords are less dense, their cells being distinct and having clear, sharp outlines, thus differing from those of the sex-cords of the pig and rabbit, in which a syncytium is formed among the pure peritoneal cells. The cells of all but the most proximal parts of the funnel-cord are elongated in the direction in which the cords extend. This elongation of the cells is so marked that they resemble the surrounding mesenchyme save for the fact that their cytoplasm is more dense than that of the latter. The cells are so closely associated that these funnel-cords stand out quite clearly from the surrounding mesenchyme.

The proximal part of each funnel-cord is met by one, or sometimes two, evaginations from the capsule of Bowman of the adjoining primary Malpighian corpuscle. These evaginations are very clearly distinguish-

able in this stage from the tissue of the funnel-cords but are in close contact with them.

In earlier stages the funnel-cords are not even in contact with the capsules of Bowman, although they lie close to them. In these stages there are no evaginations from the capsules of Bowman, although a thickening of the cells of the medio-dorsal portions of them indicates the general region where these evaginations will take place. In the much earlier stages described above, 6 mm. *C-T.*, the primary union of Malpighian corpuscle, peritoneal funnel and funnel-cord has already been described. The later union of Malpighian corpuscle and funnel-cord is a secondary one, and has nothing to do with the temporary primary union. The breaking away and reuniting of these elements seems to be a useless process which I confess I am at a loss to explain. I can merely describe it. It is, however, a most easily demonstrated fact.

In later stages, the evaginations from the Malpighian corpuscles closely fuse with the funnel-cords, and are not to be distinguished from them. As development proceeds, the primary Malpighian corpuscles are often drawn some distance laterad of the sex-gland, at the same time pulling the funnel-cords laterad and causing them to stretch. In these cases each funnel-cord becomes sharply bent at the point where the evagination from the capsule of Bowman meets it; it is then continued in a dorso-medial direction to the adrenal body. As shown above, each primary Malpighian corpuscle is connected with the sex-cord by a cord of tissue, formed by an evagination from the capsule of Bowman plus the basal portion of a funnel-cord. These strands uniting the mesonephros with the sex-gland are the rete-cords and constitute the rete-testis or rete-ovarii, as the case may be. In these later stages the funnel-cords are more elongated and slender, but far more compact than in the early stages.

Plate I, Fig. 4 shows the rete-cords and the relation that they bear to the sex-cords and primary Malpighian corpuscles. Here the base of the funnel-cord lies within the sex-gland and forms one of the sex-cords. This has been observed in many cases. In very many instances, however, the funnel-cords lie wholly outside the sex-gland, their bases being still attached at a greater or less distance from the sex-gland to the peritoneum covering the mesonephros.

It will be noticed that the two rete-cords shown in this model are united to one another by a thickening of each in the direction of the long axis of the sex-gland. This represents a tendency to form a longitudinal canal uniting the rete-cords as in the Amphibia and to a certain extent in the Elasmobranchs, and in the lizard (Braun, 77). This

longitudinal canal remains incomplete, however, although it may unite several rete-cords in the manner shown.

Young males taken at the time of hatching, show many of the rete-cords to have already acquired a lumen in places. The rete-cords of females at this age do not show a lumen, nor do they at any time, because they have already paused in development. They are, however, still recognizable. Up to this point no distinction of sex has been noted although well marked differences had begun to appear in the stage of 13 mm. *C-T.* length. Close study has yet to be made to determine the earliest evidences of sex differentiation.

It is not our aim to follow the later development of the rete-cords or sex-cords. In its general features, the further development of the sex-glands of the turtle shows many points of similarity to that in the mammals. The sex-cords degenerate in the female forming the medullary cords while the "cords of Pflüger" arise as a later thickening of the germinal epithelium. In the males the sex-cords lengthen, assuming a more regular form and arrangement. Their thorough anastomosis with one another allows the semen to be poured from several into a common rete-cord. The mesonephros degenerates leaving a number of the uriniferous tubules to function as vasa efferentia. In the adult male the rete-cords are found to be reduced in number, there being nine in the specimen studied while sixteen were counted on the right side of an embryo of *C-T.* 8 mm. length. No attempt was made to determine how or when this reduction was brought about. It is quite probable that some rete-cords are weak and become broken by shifting of the organs in the process of growth. In any case there is no systematic degeneration of the rete-cords in any particular region or regions along the sex-gland.

Summary and Conclusions.

The sex-cords are formed from irregular ingrowths of the germinal epithelium. It is not until relatively late in development that they take on the semblance of cords. They are made up of ordinary peritoneal cells, together with primitive sex-cells which are also found in the peritoneum at this stage.

The rete-testis and rete-ovarii are formed by the union of funnel-cords with evaginations from the capsules of Bowman. The funnel-cords are derived from the peritoneal funnels of the Malpighian corpuscles. They occupy a region lying along the lateral edge of the sex-gland, and not only co-extensive with the latter, but extending a short distance anterior to it. The bases of the funnel-cords may, or may not, be included in the sex-gland to form a part of the seminiferous tubules of the testis or

medullary cords of the ovary, as the case may be. The proximal portions of these funnel-cords go to form a large part of the rete-testis-ovarii, while the more distal portions join the adrenal fundament and contribute the major portion of the cortical substance of that organ.

This leads me to briefly consider the adrenal body, although this was not within the original plan of the present work. Soulié, 02, finds that in Lacerta and the chick, the cortical substance arises wholly from cords of cells proliferated from the peritoneum mediad of the sex-gland and at the base of the mesentery. He states, however, that these cords become closely applied to the capsule of Bowman of the Malpighian corpuscle. It is difficult to understand how, arising from the base of the mesentery, they could reach the Malpighian corpuscle without growing dorsad along the medial side of the V. renalis revehens to the adrenal body fundament, and thence laterad and ventrad to the Malpighian corpuscle. It is difficult to understand how they could take this course, without passing through and beyond the fundament of the adrenal body. There certainly are, in the turtle, cords of cells that arise as Soulié and others claim, near the base of the mesentery, and these contribute to the formation of the adrenal body; but certain sex-cords and the funnel cords contribute to it as well, and in even greater measure. Brauer, 02, also holds a view similar to that of Soulié as regards Hypogeophis one of the Gymnophiona. Poll, 03, reached similar results with the Elasmobranchs, Acanthias and Spinax. Be this as it may, I feel quite sure of my ground in the case of Chrysemys, and the work of Weldon, 85, and Hoffmann, 89, would lend color to this view, though they hold views in some points radically different from mine.

In this connection it may be well to state that several of Hoffmann's, 89, figures of the "Sexual Stränge" would serve fairly well to represent the funnel-cords as I have seen them. They certainly do not *prove* his contention that the cords in question, sex-cords and adrenal-cords, arise from the capsule of Bowman; although he has so interpreted them.

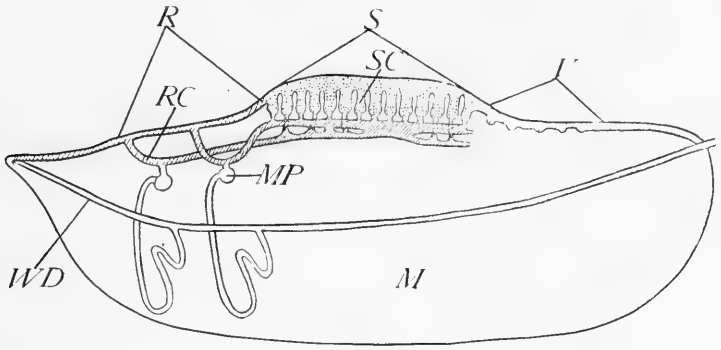
Those who held view C, probably used insufficient material and lacked the intermediate stages between the period just before the formation of the sex-cords and those subsequent to their separation from the germinal epithelium.

Janosik, 85, D, worked upon the chick. It is quite possible that future work may in large part substantiate his results for that form. My results agree with his as regards the origin of the sex-cords, but differ from his upon the origin of the rete-tissue, although even here there may be a reconciliation between our views.

In the literature upon the morphological significance of the uro-genital

system we have some melancholy examples of the futility of making rash hypotheses unsupported by a sufficient array of facts. Still it is of interest to consider the possible interpretation that may be placed upon these structures when they are viewed from the standpoint of phylogeny.

I am inclined to consider the funnel-cords as modified sex-cords. The fact that their distal extremities contribute to the formation of the adrenal bodies does not conflict with this interpretation, because that is also true of undoubted sex-cords. The funnel-cords arise just laterad of the true sex-cords and in a very similar manner. The fact that they arise from the peritoneal funnel would not be contrary to this view if the



Text Fig. F. Diagram to show essential structures of the mammalian sex-gland.

M.—Mesonephros.
MP.—Malpighian corpuscles.
R.—Rete-region.
R. C.—Rete-cord.
S.—Sex-gland region.
S. C.—Sex-cord.
V.—Vestigial portion of genital ridge.
W. D.—Wolffian duct.

funnels could be shown to be mere recesses of the peritoneum, and similar to the latter in histological character. A more careful study of the origin of the sex-glands in the Amphibia is much to be desired as it might throw new light upon this question. It will be of interest to compare the results of this paper with those of a previous paper upon the same structures in the pig and rabbit. Allen, 04.

The very schematic diagram of the testis of the pig (Text Figure *F*), shows the following points seen in a sagittal section passing through the genital ridge and the mesonephros. The genital ridge may be divided into three regions: (1) rete, (2) sex-gland, (3) rudimentary sex-gland ridge. The rete-cords are homodynamous with the sex-cords, being formed at the same time and in the same manner as the latter, and occupying

the anterior third of the genital ridge, whose middle portion is occupied by the sex-gland. As the rete-cords develop, they come in contact with slight evaginations from the Malpighian corpuscles in that part of the mesonephros which lies nearest the rete region. They then grow back to the anterior portion of the sex-gland and at a relatively late period of development advance along its entire length, giving off numerous branches (tubuli recti) which fuse with the tips of the seminiferous tubules. The rete-cords of the mammals are the peritoneal ingrowths of the anterior part of the genital ridge. Speaking in terms of phylogeny they are the sex-cords of the anterior part of the sex-gland. The *analogous* structures of the turtle, the funnel-cords, appear at intervals along the entire lateral margin of the sex-gland.

It is quite probable that the mammalian sex-gland was derived from that of some reptilian group and that some now existing groups of reptiles may show sex-gland conditions from which those of the mammals were derived. No existing group is more likely to show mammalian affinities than that of the Chelonia.

Nothing exactly corresponding to the funnel-cord has ever been found in the embryonic development of the mammals. It is true that Aichel, 00, has found that the cortical portion of the adrenal body of the rabbit (Lepus) arises from funnel-like invaginations of the peritoneum near the base of the mesentery. He is very positive in his claim that these are the peritoneal funnels of the mesonephros. Nevertheless, he does not claim to have followed these funnels back to stages in which they were actually connected with the Malpighian corpuscles. The rete-tubules that may have directly united the sex-gland proper along its entire length with the adjacent Malpighian corpuscles of the mesonephros have disappeared without leaving a recognized vestige, in any of the mammals thus far studied. The rete-region of these mammals has been evolved from that part of the genital ridge which was primitively the anterior part of the sex-gland in the ancestors of the mammals.[1]

It is scarcely possible to be more specific as regards the nature of the rete-region of the mammals. Two assumptions are possible: one, that

[1] It will be well to note that in Chrysemys, several funnel-cords occur in a well-marked region, anterior to the sex-gland, in which the sex-cords remain vestigial. Upon closer study of some sagittal sections of the sex-gland and mesonephros of Chrysemys I have been struck with the resemblance that this region bears to the rete-region of the pig and rabbit as seen in similar sections. In Chrysemys the funnel-cords of this anterior region together with those of the sex-gland region are joined to form the central canal. This shows some points of resemblance to the portions of the rete-cords lying parallel to the peritoneum anterior to mammalian sex-gland.

the sex-cords have disappeared leaving only the funnel-cords, the other, that the sex-cords which primitively existed in this region have taken on the character and function of funnel-cords. It is difficult to decide this question. I can merely say that the latter assumption seems the more probable one, because often two or more rete-cords can be seen in a single transverse section to arise from more than one point of the peritoneum covering the rete ridge. In fact the strongest and most numerous rete-cords arise from the portion of it that lies nearest the mesentery. This question might be solved with certainty by a study of the conditions in the Monotremes or even in other less primitive groups of mammals.

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The most plausible theory is that the rete-region of the mammals has not been directly derived from a condition like that in Chrysemys; but that the genital ridges of both have been derived from a type in which the anlage of the sex-cords was co-extensive with that of the funnel-cords.

To be more exact then, the rete-region of the mammals corresponds to the anterior end of the sex-gland of the turtle plus the modified region of funnel-cords anterior to it.

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EXPLANATION OF PLATE.

Ao.—Aorta.
Art.—Arterial branch passing to the Malpighian corpuscle.
FC.—Funnel-cord.
M.—Mesentery.
M. C.—Malpighian corpuscle.
P.—Peritoneum.
P. F.—Peritoneal funnel.
P. C.—Posterior cardinal vein
SC.—Sex-cord.
V. R. R.—V. renalis revehens.
W. D.—Wolffian duct.

Fig. 1. Transverse section of the mesonephros and sex-gland fundament of an embryo of 6 mm. C-T. length. × 190.

Fig. 2. Transverse section of the sex-gland fundament of an embryo of 7 mm. C-T. length (carapace 5 mm. long). × 190.

Fig. 3. Wax plate reconstruction of the indifferent sex-gland of an embryo of 7 mm. C-T. length (carapace 5 mm. long). This includes as much of the sex-gland as lies within a little more than two somites. × 190.

Fig. 4. Reconstruction of a small part of the sex-gland of an embryo of 13 mm. C-T. length (carapace 12 mm. long). × 190.

Fig. 5. Drawing of a part of a section adjacent to that shown in Fig. 1. The proximal portion of the peritoneal funnel is here better shown than in Fig. 1.

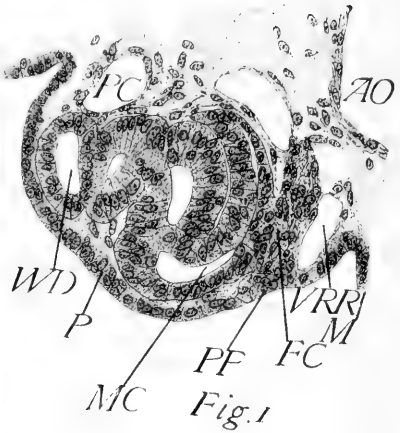

Fig. 1

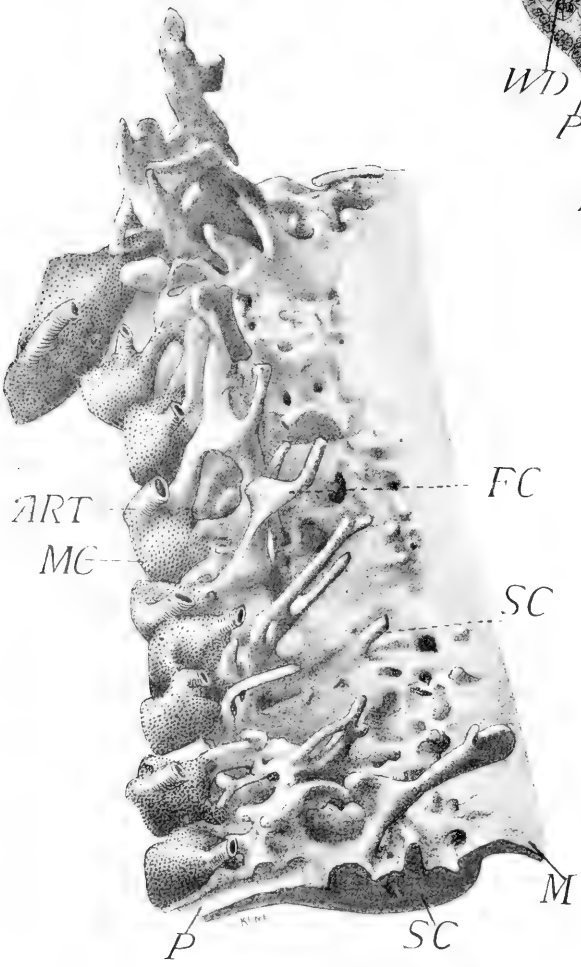

Fig. 3

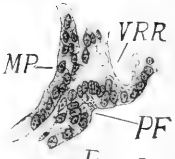

Fig. 5

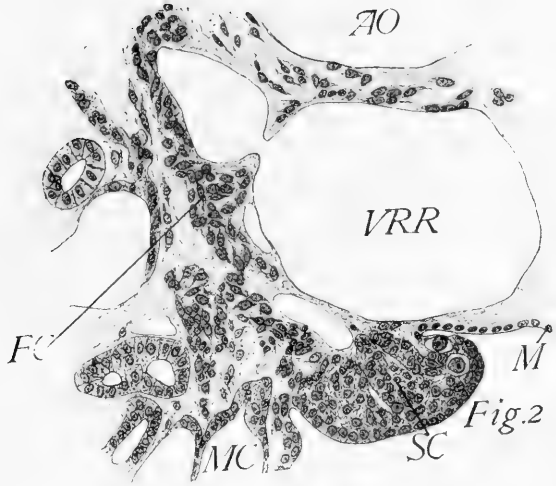

Fig.2

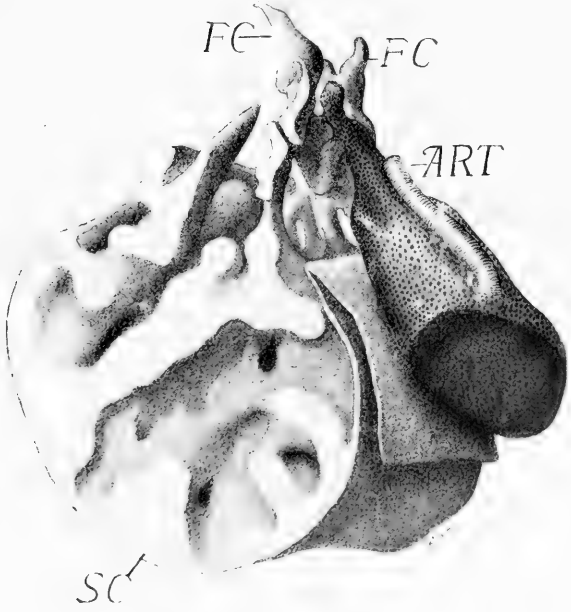

Fig 4

THE DEVELOPMENT OF THE LYMPHATIC SYSTEM IN RABBITS.

BY

FREDERIC T. LEWIS, A. M., M. D.

From the Embryological Laboratory, Harvard Medical School.

WITH 8 TEXT FIGURES.[1]

In following the transformations of the subcardinal veins in rabbits, the writer observed that a portion of those veins seemed to become detached from the venous system, and to be transformed into lymphatic vessels (**02**, p. 238). This supposition is not identical with the theory that the lymphatic system is a gland-like outgrowth of venous endothelium, always connected with the veins by means of the lymphatic ducts. It differs also from the older idea that lymphatic vessels are excavations in mesenchyma.

In favor of this mesenchymal origin, the work of Sala, **00**, is the most convincing. He observed in the chick that both the posterior lymph heart and the thoracic duct arose independently of the veins or of other lymphatics, and that their permanent openings into the veins were acquired subsequently. In the rabbit, as will be shown presently, there are many disconnected lymphatic spaces, but to their origin from mesenchyma there are four objections: 1st. The lymphatic spaces do not resemble mesenchyma even when it is œdematous, but on the contrary, are scarcely distinguishable from blood-vessels (Langer). 2d. After being formed, the lymphatics increase like blood-vessels, by means of blind endothelial sprouts, and not by connecting with intercellular spaces (Langer, Ranvier, MacCallum, Sabin). 3d. In early embryos, detached blood-vessels may be seen without proving that blood-vessels are mesenchymal spaces. These detached vessels are not far from the main trunks, from which they may have arisen by slender endothelial strands, yet often the connecting strands cannot be demonstrated. A similar supposition would account for detached lymphatic vessels. 4th. The endothelium of the embryonic lymphatics is sometimes seen to be continuous with that of the veins.

[1] This investigation, and the one which follows, were accomplished with the aid of a Bullard Fellowship, established in memory of John Ware.

The second theory, that of the gland-like origin of the lymphatic system, is supported by the remarkable injections of pig embryos, made by Prof. Sabin.[2] She considers that in mammals, this system buds from the venous endothelium at four points, forming four lymphatic ducts. The ducts are dilated to form four lymph hearts, which, though destitute of muscles, correspond with the four lymph hearts of amphibia. Starting from these hearts, lymphatic outgrowths invade the body, and those from the anterior pair unite with those from the posterior pair. Then the posterior hearts lose their original openings into the veins, but those of the anterior hearts persist as the outlets for the thoracic and right lymphatic ducts respectively. The lymph hearts themselves are said to become transformed into lymph nodes (**05**, p. 355).

According to this idea, the lymphatic vessels are true lymphatics from their earliest inception. They differ from other branches of the veins by their very oblique angle of entrance, and by failing to anastomose with arteries or veins. Anastomoses with other lymphatics are abundant, due to absorption of contiguous walls (Ranvier, **97**, p. 74).

The supposition suggested by the study of the subcardinal veins is intermediate between those of Sabin and Sala. The endothelium of the lymphatics is considered to be a derivative of that which lines the veins, since the lymphatics are at first a part of the venous system; but by becoming detached from their origins these lymphatics form closed sacs in the mesenchyma. Later they acquire permanent openings into the veins, and many connections with other lymphatics.

In studying the development of the lymphatic vessels, several methods have been employed. Sala used serial sections, generally of injected embryos, and made wax reconstructions of the posterior hearts. Sabin perfected the method of injection which had been employed by Ranvier for pigs of 100 mm., so that it was applicable to those of 20 mm. By this means she studied the large jugular lymph sacs, or "anterior hearts," which, as Saxer discovered (p. 370), are the earliest lymphatic vessels to appear. On the basis of injections she was enabled to present the first connected account of the development of the mammalian lymphatic system. This was illustrated by a series of conventional diagrams, in which the blood-vessels are shown without details. Thus the internal

[2] Ranvier described the interesting analogies, both functional and embryological, between typical glands and the lymphatic system. Sabin does not adopt the idea that the whole lymphatic system represents a few large glands. She does, however, describe it as arising from four blind epithelial (endothelial) outpocketings which ramify in the connective tissue, and this origin may be designated, after Ranvier, as "gland-like."

and external jugular veins are merged in an "anterior cardinal vein," the subcardinals are omitted, the renal and iliac anastomoses are made continuous with one another, and the sciatic and femoral veins are reversed.

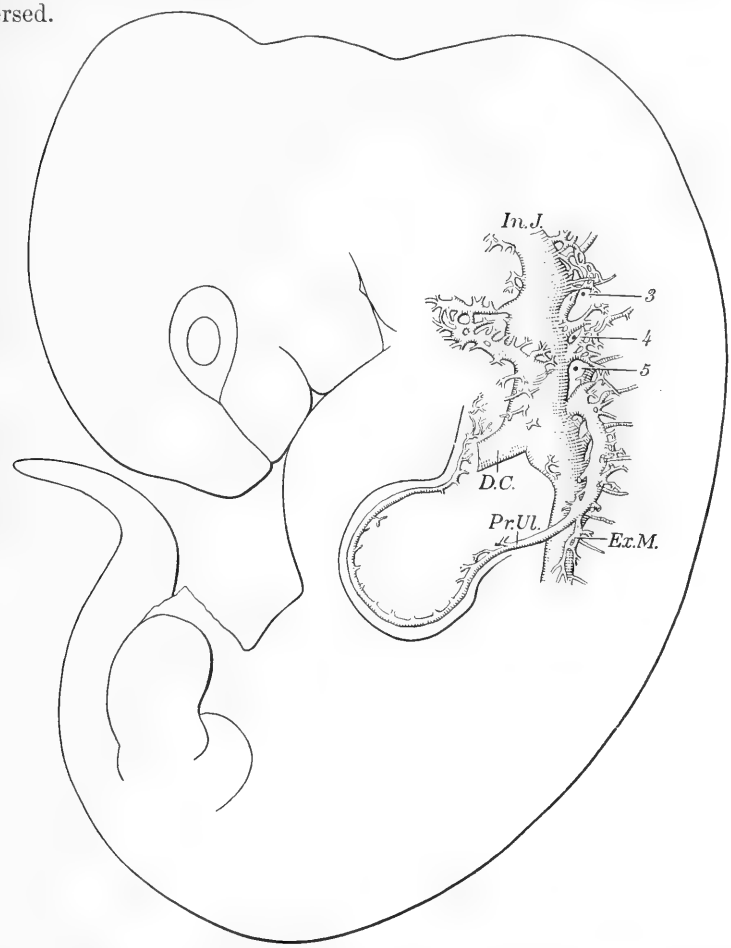

FIG. 1. Rabbit, 13 days, 9.5 mm., Harvard Embryological Collection, Series 498, × 13 diams. 3, 4, and 5 indicate the position of the corresponding cervical nerves in this, as in the following figures. The veins shown are those of the left side: *D. C.*, duct of Cuvier; *Ex. M.*, external mammary; *In. J.*, internal jugular; *Pr. Ul.*, primitive ulnar.

It was thought that more accurate figures might be obtained by the graphic reconstruction of uninjected embryos. The possibility of overlooking minute orifices guarded by valves, and the limitation of this method to small embryos are obvious disadvantages, but these are offset

by the avoidance of rupture of very thin-walled vessels and by the opportunity of seeing lymphatics too small for injection. The method has been employed with the following results.

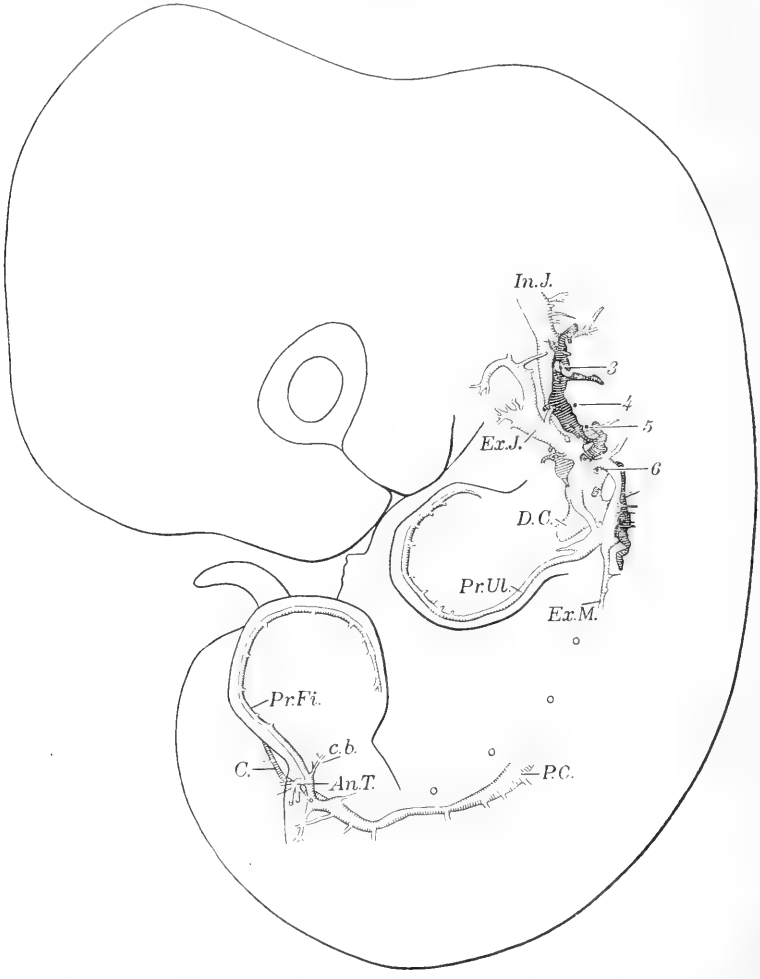

Fig. 2. Rabbit, 14 days, 10 mm., H. E. C., Series 155, × 13 diams. The lymphatic vessels are heavily shaded, as in all the following figures. The veins are those of the left side: *An. T.*, anterior tibial; *C.*, caudal; *c. b.*, "connecting branch"; *D. C.*, duct of Cuvier; *Ex. J.*, external jugular; *Ex. M.*, external mammary; *In. J.*, internal jugular; *P. C.*, posterior cardinal; *Pr. Fi.*, primitive fibular; *Pr. Ul.*, primitive ulnar.

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.

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, 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.

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 veins. No lymphatic vessels were found which did not connect with the jugular sacs. The dorsal subcutaneous extension, described in the preceding stage, occurred on both sides. In the posterior part of the embryo, no lymphatics were found. The reconstruction of the cardinal veins is that already figured in this journal, Vol. I, Plate 2, Fig. 5 (following p. 244).

The cardinal veins of the 14.5 mm. rabbit, Fig. 4, were also shown in the earlier paper (Plate 2, Fig. 7). In the plate, the lower portions of the subcardinal veins are detached from the rest, and, though colored blue

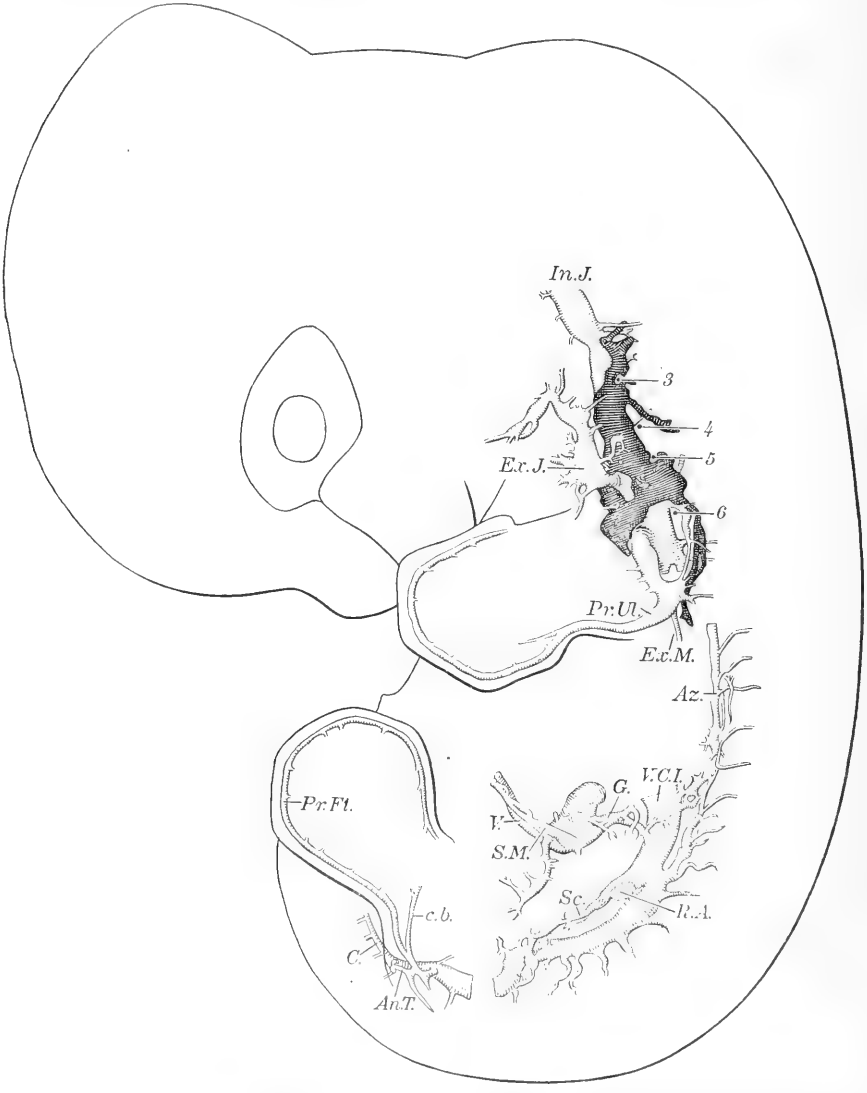

FIG. 3. Rabbit, 14 days, 11 mm., × 13 diams. The structures drawn are the same as in Fig. 2, except that in the trunk of the embryo the following veins, belonging to the median plane and to the right side, have been added: *Az.*, azygos; *G.*, gastric; *R. A.*, renal anastomosis of the subcardinal veins; *Sc.*, subcardinal; *S. M.*, superior mesenteric; *V.*, vitelline; *V. C. I.*, vena cava inferior.

like the veins, they are described and figured as "spaces in the mesentery" suggesting the lymph hearts of the chick (p. 238). It is stated that these spaces "may be subcardinal derivatives." Re-examination of this embryo has yielded no more definite information. The spaces which are undoubtedly lymphatic, as shown by their later development, seem to replace veins of the preceding stage. In the same way the lymphatic vessels in the mesentery, accompanying the superior mesenteric and the gastric veins may have arisen as the branches of those vessels seen in Fig. 3. They extend around the superior mesenteric artery, which the corresponding vein accompanies. The fused vitelline vein is destitute of small branches, and is not provided with lymphatics.

The jugular lymph sac in Fig. 4 has completely surrounded the third and fourth cervical nerves. It envelops two-thirds of the circumference of the internal jugular vein. 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. The deep subcutaneous outgrowth from the jugular sac has become greatly dilated in its distal portion. Near the beginning of the external mammary vein, a large lymph space is found wedged between two converging venous branches. This space is not connected with the veins. It may be a remnant of the lymphatic vessels which in the preceding stage accompanied the dorsal root of the ulnar vein. A few slender detached lymphatics follow the external mammary vein. Finally there are two lymphatics which appear to have arisen from branches of the azygos vein, one near the vagus nerve (Fig. 4, x) and the other along the aorta (Fig. 4, y). The former connects with a small vein, the latter ends blindly not far from one. Obviously when a connection with a vein is well preserved the structure in question would be considered a venous branch; and after becoming detached, were it not for its endothelial wall, it might be called a mesenchymal excavation. The study of this and the following specimens seems to show that the lymphatics along the aorta (thoracic ducts) are derived in part from the azygos veins; below, from the subcardinals; and above, from the jugular sacs.

In order to determine whether the lymphatic system of the rabbit differed materially from that of other mammals, reconstructions were made of a 21 mm. pig, and a 15 mm. cat. The former is of special interest as a basis of comparison between the present work and that of Prof. Sabin. The lymphatics in the pig (Fig. 5) consist of a pair of jugular lymph sacs, a pair of subcardinal sacs which fuse with one another irregularly and are variously subdivided by thin septa, and

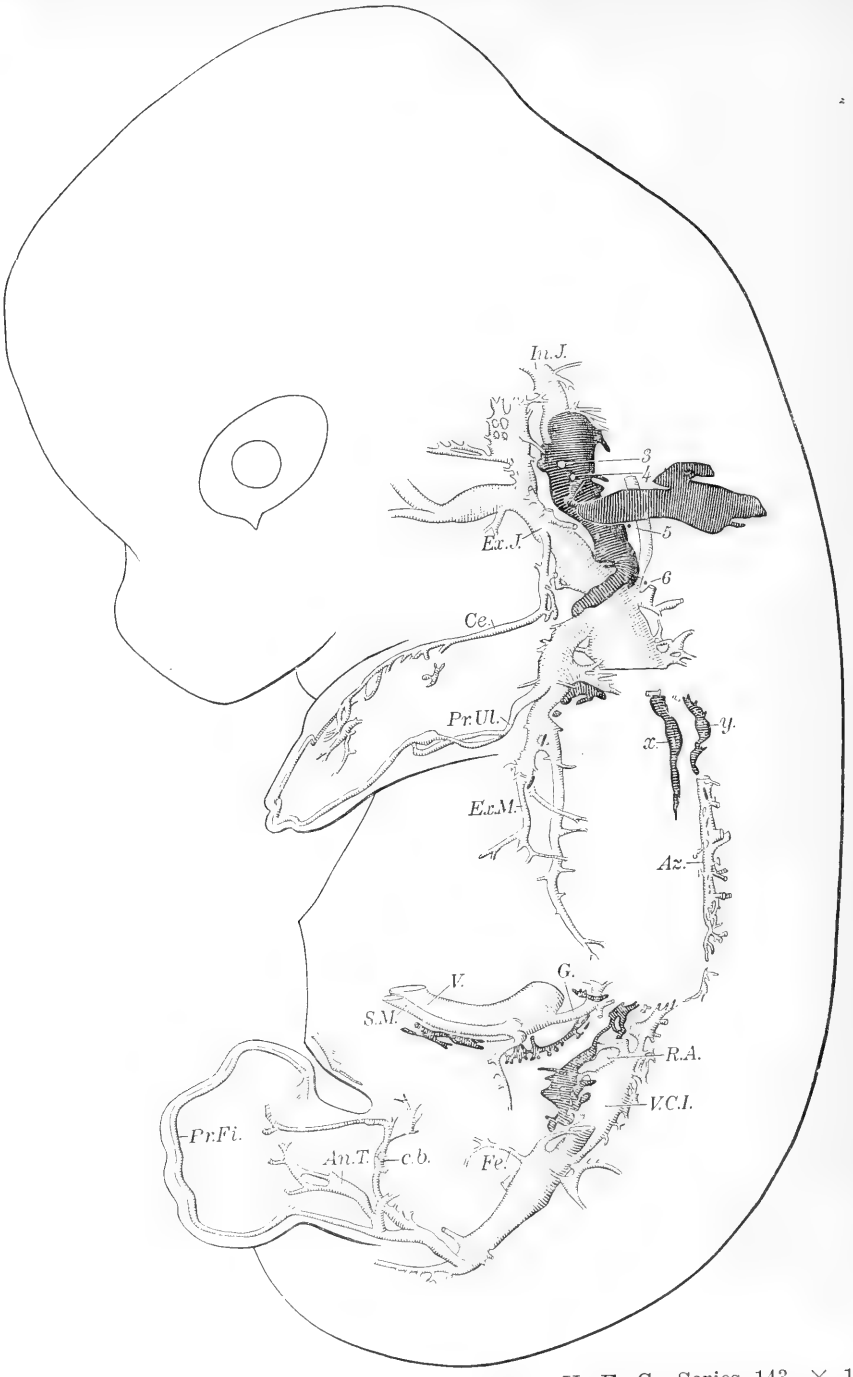

FIG. 4. Rabbit, 14 days 18 hours, 14.5 mm., H. E. C., Series 143, × 13 diams. *x* designates a lymphatic vessel accompanying the left vagus nerve; *y*, a lymphatic along the aorta. The veins of the arm are: *Ce.*, cephalic; *Pr. Ul.*, primitive ulnar. Those of the leg are: *An. T.*, anterior tibial; *c. b.*, "connecting branch"; *Fe.*, femoral; *Pr. Fi.*, primitive fibular.

finally some irregular spaces behind the aorta, probably derived from the azygos veins. These spaces also fuse across the median line at several points.

The jugular sac is shaped like a D of which the chief portion is vertical and closely applied to the internal jugular vein. Through the aperture in the D pass the third, fourth, and fifth cervical nerves, and from its dorsal arch several deeply subcutaneous sprouts pass off, corresponding with the single large sac of the rabbit. No connection between the jugular sac and the veins could be detected. Except for this point, the reconstruction agrees with, and combines, the figure and diagram presented by Prof. Sabin in this journal, Vol. 3, p. 184, and Vol. 4, p. 359. It does not agree so well with the diagram on p. 380 of Vol. I. In the latter the subcardinal lymph spaces are not shown. The posterior portion of the body contains instead two "lymph hearts" arising from the posterior cardinal veins "below the Wolffian body" but anterior to the femoral vein. In later stages, outgrowths from these hearts invade the skin of the back, and ultimately, as has already been noted, Prof. Sabin considers that the hearts become transformed into lymph nodes. From this description, it appears that the posterior lymph hearts are in the position of the ilio-lumbar veins. In the pig embryo represented in Fig. 5, however, no lymphatics were found in relation with the ilio-lumbar vessels.

Considering its lymphatic development the pig of 21 mm. is less advanced than the rabbit of 14.5 mm., since there are no lymphatic vessels along the external mammary vein nor in the mesentery. The cat of 15 mm. is more advanced than either. In this embryo the D formed by the jugular sac is almost bisected diagonally. The second, third, and fourth nerves pass through its aperture, but the fifth penetrates the posterior section of the sac by a separate opening. There are two deep subcutaneous diverticula corresponding with the single one in the rabbit and several in the pig. 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.

Where the external mammary vein joins the brachial there is a large sac, and the question arises whether or not the detached lymphatics following the mammary vein are independent formations, or are outgrowths from that sac. The occurrence of the lymphatics especially near the places where the veins branch suggests that they may have budded at such points. On the other hand, as in the rabbit, their order of appearance is from the proximal part of the vein distally. Similarly there are

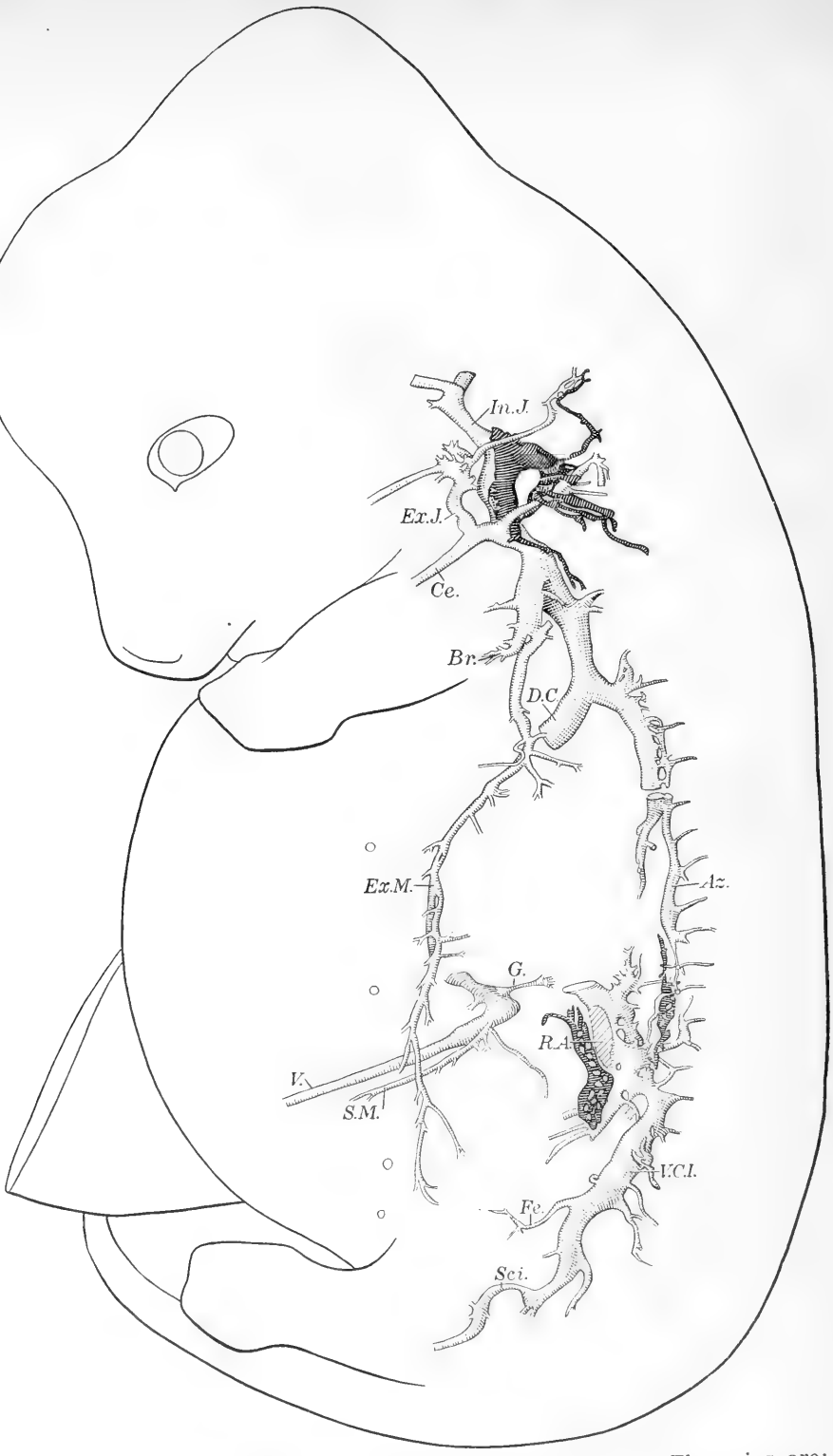

FIG. 5. Pig, 20 mm., H. E. C., Series 59, × 10 diams. The veins are: *Az.*, azygos; *Br.*, brachial; *Ce.*, cephalic; *D. C.*, duct of Cuvier; *Ex. J.*, external jugular; *Ex. M.*, external mammary; *Fe.*, femoral; *G.*, gastric; *In. J.*, internal jugular; *R. A.*, renal anastomosis of subcardinals; *Sci.*, sciatic; *S. M.*, superior mesenteric; *V.*, vitelline; *V. C. I.*, vena cava inferior.

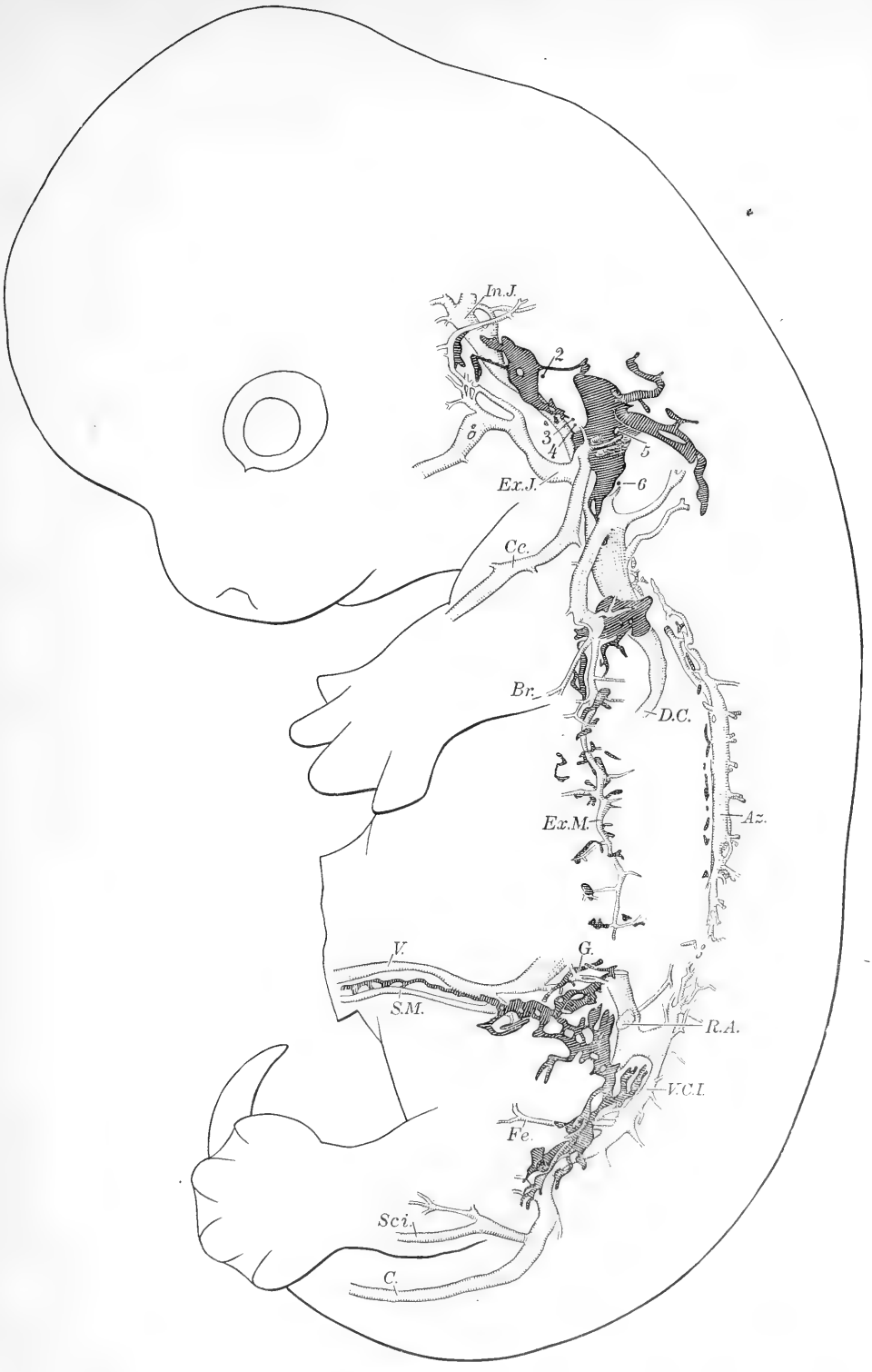

FIG. 6. Cat, 15 mm., H. E. C., Series 436, × 13 diams. The lettering is the same as in Fig. 5.

obscure spaces which appear to be lymphatic, along the aorta, and in relation with the azygos veins. An occasional apparent connection with the vein suggests their venous origin in situ. The mesenteric and subcardinal plexuses have united with one another. They do not empty into the veins. The subcardinal sacs extend from the renal anastomosis almost to the sciatic vein, connecting with one another across the median line, as in the pig. No lymphatic vessels follow the ilio-lumbar veins into the posterior body wall.

Returning to the rabbit embryos it will be seen that Fig. 7 from a 21 mm. rabbit differs from Fig. 4, the 14.5 mm. embryo, chiefly in regard to the thoracic duct. The duct is represented by a pair of vessels which connect with one another and pass on to the left jugular sac. Sometimes in the adult rabbit, as figured by Gage (02, p. 650), and occasionally in man, the thoracic duct bifurcates anteriorly and passes to the jugular sacs on either side. This did not occur in the 21 mm. embryo, which exhibited the relations figured by Sabin, Vol. I, p. 383.

In Fig. 7 scattered lymphatics are shown along the external jugular vein and its branches. One much larger than the rest occurs where the anterior and posterior facial veins unite. From its isolation it probably arose independently of the large jugular sac. Other and more isolated lymphatic centers are seen in the oldest rabbit studied, one of 20 days, 29 mm., Fig. 8, notably along the pudic and the sciatic veins. They arise near the junction of several venous branches, with which, however, they are not in communication.

In the oldest embryo the lymphatic system has invaded the skin to such an extent that it is impracticable to represent more than a small part of it. In entering the skin the lymph vessels accompany the veins, those of the head following chiefly the external jugular vein. The jugular sac has become relatively less important, and persists as the lymphatic sheath of the internal jugular vein. The deep subcutaneous extension has become covered by a thin layer of muscle, presumably the panniculus, and does not appear to connect with the more superficial vessels of the skin. There are no lymphatics in the distal part of the arm, but the subcutaneous vessels of the shoulder are attended by rich networks. These veins are the external mammary, and another which is ventral to the scapula and posterior to the shoulder joint,—a subscapular vein. The lymphatics along this large subscapular vein do not connect with the jugular sac. At the point *L. N.*, indicated in the figure, a small but very distinct lymph node has developed in relation to these subscapular lymphatics. A corresponding node is found on the opposite side of the body.

FIG. 7. Rabbit, 17 days, 21 mm., H. E. C., Series 738, × 10 diams. The veins not previously lettered in the rabbit figures are: *Il.*, ilio-lumbar; *Ss.*, subscapular; *R.*, radial; *Sci.*, sciatic.

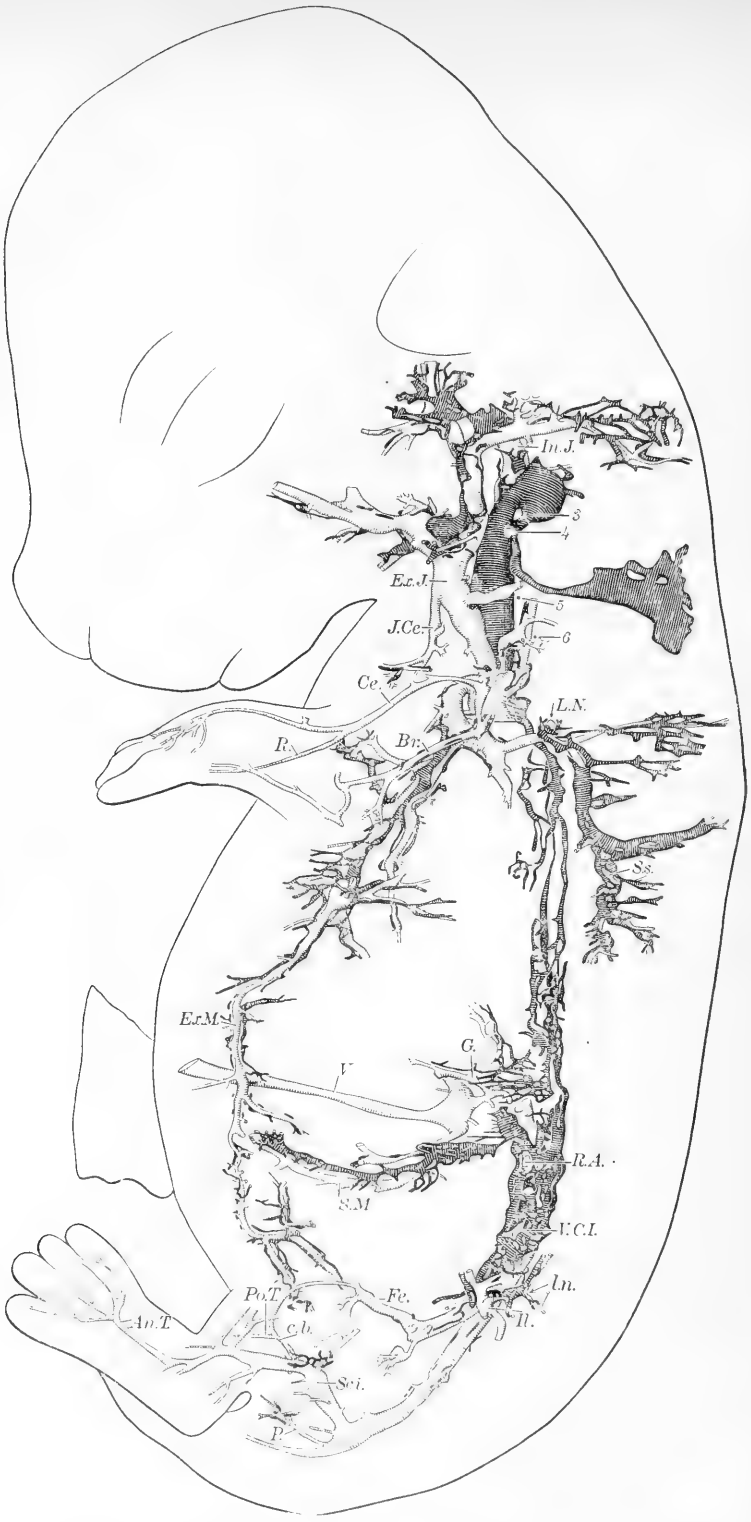

FIG. 8. Rabbit, 20 days, 29 mm., H. E. C., Series 170, × 6.9 diams. The first lymph nodes develop at *L. N.*, along the subscapular vein, *Ss.*; and at *l. n.*, along the ilio-lumbar vein, *Il.* The veins of the arm are: *Br.*, brachial; *Ce.*, cephalic; *J. Ce.*, jugulo-cephalic; *R.*, radial. Those of the legs are: *An. T.*, anterior tibial; *Sci.*, sciatic; *Po. T.*, posterior tibial; *Fe.*, femoral; *c. b.*, connecting branch between femoral and sciatic. *P.* marks the pudic vein.

The jugular 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 stages must be by osmosis, therefore, and the permanent outlets of the lymphatic system must develop later.

The left jugular sac in Fig. 8 connects with the thoracic duct, which arises from a plexus of lymphatics surrounding the aorta. Ventral to the aorta these vessels receive the lymphatics from the mesentery. There are none in the leg. The body wall is supplied by those which follow the external mammary vein in its anastomosis with the superficial epigastric, and by vessels accompanying the ilio-lumbar vein. The ilio-lumbar vein of Krause, which Hochstetter named the posterior transverse lumbar, supplies the subcutaneous tissue of the back, and seems to be inversely homologous with the much larger subscapular vein. At the position *l. n.,* indicated in Fig. 8, a node is found among the lymphatics accompanying this vein. A similar node exists on the opposite side, and the pair was identified in a duplicate series of a 20-day rabbit. These superior inguinal nodes (Krause) develop almost simultaneously with the subscapular nodes already described. The early appearance of the inguinal nodes further identifies the lymphatics of the ilio-lumbar vein with the "posterior lymph heart" of Prof. Sabin. It is my opinion that an identification of this structure with the amphibian or avian lymph heart is, at present, not justified. The posterior heart of the bird empties into the coccygeal veins (Sala), and that of the frog into the transverse iliac vein, a vessel connecting the femoral with the sciatic vein (Gaupp). The ilio-lumbar vein is more anterior than either. Its lymphatics do not differ in form, from those accompanying other veins, and they are presumably non-contractile. If the first lymph nodes can be utilized in making comparisons, then this "posterior heart" of the rabbit should be compared with the lymphatics of the subscapular vein, and not with the jugular sac. The jugular sac itself does not empty into the vertebral vein, like the anterior heart of the frog. It is non-contractile, so far as known. If it shall be found that the anterior heart of the frog develops from the first lymphatics which are formed in that animal, a comparison between the jugular sac and a lymph heart may be possible. At present it is not evident that mammals possess any lymph hearts.

Summary.

The lymphatic system of rabbits begins along the internal jugular vein as a detached sac formed by the coalescence of several venous outgrowths.

Similar though smaller sacs arise from the subcardinal and mesenteric veins at a slightly later date.

Subsequently lymphatic vessels develop along the courses of the azygos and cutaneous veins, apparently from independent venous outgrowths. All of these vessels unite with one another to form a continuous system, which acquires new and permanent openings into the veins near the subclavian termination.

The first lymph nodes observed are two pairs, one beside the subscapular vessels, and the other beside the ilio-lumbar vessels.

In order to facilitate comparison with Prof. Sabin's work, the following conclusions may be added:

The lymphatic system does not arise from the venous system by four outgrowths, but by several. It is not always in communication with the veins. The outlets of the thoracic and right lymphatic ducts are not persistent primary openings. An identification of mammalian lymph hearts, comparable with those of the amphibia, should not be made, on the evidence now available. Judged by their relation to the early lymph nodes, the jugular sac is not comparable with the lymphatics along the ilio-lumbar vein. However, the study of rabbit embryos confirms the chief conclusion established by Prof. Sabin, that the lymphatic system is a derivative of the venous system.

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THE DEVELOPMENT OF THE VEINS IN THE LIMBS OF RABBIT EMBRYOS.

BY

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WITH 1 TEXT FIGURE.

In connection with the preceding study of the lymphatic system it was necessary to reconstruct the veins of the shoulder and hip in a series of rabbit embryos. The reconstructions were then extended to include the distal portions of these vessels, complete figures of which had never been published. Hochstetter, in 1891, had observed the veins in the limbs of living rabbit embryos, and had studied them in serial sections. His drawings, however, show only detached portions of the veins such as could be seen under most favorable conditions, in living embryos. Ten years later Grosser described but did not reconstruct, the developing veins in the extremities of bats. To these two investigators embryology is indebted for the present knowledge of the veins in mammalian limbs. It is proposed to review their work, while describing the reconstructions, considering first the veins of the anterior extremity, then those of the posterior extremity, and finally the homologies which exist between the two sets.

VEINS OF THE ANTERIOR EXTREMITY.

In the youngest rabbit figured, an embryo of 13 days, Fig. 1, p. 97, the small vessels along the radial or anterior border of the arm unite to form a vein which follows the periphery of the limb to its posterior or ulnar border, and then ascends behind the brachial plexus to terminate near the junction of the anterior and posterior cardinal veins. It receives a branch which at this stage is not well defined, ascending in the body wall. This is the *Seitenrumpfvene* of Hochstetter, and becomes the external mammary vein of the adult.

According to Hochstetter, in rabbits of 12 and 12½ days, the "border vein" makes a complete circuit of the limb, and its radial part either empties into the ulnar vein near its termination or connects with the cardinal vein directly. But this radial vein is said to be hard to follow

because "attended by several venous twigs of nearly the same caliber, and only shortly before its termination is it recognizable as a distinct (stärkeres) vessel" (p. 24). In a 13-day rabbit the radial vein had disappeared, "since it had been but imperfectly marked out." Similarly Grosser found a radial vein emptying into the anterior cardinal close to the ulnar vein, in the youngest bat which he studied (4¾ mm.). In the next stage (6¼ mm.) it had vanished (p. 136). An examination of rabbits of 12 and 12½ days, together with younger ones in the Harvard Collection, shows that the first vein of the arm develops along its ulnar margin, extending distally around the border to the radial side. Small and variable vessels such as Hochstetter described as a radial vein may occur, as shown in Fig. 1, p. 97, but they do not form a structure comparable with the primitive ulnar vein. The latter may be called the primary vein of the arm.

The rabbit of 14 days, Fig. 2, p. 98, presents the condition described by Hochstetter in embryos of 13 days. The primitive ulnar vein has acquired a new outlet ventral to the brachial plexus, so that, by the persistence of the original dorsal termination, most of the plexus and the brachial artery are surrounded by a loop of vein. In the following rabbit, Fig. 3, p. 100, the ventral outlet of the ulnar vein is the chief one. This specimen shows a small vessel extending from the external jugular vein toward the radial border of the arm.

The next embryo, Fig. 4, p. 102, is considerably more advanced. The dorsum of the hand, which was previously its external surface has rotated and become anterior; the arm is in pronation. The differentiation of the fingers is indicated by the sinuous terminal border of the hand, and by shallow interdigital depressions on its dorsum. Beneath these, interdigital veins have been formed, probably from branches of the primitive ulnar vein. A new vein has grown from the external jugular down the anterior or radial border of the arm, and has united with the independently formed interdigital veins. This is the cephalic vein of the adult. It is embryologically the second vein of the arm.

Hochstetter states that the cephalic vein in rabbits develops toward the body from the back of the hand, connecting with the ulnar vein at the elbow, and later continuing up the arm to the external jugular vein. The preceding reconstructions of the rabbit agree better with Grosser's description of the bats. He failed to find a stage in which the cephalic vein emptied into the ulnar. In the earliest specimen in which the cephalic vein was found, it connected with the external jugular vein.

The cephalic vein of the 17-day rabbit is the chief vein of the limb, and has developed a branch which follows the radial artery, the deep radial

vein, Fig. 7, p. 107. At 20 days, Fig. 8, p. 108, the cephalic vein has acquired its new and permanent orifice near the axillary vein. The jugulo-cephalic vein marks its former outlet.

With the differentiation of the digits, the primitive ulnar vein becomes greatly reduced by the loss of its distal portion. This is shown in Fig. 4. At 17 days, Fig. 7, p. 107, the continuity of the primitive ulnar vein has been interrupted at the elbow, resulting in further reduction. The vein then extends from the elbow to the superior vena cava, following the brachial artery, from around which it receives small branches. In the 20-day embryo, Fig. 8, p. 108, the brachial vein (proximal part of the primitive ulnar) is continued down the forearm following the ulnar artery. If we may judge from the position of this vessel, there has been a re-establishment of the course which was interrupted in the younger embryo. Hochstetter, however, states (p. 28) that in rabbits the forearm section of the primitive ulnar vein seems to disappear, although in man (p. 33) the corresponding vessel is preserved throughout, and forms the basilic vein of the forearm and arm, the axillary and subclavian veins.

The question arises whether the primitive ulnar vein should be described as producing the deep ulnar, brachial, and axillary veins, naming it for the adjacent arteries, or as forming the basilic and axillary veins, considering the cutaneous vein of the corresponding region as its more direct derivative. This uncertainty calls attention to the fact that both the superficial and deep sets of veins have a common origin, and that before their separation the embryonic vein may properly be called either brachial or basilic. The rabbit of 20 days is characterized by the establishment of this brachial (or basilic) vein.

In the development of the veins of the arm three stages have been distinguished:

1st. The stage of the primitive ulnar vein.
2 d. " " " " cephalic vein.
3 d. " " " " brachial vein, the cephalic vein persisting.

Veins of the Posterior Extremity.

A rabbit of 10½ days (Harvard Collection, No. 199) has a very large umbilical vein which sends branches into both limbs. Those in the leg form a net which connects with the posterior cardinal vein, still a minute vessel in the caudal end of the body. From the network a vein is developed, which after following the periphery of the limb and passing along its posterior or fibular border, empties into the cardinal vein. This vessel may be called the primitive fibular vein. The original connections of the

net with the umbilical vein do not form a well defined vessel and soon disappear. Although Hochstetter recognizes this, he refers to the connection with the umbilical vein as a tibial border vein. Grosser could not identify such a vessel in any of his three youngest bats (p. 149).

The primitive fibular vein as shown in Fig. 2, p. 98, is a vessel readily comparable with the primitive ulnar vein. Both course along the posterior borders of their respective limbs, in which they are the first veins developed. They are undoubtedly homologous. In later development, however, they constantly diverge from one another. Even at 14 days the fibular vein has two small branches which are not matched by any belonging to the ulnar vein. One of these, coming from twigs on the outer and caudal surface of the leg, becomes the anterior tibial vein, *An. T.* The other which extends mediad toward what at this stage is the inguinal line, may be referred to as the "connecting branch," *c. b.* In the more advanced embryo, Fig. 3, p. 100, the same branches appear in similar relations. They have become much larger at 14 days 18 hours, Fig. 4, p. 102. Here the anterior tibial branch has extended diagonally down the limb to the dorsum of the foot. The connecting branch has sent its twigs into the abdominal wall and the adjoining tibial border of the limb. The primitive fibular vein is still the chief vein of the leg.

In the older rabbit, Fig. 7, p. 107, the differentiation of the toes has broken up the distal portion of the primitive fibular vein, which has disappeared almost to the point where it receives its anterior tibial branch. This branch now arises from the interdigital veins on the dorsum of the foot and its main trunk appears continuous with the proximal part of the primitive fibular vein. The anterior tibial and primitive fibular veins together, now constitute the sciatic vein, which is embryologically the second vein of the leg.

The reconstructions to which we have referred agree with Hochstetter's description of the development of the sciatic vein except in one detail. They do not show that a part of the primitive fibular vein distal to the anterior tibial branch persists as the small saphenous vein.

In the rabbit of 14 days 18 hours, a third vein of the leg has begun its development. This is the femoral vein which terminates in the posterior cardinal anterior to the sciatic vein. It advances toward the tibial border of the limb. At 17 days, Fig. 7, p. 107, it is seen approaching the external mammary and the connecting branch of the sciatic vein. In the embryo of 20 days, Fig. 8, p. 108, it has anastomosed with both and passes down the leg as the posterior tibial vein, *Po. T.*

Just as it is questionable in the arm whether the parent vessel should

be designated brachial or basilic, so in the leg there is the choice between femoral and large saphenous vein. Both of the latter spring from the vessel which we have called femoral. The close relation between the two is shown by Krause's description of the veins in the adult rabbit, where the posterior tibial is considered to be the distal continuation of the large saphenous vein. It seems probable also, that the anteror tibial vein, which is quite superficial at 20 days, though it accompanies the artery, should give rise to the small saphenous vein, with which it anastomoses in the adult. Hochstetter, as already noted, assigns a somewhat different origin to the small saphenous vein.

The condition found in the rabbit at 20 days, is essentially that of the adult. The sciatic vein remains a large vessel. In man, assuming that the embryological history is similar to that of the rabbit, the proximal section of the sciatic vein dwindles after the formation of the femoral anastomosis near the knee. The sciatic vein is represented, therefore, merely by the collateral circulation of the thigh, as figured by Charpy (Poirier's Anatomie, Vol. 2, p. 1052), and by Spalteholz (Handatlas, Vol. 2, p. 469).

The preceding observations seem to establish three stages in the venous development of the leg, comparable with those in the arm.

1st. The stage of the primitive fibular vein.
2d. " " " " sciatic vein.
3d. " " " " femoral vein, the sciatic vein persisting (in man, very much reduced).

Homologies between the Veins of the Anterior and Posterior Extremities.

Bardeleben's view that the primary vein of the arm consisted of the vena cephalica antibrachii, vena mediana cubiti, and vena basilica brachii, and that this was homologous with the vena saphena magna of the leg was rightfully criticized and condemned by Hochstetter. Nevertheless it is referred to somewhat favorably by Charpy.

Krause finds that the cephalic and sciatic veins are analogous (p. 210). Hochstetter denies this, and arrives at the following conclusions. Since the ulnar and fibular borders of the limbs are homologous, the primitive veins which follow them are also homologous. The small saphenous vein and the basilic vein of the forearm, being presumably persistent portions of the primitive veins, are therefore homologous. The cephalic and large saphenous veins are secondary formations, and any comparison between

them is uncertain. The femoral and brachial veins "show no agreement either in position or in origin" (p. 35).

These conclusions clearly depend upon the serial homology of the limbs. If we should accept the idea of inverse homology, advocated by Wilder, Wyman, and others, according to whom the thumb is comparable with the little toe, and the radial border with the ulnar, then conclusions almost the reverse of Hochstetter's would be expected. A third basis for comparison

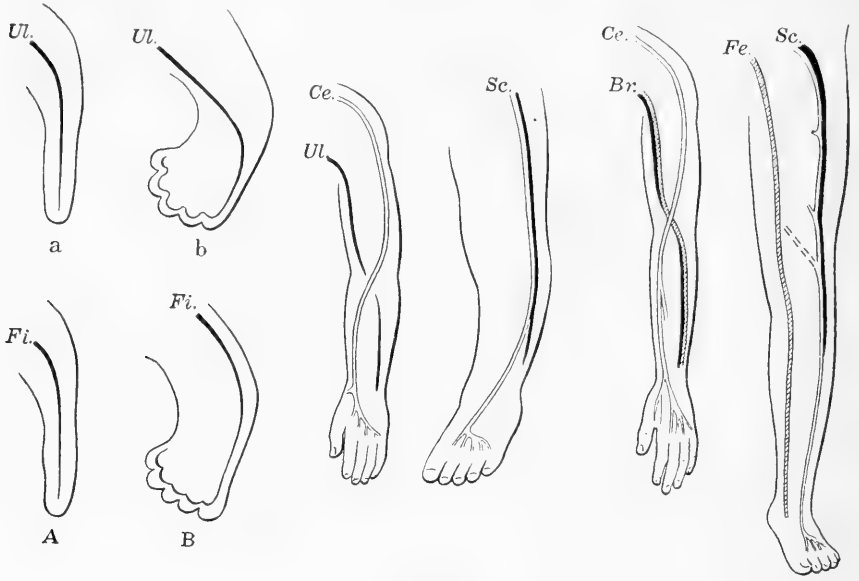

Diagram 1. Anterior view of the arm and leg in their three stages of venous development. In Stage 1, *a* and *A* show the arm and leg, respectively, before rotation; *b* and *B*, after rotation. The primitive ulnar and fibular veins are in solid black. The secondary cephalic and sciatic veins are drawn as double lines, and the tertiary brachial and femoral veins have transverse shading. The black lines in contact with the secondary and tertiary vessels indicate the portions of those veins which are formed from the primitive vessels of Stage 1.

is supplied by the familiar rotation theory. According to it, the limbs are at first serially homologous. The thumb and great toe, the ulnar and fibular borders correspond. The external surfaces of both limbs are to be extensor and the inner surfaces flexor. Later a rotation of approximately 90° occurs in both limbs, but in opposite directions. The extensor surface of the arm becomes posterior, and that of the leg becomes anterior. The knee and elbow are thus brought to bend in opposite directions. The foot is rotated with the leg and its extensor surface

(dorsum) is directed anteriorly. The hand is not rotated with the arm, but ordinarily in the reverse direction, so that its extensor surface is directed anteriorly like that of the foot. Since the arm and hand are rotated in opposite directions, a crossing of the bones of the forearm is produced. In man the hand may, in later development, be rotated with the arm so that its dorsum looks posteriorly and the bones of the forearm are not crossed. In this position the inverse symmetry of the arm and leg is complete.

The embryonic rotation of the limbs is not to be compared with their voluntary rotation in the adult, for the former is a complex shifting of tissues involving modifications in the shapes of the bones. These changes in the human leg are clearly shown by Bardeen's reconstructions in Vol. 4 of this journal. (Compare Figs. 3, 5, 9, 12, and 13, following p. 302.) The external appearances during rotation may be observed in the rabbit embryos figured by Minot and Taylor for Keibel's Normentafeln. From these it will be seen that rotation does not occur with mathematical precision.

Interpreted according to the rotation theory, the fundamental veins of the arm correspond with those of the leg. Their homologies are shown in the accompanying diagram which is based upon the reconstructions previously described. The diagram presents throughout anterior views of the left limbs, the veins being drawn as they would appear if the limbs were transparent. In Stage 1, at *a* and *A* respectively, the arm and leg are shown before rotation. Serial homology between the primitive ulnar and fibular veins is complete. Then rotation occurs, whereby the fibular vein is carried from the posterior to the outer border of the leg, as shown at *B*. The arm, on the contrary, turns so that the ulnar vein is carried from the posterior to the inner border, as in *b*. The forearm rotates in the opposite direction from the upper arm, so that the ulnar vein crosses from the inner side above to the outer side below. Were the forearm in supination, the ulnar border would be internal throughout. After rotation the ulnar border is no longer homologous with the fibular, but corresponds with the tibial border.

In Stage 2, veins are established along the inversely homologous external borders of the limbs, the radial and fibular respectively. As shown in the diagram, the cephalic vein must be a new formation throughout, but the course of the sciatic vein is already partially occupied by the primitive fibular vein. Consequently the sciatic may incorporate a portion of the fibular vein. Thus it appears that a real homology exists between the cephalic and sciatic veins, although, as Hochstetter pointed out, they differ in their relations to the primitive veins of the limbs.

In Stage 3, the brachial and femoral veins develop along the inversely homologous ulnar and tibial borders. In this case the vein of the arm may incorporate the remains of the primitive ulnar vein, as was found to occur in rabbit embryos. The femoral vein on the contrary must be new throughout.

Thus the primitive ulnar and fibular veins, which develop before rotation, are serially homologous. The veins arising after rotation may be considered inversely homologous, the cephalic with the sciatic, and the brachial with the femoral.

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FURTHER EXPERIMENTS ON THE DEVELOPMENT OF PERIPHERAL NERVES.[1]

BY

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WITH FIVE FIGURES.

Two main questions have arisen in connection with the study of the development of the peripheral nerves. The one concerns the constitution of the nerve fiber, *i. e.*, whether it is a process of a single cell or derived from a chain of cells. The other has to do with the manner in which the connection between center and periphery is established, whether there is a continuity *ab initio* (protoplasmic bridges) or whether the connection is secondarily brought about by outgrowth from the center towards the periphery.

Prior to the year 1904 all attempts to solve these problems were based on observations made upon successive stages of normal embryos. When one compares the careful analyses of their observations, as given by various authors, one cannot but be convinced of the futility of trying by this method to satisfy everyone that any particular view is correct. The only hope of settling these problems definitely lies, therefore, in experimentation.

The question of the constitution of the nerve fiber, whether a cell process or a cell chain, may here be considered first.

If one examines a developing nerve, one sees that there are numerous spindle shaped cells (cells of Schwann, sheath cells) throughout its course, and that these are very closely attached to the young nerve fiber; on the other hand, it is also found that the nerve is connected with ganglion cells. The disputed point with which we here have to deal concerns primarily the respective rôles played by these two kinds of cells in the genesis of the fiber. Some time ago I described a series of experiments[2]

[1] Read before the Association of American Anatomists at the meeting held at Ann Arbor, Mich., December 29, 1905.

[2] Harrison, Neue Versuche und Beobachtungen über die Entwicklung der peripheren Nerven der Wirbeltiere. Sitzungsber. d. niederrheinischen Ges. f. Natur u. Heilkunde. Bonn, 1904.

in which the spindle shaped sheath cells were eliminated by the removal of their source, at least their principal source, in an early embryonic stage, before nerves of any kind are visible. The experiment consisted in removing the ganglion crest. This was done by cutting off a thin strip from the dorsal side of the body of embryos (*Rana esculenta*) from 2.7 to 3 mm. in length (Fig. 1). Since this operation removes the source of the spinal ganglia also, the embryos develop without sensory nerves and ganglia, but the motor nerves do develop, and instead of being cellular in structure, as is the case in normal specimens (Figs. 2 and 3), they consist of naked fibers, which can be traced in a number of cases as far as the extreme ventral part of the musculature, *i. e.*, as far as the nerves extend in the adult organism (Fig. 4).[3]

The first experiments were made upon *Rana esculenta;* they have since been confirmed upon the embryos of two American species, *R. sylvatica* and *R. palustris.* These experiments concerned only the spinal nerves.

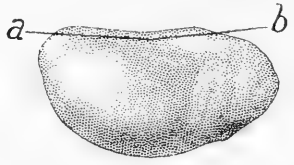

FIG. 1. Profile view of frog embryo (Rana esculenta, 2.7 mm. long) at the stage of operation; the line (*ab*) indicates the incision.

Last season an attempt was made to corroborate the results in cranial nerves. For this purpose the cranial ganglia, the skin covering the side of the head, and the dorsal part of the brain were excised from one side of the embryo before closure of the medullary folds. With one exception

[3] The experiment was based upon the assumption that the ganglion crest is the source of the sheath cells. The result of the experiment proves this to be true, as far as the early stages of development are concerned. In certain lower vertebrates, particularly in Elasmobranchs, it has been shown that large numbers of cells are given off from the ventral part of the medullary cord, wandering out along the motor roots of both the cranial and spinal nerves, and giving rise at least in part to the nerve sheaths. The literature bearing upon this subject has recently been considered by Neal (Mark Anniversary Volume, New York, 1903). In the frog such cells are not given off until the yolk is nearly gone but after this period cells do wander out singly along the motor roots, and in these features the frog embryo resembles closely the salmon (Harrison, Archiv f. mikrosk. Anat., Bd. 57, 1901). The cells do not, however, begin to come off until the motor nerves are well developed and have reached the extreme end of their course. Thus it happens that in the experiment the nerves are developed without sheath cells.

these experiments gave inconclusive results, as small ganglia were always found later, showing either that their normal rudiment had not been entirely removed, or that they had regenerated from some other source. In one experiment, however, in which the embryo was preserved four days after the operation, an examination of the serial sections revealed no ganglia except several sporadic cells on the n. facialis and n. vagus. These nerves consist of naked fibers, except that several sheath cells are present near their origin. A nerve in front of the facial, probably the oculo-motor, but perhaps the motor part of the trigeminus [4] is entirely without sheath cells and the naked fibers may be traced from the brain to a mass of mesoderm cells in the region of the eye. The results

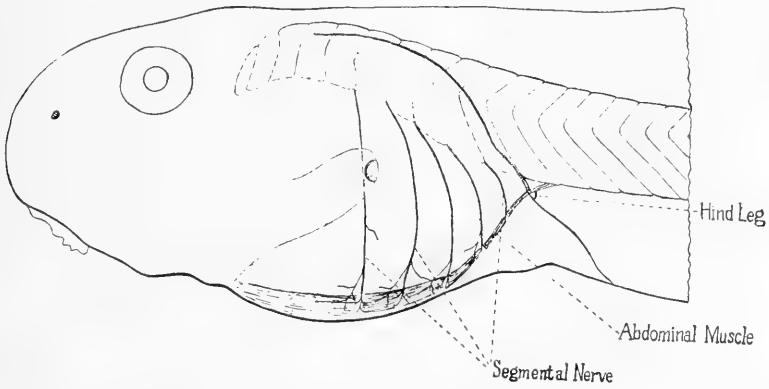

FIG. 2. Profile view of frog larva (Rana palustris, 12 mm. long) after complete resorption of yolk. The relation of the segmental nerves and the primary abdominal muscles are shown.

of this experiment, therefore, confirm the first series, showing that the cranial nerves may develop without the aid of the sheath cells.

But while the sheath cells are thus demonstrated not to be a necessary factor in the formation of the nerve, it may still be urged that they, as well as the ganglion cells, might normally form some of the fibers. During the past year an effort was made to solve this question by studying the behavior of the sheath cells in the absence of processes from the nerve centers. The source of the motor nuclei (ventral half of the medullary cord) was removed from embryos of the same age as in the previous experiments, leaving the dorsal part of the cord together with the ganglion crest intact. The object was to ascertain whether the sheath

[4] Owing to the absence of most of the important landmarks on the injured side the exact determination of this nerve is doubtful.

cells from the ganglion crest would be able in the absence of the motor ganglion cells to form the purely motor rami of the spinal nerves. There are difficulties in the way of making this experiment because it is first necessary to cut off the dorsal half of the cord, leaving it attached at one end, then by a second cut to remove the ventral half entirely, and

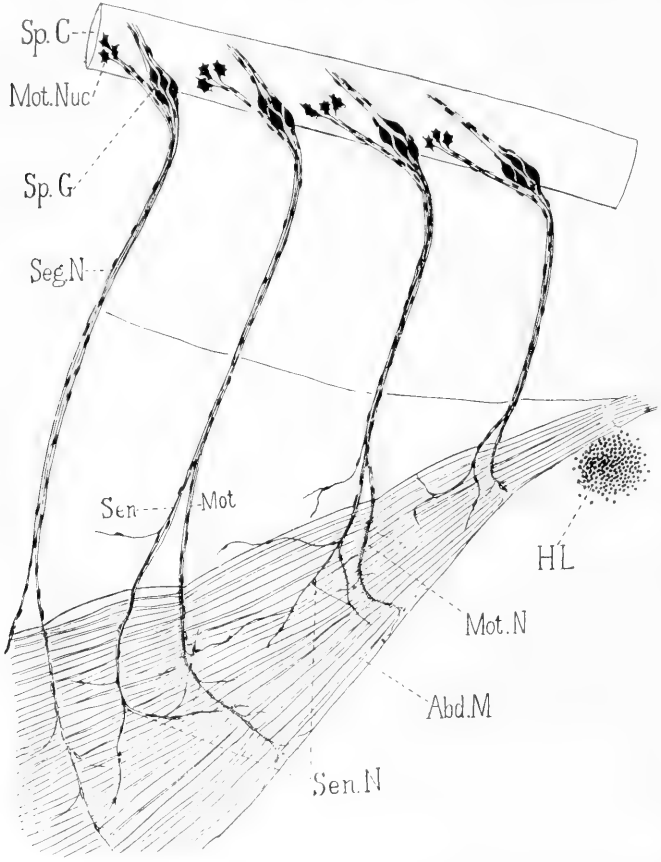

FIG. 3. Semidiagrammatic view of the nerves of the abdominal walls of the frog larva (normal specimen). *Abd. M.*, abdominal muscle; *HL.*, rudiment of hind leg; *Mot. N.*, motor branch of segmental nerve running in inscriptio tendinea of the primary abdominal muscle; *Mot. Nuc.*, motor nucleus (ventral horn cells) in spinal cord; *Seg. N.*, segmental (spinal) nerve; *Sen. N.*, sensory branch of spinal nerve running to integument outside of muscle; *Sp. C.*, spinal cord; *Sp. G.*, spinal ganglion.

finally to heal the first strip, which is very thin, back in place. Even if this is done successfully, a scar is left, which lies in the path of the

spinal nerves, and which no doubt serves as a hindrance to their development. Again it seems to be practically impossible to remove entirely the motor elements from all regions of the cord. After several days the larvæ, although almost completely paralyzed, regain some power of movement, showing usually a slight tremor in some part of their axial musculature, when stimulated mechanically. Sections show that in some

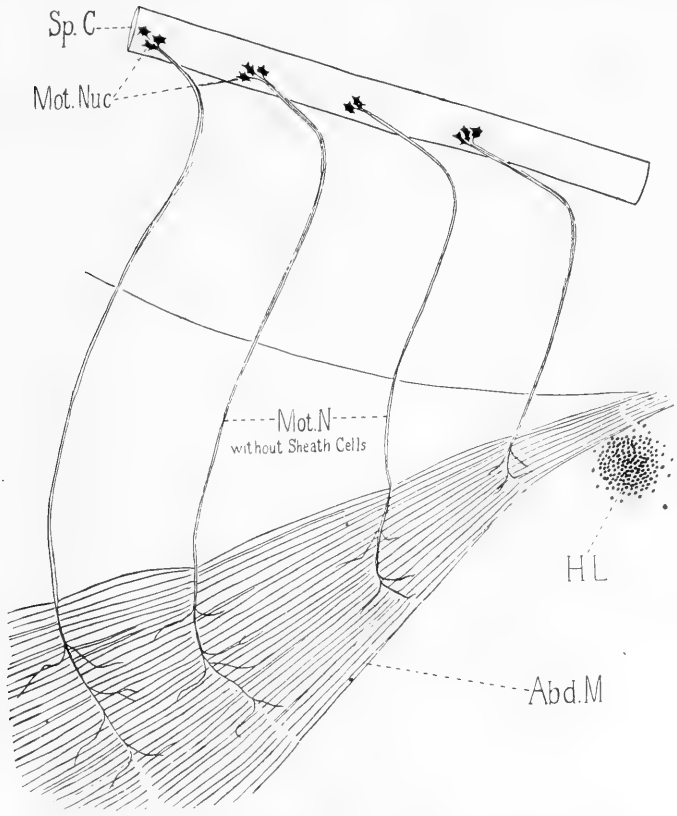

Fig. 4. Semidiagrammatic view of the nerves of the abdominal walls of a frog larva from which the ganglion crest had been removed as shown in Fig. 1. Only motor nerves are present and these consist of axis cylinders without sheath cells.

segments very fine motor roots are present, and the ventral part of the remaining medullary cord contains in these regions a few large motor cells. These motor fibers supply, as the movements indicate, merely that part of the musculature lying close to the spinal cord. With the two exceptions below noted, no motor fibers whatever were found in the

abdominal walls, which were used especially for study, because it is only there that one can distinguish clearly between motor and sensory rami (Fig. 3). The results of ten [5] experiments were as follows: In seven cases sensory nerves were found in the abdominal walls, but no motor (Fig. 5), although the sheath cells, as shown particularly in one case, were in very

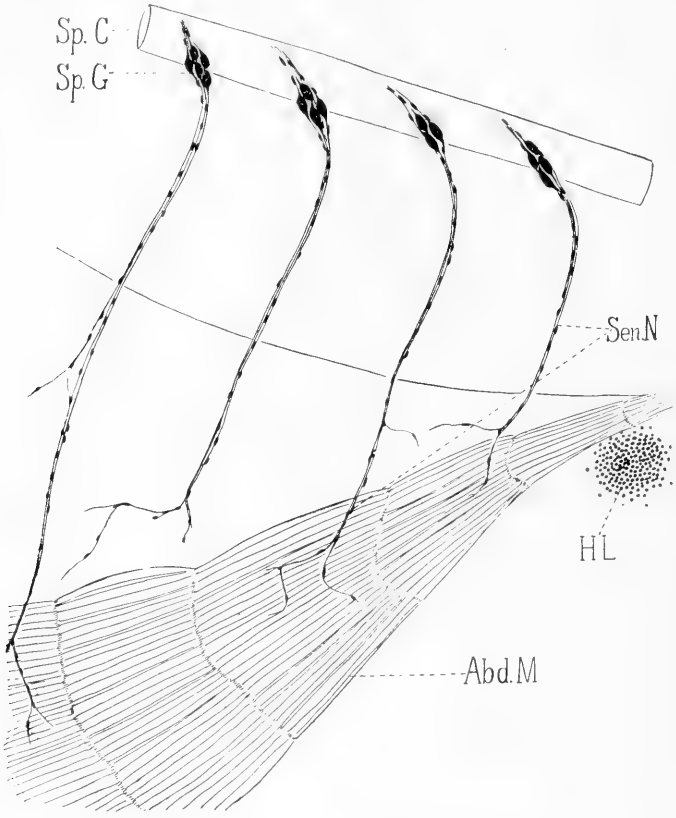

FIG. 5. Semidiagrammatic view of the nerves of the abdominal walls of a frog larva from which the ventral half of the spinal cord had been removed at the stage represented in Fig. 1. Absence of the purely motor rami, which normally run in the inscriptiones tendineæ.

close proximity to the point where the terminal motor rami normally arise; often, however, the sensory nerves were not so well developed as in

[5] Each side of each individual specimen is counted as a case, because, as far as the factors in the experiment are concerned, the two sides of the body are mutually independent.

normal specimens. In two cases motor as well as sensory nerves were found; once in one, and once in two segments, though in other segments only sensory nerves were present. Here the motor nuclei had been less completely removed than in the other cases. In one case neither sensory nor motor branches were found. The last named case is to be explained as due to imperfect union of the parts, as is also the fact that in other cases the sensory nerves were often scantily developed. The results of these experiments show, therefore, that during the period in which the specimens were kept under observation, the sheath cells are unable by themselves to form nerve fibers. The negative character of the result renders it necessary, however, to secure further cases before this conclusion can be regarded as established beyond all question. Should it be urged that time enough was not given the sheath cells to form the nerves, it may be pointed out that in normal specimens the motor fibers develop at a much earlier stage and that if the sheath cells *normally* contribute to their formation they should unquestionably act in the period allotted. The purpose of the experiment was to determine the normal behavior of the cells and not any possible regulative action on thir part, which might take place later.

The above experiments deal only with the motor nerves, and it has not been found practicable to experiment systematically with the sensory nerves because in the latter the ganglion and the sheath cells have a common place of origin. In studying the normal development of the sensory nerves in the amphibian embryos, we find important evidence bearing upon the question. For instance, the nerves derived from the dorsal (giant) cells of Rohon-Beard are formed without sheath cells. These fibers consist, in fact, of naked axis cylinders, which branch and form a delicate plexus of nerves under the skin of the frog larva, and are entirely devoid of cells (or nuclei). Again in the Triton larva, even some of the nerves derived from the spinal ganglia of the tail are for a short time devoid of sheath cells; these, together with the nerves from the dorsal cells form a non-cellular plexus in the fin folds.[6] In the frog larva the nerves derived from the spinal ganglia have sheath cells from the beginning. Comparison of these instances show that these cells are a variable element in the young nerve fiber; it may therefore, be concluded that they play no necessary part in

[6] Since this fact was disputed by O. Schultze (Archiv f. mikrosk. Anat., Bd. 66, 1905, p. 68), I have again examined the specimens in question and have nothing to correct in my former statement. It may be added, however, that I did not intend to include the n. lateralis, which is independent of the cutaneous plexus. This nerve of course has sheath cells at this stage.

its formation. In addition to these normal cases there are several experiments at hand, which show that even in the frog embryo the spinal ganglion cells are by themselves capable of forming long peripheral nerve fibers. The cases in question are those in which relatively small fragments of ganglia had been dislocated or transplanted. In one such case four ganglion cells, transplanted to the abdominal wall, were found giving rise to a long nerve, which ran free through the peritoneal cavity of the larva. This nerve consisted solely of bundles of fibrillæ without cells and could be traced for a distance of nearly two millimeters.

These results differ from those recently reported by O. Schultze (Op. cit.) based also upon the study of the amphibian larva. It is not possible to discuss this work in detail here, but it may be pointed out that by confining his studies to relatively late stages, Schultze has missed the early and fundamental phases of development and thus is led to consider the purely secondary connections of the sheath cells with the nerve fibers as a primary genetic relation.

We may now take up the second great question, viz., the origin of the connection between ganglion cell and end organ. According to the one view a protoplasmic process grows out from the ganglion cell, makes its way through tissues and ultimately reaches its end organ, gradually differentiating into a nerve fiber. According to the second view (Hensen's[1] hypothesis) protoplasmic connections remain between cells after division; those that are used, i. e., that function as conducting paths, persist and differentiate into nerves, the remainder disappearing.

According to Hensen's hypothesis the nerve paths are thus developed much earlier than they seem to be, and they are present for some time before they become visible. If we consider the embryo of a stage just before the nerves do become visible, then the two theories might be distinguished as follows: according to the one, the center (ganglion cells) is the all important factor in forming the nerve; according to the other the nerve is formed *in situ* in the peripheral path. This difference affords the basis for experimentation, though unfortunately the distinction is not so clearly cut as could be desired, for the first view does not deny the importance of the periphery in forming paths along which the developing nerve grows, nor does the second altogether disclaim the influence of the ganglion cell upon the differentiation of the primitive protoplasmic connections into nerve fibers.

The first set of experiments consisted in the extirpation of the center.

[1] Virchow's Archiv, Bd. XXXI, 1864. Die Entwickelungsmechanik der Nervenbahnen im Embryo der Säugetiere. Kiel und Leipzig, 1903.

This was done by removing the medullary cord of the trunk shortly after its closure. The result is always the total absence of peripheral nerves, except the cranial. In the second set of experiments the peripheral path was altered. The simplest way to accomplish this is to remove the spinal cord before any nerves are visible. After this the wound heals readily and during the next week at least no regeneration takes place. Above the notochord in the trunk of the embryo there is thus left a small space which becomes filled with mesenchyme. Into this the longitudinal bundle fibers arising in the brain grow, and after a few days they may be followed as far as six or eight segments from the cut end of the medullary tube. In other words, fibers which normally develop in the walls of the latter, develop here within the mesenchyme, which is a tissue as unlike that forming the normal path as it could possibly be.

The third mode of experimentation, which is not formally different from the preceding, consisted in the transplantation of parts of the central organ. In one series of experiments the spinal cord of the embryo was extirpated, and in each case a small piece of the cord was transplanted under the skin of the abdominal walls. The normal nerves of the body of course do not develop in such cases, but small nerve trunks do arise from the transplanted pieces and run for some distance in various directions, usually remaining in the abdominal walls. Sometimes portions of the ganglion crest were transplanted with the cord, resulting in the formation of small ganglia. In one of these instances, already referred to above, the nerve fibers, which were sheathless, ran free through the peritoneal cavity. While the great length of this nerve is due largely no doubt to the shifting of its peripheral attachment, it is nevertheless quite impossible that preformed bridges could have been present in its course.

The foregoing results can be interpreted in but one way. The nerve center (ganglion cells) is shown to be the one necessary factor in the formation of the peripheral nerve. When the former is removed from the body of the embryo the latter fails to develop. When it is transplanted to abnormal positions in the body of the embryo it then gives rise to nerves which may follow paths, where normally no nerves run, and likewise when the tissues surrounding the center are changed entirely, nerves proceeding from that center may develop as normally. The nerve fiber is therefore a product of the ganglion cell. The histological findings indicate that it is an outflow of the substance of the ganglion cell and not a mere activation by contact of indifferent extra ganglionic substance.

While Lewis's [8] experiments upon the olfactory and optic nerves afford

important additional evidence for this view, the conclusion of Braus,[9] who was the first to experiment upon this phase of the question of nerve development, are diametrically opposed to it. Braus interprets his results in accordance with Hensen's hypothesis. While one cannot but admire his ingenuity of experimentation and argument, his results are not, in my opinion, in any way inconsistent with the outgrowth theory. The growth of strange (facial or pelvic) nerves into a transplanted fore limb can be accounted for on the assumption, for which there is good evidence, that the configuration of the various organs and tissues plays an important part in determining the course taken by growing nerve fibers. The failure of the nerves of the host to grow into "aneurogenic" buds, while they do grow into "euneurogenic" transplantations, might be due to the absence of the attraction afforded in the latter by the cut ends of the nerves.[10] The large size of the nerves in the transplanted limb as compared with the nerves connecting them with the center, may be due partially to the presence of the sheath cells transplanted with the bud, and partially to an abnormal number of dividing fibers. Braus does not exclude beyond doubt the possibility of the latter. In any case the evidence for autogeneration of fibers could be regarded as crucial only if nerves having no nervous connection whatever with the center are developed in the transplanted part. This condition Braus has failed to demonstrate.

While the facts necessitate our deciding against the validity of Hensen's view, as far as the question of *primary* continuity is concerned, it should be pointed out before closing that this view is in so far correct as in many instances the nervous connection between center and end organs is established when the two are very close together, and the long nerve paths originate in such cases by the moving apart of center and innervated organ after the establishment of the connection. The best example of this is seen in the lateral line. Here the ganglion is practically in contact with the rudiment of the sense organs when the first nerves are developed. The cell processes have merely to grow out for a distance less than the diameter of a cell in order to make connection. Yet by the wandering of the sensory epithelium from the head to the tip of the tail the lateral branch

[8] W. H. Lewis, Proceedings Ass. Am. Anatomists. Am. Journ. of Anat., Vol. V, No. 2, 1906.

[9] H. Braus, Verhandl. d. Anatom. Gesell., Jena, 1904. Anatom. Anz., Bd. XXVI, 1905.

[10] Forssman (Ziegler's Beiträge, Bd. 24, 1898, and Bd. 27, 1900), has shown beyond question that a tropism of this kind does play an important part in the regeneration of peripheral nerves.

of the vagus is ultimately drawn out to this enormous length. The observations of Kerr[11] upon the motor nerves of Lepidosiren are, in my opinion, capable of a similar interpretation and are a valid support of Hensen's view only in the above modified sense. In other words, the nervous connection, though formed very early, is by no means primary.

The results of the foregoing may be summarized as follows: The axis cylinder of the nerve fiber is the outgrowth of a single ganglion cell, with which it remains in continuity throughout life. It grows gradually from the center towards the periphery establishing secondarily connection with its end organ. The other elements, the cells of Schwann, which are found upon the developing nerve have nothing to do with its genesis, though they may play an important part in the nutrition and protection of the fibers.

[11] J. Graham Kerr, Trans. Roy. Soc. Edinburgh, Vol. XLI, 1904.

THE GASTRULATION AND EMBRYO FORMATION IN AMIA CALVA.

BY

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WITH 4 DOUBLE PLATES.

In an earlier paper entitled "The Egg of Amia and Its Cleavage," Whitman and Eycleshymer, 96, described the development from the time of fertilization up to and including the late blastula. The present paper is a continuation of the earlier study, and deals with the changes taking place between the late blastula and the time when the tail of the embryo becomes free from the yolk; that is, from the time of the late blastula until the time when most of the organs are laid down.

The material was killed in Flemming's fluid, Perenyi's fluid, chrom-osmic acid, picro-acetic acid, picro-sulphuric acid and corrosive sublimate-acetic acid. For surface views, chrom-osmic acid gives most perfect pictures; the osmic acid blackens the lines of cleavage so that they stand out in bold relief. Another excellent method for surface study is faint staining with Delafield's hæmatoxylin which may be employed after any of the above-named fixing solutions. The best serial sections have been obtained after fixation in picro-acetic acid. Owing to the crumbling of the yolk we have been compelled to use celloidin as an imbedding mass. Serial sections were made after the method described elsewhere by the senior author, 91. Staining *in toto* is best accomplished by using Czoker's alum-cochineal for from twenty-four to forty-eight hours. Staining in section with Mayer's hæmalum and alcoholic carmine has proved very satisfactory.

So far as the writers are aware, but two papers have been published dealing with the phases of development under consideration. Both of these appeared in 1896. The first was published by Sobotta and contains a fairly accurate, but incomplete, description of the gastrulation stages. The illustrations, however, are few and highly diagrammatic. The second was written by Bashford Dean and is more extended, but less accurate. Dean's descriptions unfortunately were based upon the errone-

ous assumption that the egg of Amia is meroblastic. In view of these facts, the present writers have thought that a renewed study of these phases of development might be profitable.

The ages given in the following description of stages have been determined from material taken from a single nest. The eggs were taken from the nest and placed in dishes which were submerged in the lake, a constant temperature of about 16° C. being thus maintained. It is well known that no two spawnings progress at precisely the same rate. It is thus obvious that the ages designated are only in a general way indicative of the degree of development. We have, therefore, given measurements of the extent of the blastodisc and embryo in addition to the age.

The description of the latest stage studied by Whitman and Eycleshymer reads as follows: "The calotte, which has now begun to extend over the yolk, consists of thickly crowded spherical cells which marginally pass abruptly into the large yolk segments, while in the central portion they gradually increase in size and lie loosely scattered. The outer layer of the calotte is distinctly differentiated in that the cells are elongated and more densely granular. The entire yolk is irregularly cleft, the cells forming the lower portion are roughly polygonal and grade off into the larger yolk spheres which lie at the center." This stage of development indicates the beginning of our study.

DETAILED DESCRIPTION OF STAGES.

Egg Nine Hours After Fertilization. Blastodisc Covers About 100° of the Circumference of the Egg.—A profile view of an egg of this age is shown in Fig. 1. An examination of the surface of the blastodisc shows an area at the upper pole of the egg in which cell division is most rapid. In addition to this, there are frequently found other areas in which cell division is accelerated. Often one side of the blastodisc is distinctly in advance of the other. Again, the most careful search results in a failure to detect such areas. We, therefore, are unable to say what relation, if any, these areas bear to the future embryo.

In all eggs of this stage the surface of the yolk is cleft by thirty to forty furrows which pass in meridional planes. Many of these grooves have not as yet reached the vegetative pole. Some never reach the pole, but pass obliquely into the longer ones. Through this process a number of long triangular segments are cut off at the upper margin of the yolk, as shown in the figure. At the lower pole, where fifteen to twenty grooves converge, the yolk is irregularly cleft. In general it may be said that the cleavage of the yolk as compared with the cleavage of the blastodisc is exceedingly slow.

A study of meridional sections of many eggs in this stage shows that the blastodisc takes on different forms. In most eggs it is distinctly crescentic, but in some it is lenticular. When it takes on the crescentic form, as shown in Fig. 21, there is often a very distinct segmentation cavity (*s. c.*) present. The roof of the cavity is here made up of five or six layers of cells. The cells of the blastodisc contain finer granules than those contained in the large yolk segments. At the margin of the blastodisc, the cells pass over into those of the yolk by such imperceptible gradations that no sharp line of demarcation can be seen. The outermost layer of the blastodisc may be designated as the superficial layer of the ectoblast (*s. ec.*) and as stated by Whitman and Eycleshymer it early appears quite unlike the deeper ectoblastic layers (*d. ec.*), in that it possesses granules which stain more intensely than those in the other layers.

The upper ends of the yolk masses (*y. m.*) are, in the egg shown, smooth and only at the margin are the cells being cut off. Other eggs, however, show that the large yolk masses at the center of the egg are actively contributing to the blastodisc. In the section shown (Fig. 21) the yolk nuclei lie near the upper margin of the large masses and this upper portion is probably to be considered as homologous with the periblast of bony fishes. Not more than one-third of the cleavage grooves observed on the surface of the yolk have reached the center of the egg, leaving the yolk masses incompletely cleft and thus forming a great syncytium.

Egg Twelve Hours After Fertilization. Blastodisc Covers About 110°.—The surface view (Fig. 2) shows that the rapid multiplication of the cells in the margin of the blastodisc has now given rise to greater uniformity in the size of all the cells of the blastodisc. Beyond this feature, surface views show no points worthy of special mention. Meridional sections (Fig. 22) show that the cells forming the superficial ectoblast (*s. ec.*) are smaller and more elongated than in the preceding stage. The lower layers of cells of the blastodisc are scattered through the upper portion of the segmentation cavity. The entoblastic cells, which have been cut off from the large yolk masses, are distinguished by their coarser granules and are also scattered through the segmentation cavity, many of them being found in its upper portion. Through these changes the segmentation cavity is more or less obscured.

Egg Twenty Hours After Fertilization. Blastodisc Covering About 120°.—An egg of this stage (Fig. 3) shows a well-defined blastodisc with a sharply delimited margin in which, under the magnification given, cell boundaries are no longer distinguishable. No features have been observed which enable us to recognize the embryonic anlage. The yolk shows little advance in cleavage beyond that described in the preceding

stage. In this particular egg the grooves, instead of following meridional lines as usual, diverge more widely than those in the eggs shown in Figs. 1 and 2.

Sections show, although none are figured, that the blastodisc in this stage is made up of eight to ten layers of cells which gradually pass over into the yolk derivatives. The outermost layer of the blastodisc has undergone still further modification in that its cells are more elongated, closely apposed and more deeply stained. The large yolk masses are actively budding off cells not only around the margin of the blastodisc but also in the central portion of the yolk. The yolk nuclei, which in the earlier cleavage stages, were confined to the upper portion of the yolk masses are now frequently found more deeply situated.

Egg Forty Hours After Fertilization. Blastodisc Covers 130°.—Although the surface of the egg, as shown in Fig 4, presents no features worthy of special comment, changes are going on in its interior which merit consideration. If a meridional section of an egg in approximately the same stage (Fig. 23) be examined it will be seen that the blastodisc proper is made up of from ten to twelve layers of cells so closely apposed that they make a compact stratum. In addition to these cells, there is a large number of loosely scattered cells which lie in the space which we have hitherto designated as the segmentation cavity. These loosely scattered cells gradually pass over on the one hand into the cells of the blastodisc proper and on the other into the large yolk masses. The cells of the ten or twelve layers forming the upper portion are smaller, more uniform, more closely compacted and contain very fine granules as shown in the figure; while the loosely scattered yolk cells and those being budded off from the large yolk masses are larger, more irregular in outline and contain coarser yolk granules. These two portions cannot be considered as sharply marking off ectoblast and entoblast, since one finds in the portion which is largely ectoblastic, large cells which are filled with coarse granules; and if granules be the criterion for the separation of layers these cells must be regarded as recent derivatives from the large yolk cells which have wandered up from the lower portion of the blastodisc. If this interpretation be correct, it is a fact of some importance, since the part hitherto considered as *exclusively* ectoblast contains a considerable number of cells derived from the large yolk masses.

It has already been pointed out that the outermost layer of the ectoblast (*s. ec.*) can be readily distinguished from the underlying layers. A glance at Fig. 23 shows that in the locality where this layer passes over into the large yolk cells there is a marked proliferation of its elements. A study of the remaining sections shows that as yet there is no invagination.

This thickening of the superficial layer marks the anlage of the forthcoming dorsal lip (*d. l.*) of the blastopore. The margin of the blastodisc in the embryonic region has a more rounded contour than at the opposite margin. It is also thicker and possesses a greater number of cells with fine granules; the periblast, too, is more actively engaged in budding off derivatives in this region than at the opposite margin. These several factors enable us at this time to orient the forthcoming embryo.

The so-called periblastic nuclei are no longer confined to the upper margin of the large yolk masses, but are often widely scattered. These yolk masses sometimes contain several nuclei and the same is true of the scattered entoblastic cells. In other words, nuclear division here goes on far in advance of cytoplasmic division.

Egg Fifty Hours After Fertilization. Blastodisc Covers About 180°. —If the surface of the egg be carefully examined in a stage intermediate between Figs. 4 and 5, it will be found that just above the equator on the side of the blastodisc which is least transparent there is a slight indentation which indicates the beginning of the blastopore. As development progresses this indentation becomes a groove which extends in a latitudinal plane until it reaches the condition shown in Fig. 5, where it extends around some 20° of the egg's equatorial circumference.

The meridional section represented in Fig. 24 is from an egg intermediate between those represented in Figs. 4 and 5. At this time, the blastodisc has taken a more definite form owing to the greater compactness of its layers. In extent it covers very nearly one-half of the egg's surface. It is noteworthy that at the time the blastopore appears the blastodisc reaches its maximal thickness.

In the particular egg described, the entoblastic cells are less densely aggregated than usual, with very large intercellular spaces, while the large yolk masses extend well up towards the lower layers of the blastodisc. In this respect we find considerable variation. In some eggs they are even less densely aggregated than shown in Fig. 24 so that a well-marked segmentation cavity (*s. c.*) is shown between the large yolk masses and the blastodisc.

In the stage under consideration we have the first actual appearance of the invagination to form the archenteron. As to the factors which initiate this process, we are as much in the dark as ever. Without attempting to discuss the various theories, we may simply say that thus far there is an infolding of the external layer and that this infolding is not in the locality where the transition between large and small cells is most abrupt, but in a locality where the superficial cells are largest and of fairly uniform character. One would at first glance think the in-

vagination were wholly in the part of the surface layer which belongs to the epiblast. A comparison with other forms, however, such as the various Amphibia, leads one to hesitate in such an interpretation. The crucial factor is the determination of the limits of the ectoblast. If the ectoblast be considered as extending to the point where the smaller cells pass over into the large yolk masses, then the invagination is in the ectoblast. If it does not extend to this point, there are no features which will enable us to determine how far it does extend.

A more highly magnified view of the blastoporic region is shown in Fig. 25. The section is taken from an egg of the same age as that shown in Fig. 24. In many eggs of this stage, there is a stratum or tongue of cells which is somewhat peculiar. This stratum is directly continuous with the deep ectoblast at the dorsal lip of the blastopore. Anteriorly its cells are separated from the deep ectoblast by the segmentation cavity above, while below they pass over into the entoblastic cells. In this stratum which is from four to five layers thick, two kinds of cells are present. The more numerous are cells which conform in structural peculiarities to those of the deep ectoblast. The less numerous are cells which possess the structural features of the entoblast. This layer of cells Sobotta, **96,** has described as mesoblast. Since this layer not only contains mesoblast but also entoblast, we have decided to designate the layer as mes-entoblast (*m. en*).

In Fig. 26 there is represented a meridional section of an egg somewhat older than that just described, but younger than that shown in surface view in Fig. 5. The archenteron or gastral cavity is more extended and its dorsal wall is formed of cells which are so much like those of the superficial ectoblast that one is inclined to regard invagination as still playing the more important rôle. The rounded cells at the end of the gastral cavity are further confirmation. In short it may be said that thus far there are no reasons for considering delamination as a factor of any importance in the formation of the gastral cavity.

No particular changes are noticed in the character of the cells in the region where the head of the embryo is about to appear. The yolk derivatives are widely scattered in the segmentation cavity and many cells which, from the character of their granules, would be called yolk derivatives are still to be found scattered among the ectoblast cells. The large yolk masses are still actively budding off cells and this process has gone on so rapidly in this particular egg that these masses have become greatly reduced in size.

Egg Fifty-three Hours After Fertilization. Blastodisc Covers About 200°. The anlage of the embryo can now be faintly recognized in sur-

face view (Fig. 5). It first appears as a light area with ill-defined outlines extending over some 80° towards the upper pole, where it shades off imperceptibly into the remainder of the blastodisc. That portion which is invaginated to form the dorsal lip of the blastopore stands out more prominently from the yolk than elsewhere. The line of invagination now extends some 60° along the margin of the blastodisc and appears as a crescentic fissure.

A meridional section of an egg in a stage closely corresponding to that described above is represented in Fig. 27. Although this egg is but slightly older than the one shown in Fig. 26, some interesting changes have occurred. In the preceding stage the deeper ectoblast was seven or eight layers of cells thick; now it is only two or three. The embryonic margin of the blastodisc has likewise undergone a reduction, while the opposite side of the blastodisc is reduced to one-half the number of layers present in the preceding stage. In addition to these changes, the stratum of cells which we have designated as mes-entoblast extends well up toward the upper pole of the egg and it is probably through the extension of this layer that the surface views show faintly the anlage of the embryo.

In most eggs in this and subsequent stages, there are relatively few entoblastic cells as compared with the earlier stages. The space which in most of the earlier stages was filled with small cells is now filled with large yolk masses with a few smaller cells scattered among them. It is possible that many of the entoblastic cells have found their way into the rapidly extending blastodisc.

Fig. 28 represents a section of the blastoporic end of the embryo under much higher magnification. The section is taken from another egg in about the same stage of development as that shown in Fig. 27. It will be here noted that the gastral cavity is lined above by a single layer of cells which strikingly resemble those of the superficial ectoblast in size, granular contents and staining capacity. The same can be said of the cells forming the ventral wall. The cells of this wall rest in this section upon the large yolk masses whose margins are regular and clearly delimited. A peculiar feature which was noticed in the preceding stage, but is here more clearly shown, is the striking differentiation of the innermost layer of the deep ectoblast. These cells stain more deeply than the remaining cells of this stratum.

Fig. 29 represents a meridional section of an egg in a stage somewhat later than that last described, but earlier than the stage shown in Fig. 6. The blastodisc has undergone continual thinning at the upper pole until at present it is but two or three layers of cells thick. At the blastoporic margin the blastodisc is thickened, while on the opposite side of the egg

a slighter thickening of the margin gives rise to a condition which reminds one of the germ-ring of the teleost.

An equatorial section, taken just above the equator of an egg in the same stage, is shown in Fig. 30. The ectoblast in the embryonic region is much thicker than elsewhere, and from this region of greatest thickness it shades off gradually on either side, showing that at this time there are no well-defined lateral boundaries of the embryonic anlage. Just beneath the median portion of the embryonic anlage there is a compact arrangement of the mes-entoblastic cells which represents the beginning of the notochord (*ch*).

Egg Fifty-five Hours After Fertilization. Blastodisc Covers About 240°. Embryo Extends Over 110°-120°.—The embryo now presents a profile (Fig. 6) which may be spoken of as somewhat triangular. Its anterior portion fades out in the region of the upper pole of the egg. Its posterior portion, however, is more sharply defined owing to its being deeply infolded at the blastoporic margin. In many embryos of this stage, there is present a median thickening in the blastoporic margin which may doubtless be considered as the homologue of the caudal knob of the teleosts. The lower portion of an egg in about the same stage is shown in Fig. 7. It will be noticed that the margin of the blastodisc is not only deeply infolded along the base of the embryo, but also slightly infolded on the opposite side of the egg. A comparison of Figs. 6 and 7 with Fig. 5 shows that the surface cleavage of the yolk is very slow.

A meridional section of an egg in this stage is shown in Fig. 31. The embryonic anlage here shows as a thickening of the ectoblast. The area of maximal thickness (*h*) near the upper pole of the egg represents the anlage of the head. In this region the superficial ectoblast shows no changes, the thickening being due to the proliferation of the deeper ectoblast which is now twelve to fourteen layers thick as compared with six to eight in the preceding stage. The deeper layers decrease in number throughout the anterior trunk region and again increase at the blastoporic margin. In front of the anlage of the head (*h*), the deep ectoblast becomes thinner until, in the region of the equator, it is but a single layer thick; beyond this region it again thickens and at the blastoporic margin is three or four layers deep.

The segmentation cavity, which in the preceding stage extended over the greater portion of the upper hemisphere, is no longer present above the level of the equator. The layer of ectoblastic cells forming its roof is still sharply diffrentiated from the other layers of the ectoblast.

The gastral cavity (*g. c.*) has extended cephalad to the level of the posterior third of the embryo. Behind the dorsal lip of the blastopore, it

extends around on either side of and behind the large yolk plug where it is continuous with that part of the cavity which is everywhere lined externally by a sharply differentiated layer of hypoblast. At the blastoporic margin, this layer of hypoblastic cells changes in character from the small elongated cells with deeply staining granules to larger cuboidal cells and these in turn shade off into the smaller elongated cells of the superficial ectoblast. The floor of the anterior portion of the gastral cavity is made up of entoblastic cells which are heavily laden with large yolk granules. Toward the exterior these cells increase in size as they extend over the sides of the yolk plug until they finally become continuous with the great yolk masses (*y. m.*)

The layers of mes-entoblast (*m. en.*) not only extend much farther forward in the embryonic region but also become well differentiated in the extra-embryonic portion of the blastodisc. By tracing these layers in serial sections it is readily found that the anlage of the mesoblast is peristomal.

Embryo Sixty Hours After Fertilization. Blastodisc Covers About 245°. Embryo Extends Over 130°.—The outline of the embryo as yet is indistinct in the anterior region, but fairly well defined posteriorly (Fig. 8). The entire margin of the blastodisc is deeply infolded around the projecting yolk plug. In the posterior portion of the embryo, there is a shallow groove present. A comparison with other embryos in this same stage shows that this groove is variable, being sometimes more and sometimes less pronounced. Sometimes it terminates posteriorly in a deep indentation in the margin of the blastopore much like the condition observed in Batrachus or Ameiurus; at other times there is a well-defined caudal knob.

The sagittal section represented by Fig. 32 is from an egg in the same stage. The deep ectoblast in the head region is notably thickened, being now twelve to sixteen layers in dorso-ventral thickness. In the trunk region these layers are further reduced while at the blastopore they remain practically unchangd. Anterior to the region of maximal thickness the deep ectoblast gradually thins until, as in the preceding stage, it is but one or two layers thick in the equatorial region; finally at the ventral lip there are four or five layers.

The mes-entoblast has extended farther toward the upper pole, but to just what extent it is impossible to say since the cells are here indistinguishable, on the one hand, from those of the deep ectoblast, and on the other, from those of the yolk. At the blastoporic margin where the cells of the mes-entoblast and the deep ectoblast unite, they form a sharp angle. In this angle there now appears a peculiar group of cells

which has been derived from the mes-entoderm. We were at first inclined to regard these cells as exclusively mesodermal but since they later lose their distinctive character the question cannot be definitely settled.

A sagittal section of an embryo slightly later than the preceding is shown in Fig. 33. The principal changes are the further extension of the blastodisc and the corresponding reduction in the diameter of the yolk plug. The peculiar differentiation of the inner layer of the deep ectoblast is here prominent. The segmentation cavity is vanishing, the gastral cavity enlarging. The yolk is being rapidly segmented, especially at its periphery.

Embryo Sixty-five Hours After Fertilization. Blastodisc Covers About 355°. Embryo Extends Over About 140°.—The surface view of an egg in this stage is represented by Fig. 9. The embryo is now much better defined. The anterior portion is somewhat broader than the trunk, which in turn becomes narrowed towards the blastoporic end. The blastopore is almost closed. In its closure one rarely finds the condition so frequently found in the amphibia where the lateral lips approximate so much faster than the dorsal and ventral that a slit-like blastopore arises.

Embryo Seventy Hours After Fertilization. Embryo Extends Over About 154°. The next surface view (Fig. 10) represents an embryo about five hours older than that shown in Fig. 9. The features noted in addition to those described in the preceding stage are the further elongation of the embryo; the presence of a well-marked neural trench; the further closure of the blastopore. At this time there are no external evidences of optic vesicles, protovertebræ, or pronephric ducts.

A sagittal section of the posterior portion of an embryo in this stage is shown in Fig. 34. At this time the blastopore is nearly closed. The external epiblast, as in the earlier stages, is a single layer of cells which still retain their peculiar coarse granules and deep staining capacity. These cells are in direct continuity with the single layer of cuboidal cells lining the blastopore. These cuboidal cells in turn pass over into the elongated layer of hypoblastic cells which form the dorsal wall of the gastral cavity. The floor of the gastral cavity is made up for the most part of a single layer of entodermal cells. The appendicular portion of the gut (*a. g.*) is lined by cells similar to those just described.

A transverse section through the blastopore of an embryo in the same stage is shown in Fig. 35. The relation of the layers is here more clearly shown. It will be noted that the section shows especially well the great lateral sheets of mesoblast (*mes.*)

A median sagittal section of an embryo a few hours later is shown in Figs. 36 and 37. The anterior and middle portions are shown in Fig. 36,

the posterior in Fig. 37. The general contour of the embryo shows some advance beyond the conditions shown in Figs. 34 and 35. The head region shows a considerable increase in the number of ectoblastic cells, in the trunk region but two or three layers are present, while posteriorly they again show a marked increase in number. Just beneath the superficial ectoblast (*s. ec.*), there is now differentiated a second layer of elongated cells. These cells, however, possess granules which are similar in staining capacity to those of the deep ectoblast and have thus been considered as derivatives from the deeper layer.

In the mass of cells which makes up the anlage of the future brain, there is now observed a slight cavity (*br. c.*) which is the first appearance of a cavity in the central nervous system. In front of this mass of cells is a second thickening which has been designated as the pre-cerebral mass (*p. cb*).

The notochord (*ch.*) is now well differentiated, being readily distinguished from the surrounding tissues by the loosely scattered arrangement of its cells. It extends from the undifferentiated caudal mass of cells to the anlage of the future optic vesicles.

The gut has increased through both forward and lateral extension. In its anterior portion its dorsal and ventral walls are closely apposed, yet they can be readily traced as distinct layers to a point somewhat beyond the anterior end of the brain. In its middle and posterior portions it is widely open. Just anterior to the line of large yolk cells (*bl.*) which represent the closed blastopore, there is a dorsal diverticulum of the gut which has been regarded by others, as well as ourselves, as the homologue of Kupffer's vesicle.

Embryo Seventy-five Hours After Fertilization. Embryo Covers About 150°.—In Figs. 11 and 12 are represented the anterior and posterior portions of an embryo of this age. The anterior trunk region is narrower and two or three protovertebræ are now present. Lateral thickenings at the anterior end represent the beginnings of the optic vesicles. On either side of the anterior end, there is a darkened area which represents the lateral extension of the mesoblast.

An oblique section through the optic thickenings (*op. t.*) is shown in Fig. 38. The superficial ectoblast which is now double layered passes over these thickenings unmodified. No lumen is present in the central nervous system at this level, but in sections intermediate between those represented in Figs. 38 and 39 there is a slight fissure present. The mesoblast shows as two wide lateral bands (*mes.*) on either side of the neural rod or keel. The foregut is present, but the close approximation of its walls makes its lumen obscure.

Another section of the same embryo through the posterior portion of the hind brain is represented in Fig. 39. On either side of the neural keel and in close proximity to its dorso-lateral margin, there are deeply staining groups of cells which are probably spinal ganglia; although it should be said that in some preparations they appear to be proliferations of the inner layer of the superficial ectoblast. The notochord (*nc.*) is well differentiated at this level. Beneath it the layers forming the walls of the gut are in contact so that the lumen is here obscured. On either side of the notochord, however, the layers separate and the laterally extending gut cavity (*g. c.*) is obvious.

Another transverse section at the level of Kupffer's vesicle is represented in Fig. 40. In the median line there is a groove in the superficial epiblast (*n. t.*) which we have interpreted as a neural trench. It extends backward to the point where the scattered coarsely granular cells indicate the line of closure of the blastopore (*cf.* Fig. 37). The deeper epiblast has not yet taken on the form of a neural keel, but extends laterally to a considerable distance. The notochord is well differentiated and consists of cells whose character lends confirmation to the view that they are derived from the mesoderm rather than the gut hypoblast. At any rate we have not observed the coarsely granular cells of the hypoblast participating in its formation.

The section represented by Fig. 41 is taken through the posterior portion of an embryo of about the same age. In this embryo a deeper neural trench (*n. t.*) is present than in the preceding. The posterior end of the notochord, as it passes over into the mass of undifferentiated cells is barely defined by the peculiar arrangement of its cells. Kupffer's vesicle is smaller than in the preceding embryo. In this structure there are wide variations in size as may be inferred by glancing at the different figures.

Embryo Eighty Hours After Fertilization. Embryo Covers About 160°.—The surface views (Figs. 13 and 14) show that the embryo is considerably advanced beyond the stage represented in Figs. 11 and 12. The body of the embryo is narrower; the optic vesicles are more prominent; seven to nine protovertebræ are differentiated; the pronephric ducts are forming. In the anterior portion of the embryo there are three fairly well defined regions which represent the primary divisions of the brain. Anterior to the optic vesicles the nervous system is continued into a conical process, the homologue of the structure which Salensky found in Acipenser and to which he gave the name "Stirnforsatz." This precerebral portion of the head is the anlage of several structures to which we shall hereafter refer in greater detail. The mid-brain is marked off by a constriction posteriorly and behind this constriction is a

marked enlargement which forms the basis of the anterior portion of the medulla. In this region, as Keibel has pointed out, the anlage of the otic vesicles will later appear. The darkened zone around the anterior end of the embryo represents the extent of the mesoblast. Posteriorly the lateral boundaries of the mesoblast are poorly defined so that in surface views it is impossible to indicate them.

Embryo Ninety Hours After Fertilization. Embryo Covers About 180°.—Figs. 15 and 16 represent the anterior and posterior portions of an embryo in this stage. Many striking changes have occurred. The subdivisions of the brain are more clearly defined and are more prominent. The optic vesicles are better defined. The precerebral portion extends forward as a distinct process. The first visceral arch has formed and, just behind it, is the first visceral cleft. The protovertebræ have increased to sixteen or more pairs. The pronephric ducts have extended both anteriorly and posteriorly. There are at this time, however, no external indications of the olfactory, auditory or adhesive organs.

Fig. 42 represents a section passing close to the median sagittal plane of an embryo slightly younger than that shown in Fig. 17. The superficial ectoblast (*s. ec.*) consists of two layers of cells which are invaginated at a point lying between the anterior margin of the fore brain (*f. b.*) and the median portion of the adhesive organs.

The brain cavities are now well defined. There is, however, as yet no indication of the infundibular or epiphysial evaginations. The notochord extends nearly to the level of the middle portion of the brain, as shown in the figure. The gut cavity is well defined beneath the posterior portion of the brain; it is greatly reduced in size anteriorly. After reaching the level of the epiblastic invagination described above, it again expands into a wide cavity (*g. d.*). The walls of the gut show little change until the head region is passed when the dorsal wall is greatly thickened to form the beginnings of adhesive organs (*a. o.*).

A transverse section through the extreme anterior end of the brain is represented in Fig. 43. The superficial ectoblast shows no modification in this section. Just beneath this layer the deep epiblast extends over the surface, but in the median line it is lost in the mass of cells which are radially disposed and which represent the anterior end of the fore brain. Below the fore brain is a wide layer of mesoblast (*mes.*) which extends upward on each side. On either side of the median line the fore gut is greatly expanded. The layer of columnar cells covering these expanded portions, even at this early stage, is different from that forming the dorsal wall of the gut in other portions of the body. On either side the hypoblast extends peripherally, its cells take on the cuboidal form,

and become continuous with the yolk. In the figure given the dorsal hypoblast has been too deeply shaded so that it is brought out in too strong contrast with the layer of yolk-bearing cells which form the floor of the fore gut. On either side of the gut the anterior extremities of the cœlomic cavities (*c.*) are present.

The next section described is represented in Fig. 44. The section passes through the optic vesicles (*o. v.*) which at this time are hollow and in wide communication with the fore brain. On either side there are slight depressions of the superficial epiblast which are probably artifacts due to killing reagents. The loosely scattered cells of the mesoblast (*mes.*) form two large masses which extend laterally from the region where the floor of the fore brain rests directly upon the gut hypoblast. Just beyond the lateral boundary of the fore gut the mass separates into two layers, an outer somatic which is closely united with the ectoblast and an inner splanchnic which lies close to the gut hypoblast. Between these two layers are the cœlomic cavities (*c.*). The gut (*f. g.*) is here widely expanded and, on either side, are seen the hypoblastic cells as they pass over into the anlagen of the adhesive organs.

A section through the region where the mid brain passes over into the medulla is show in Fig. 45. The pharyngeal portion of the gut is here widely open and slight evaginations indicate the first appearance of the visceral clefts. Just external to these are mesoblastic masses (*v. a.*) which are the beginning of the visceral arches. The mesoblast extends down around the brain until it comes in immediate contact with the notochord. The walls of the cœlomic cavities (*c.*) are separated widely and are lined by a single layer of cells.

Passing still further back we have selected a section (Fig. 46) through the region of the auditory vesicles. The cavity of the medulla is here widely open. Its lateral walls are made up of elongated epithelial cells arranged in one or two layers. Its roof, however, is very thin, so that when viewed from the surface it is very transparent. On either side are the auditory vesicles (*a. v.*) which have formed from thickenings of the deeper ectoblast. Above these the two layers of the superficial ectoblast are continuous. The vesicles which were earlier solid now show very small lumina. Just external to the vesicle on the right side there is a diverticulum of the gut, the pharyngeal portion of the third cleft, while just outside this, a thickening in the mesoblast is the third arch. The cells of the hypoblast forming the dorsal wall of the gut are flattened, but in the region of the clefts they become cuboidal, which character they retain until they pass over into the yolk cells. The ventral wall of the gut is

still formed by a loosely scattered layer of entodermal cells which lie above the large yolk masses.

The last section of this embryo, which we have represented in Fig. 47, is taken at the level of the last protovertebra. In this section we see that the neural keel of the earlier stages has taken on a cylindrical outline and has acquired a large well defined lumen in which there are no traces of cell degeneration. Below the neural tube and in contact with it, is the large notochord, and between the notochord and the gut hypoblast, is the sub-notochordal rod (*h. ch.*) which, from the character of its cells, seems to have arisen from the hypoblast of the gut.

On either side of the notochord, are the large masses of mesoblast which form the last protovertebræ. At this level, the mesoblast shows no line of division between its somatic and splanchnic portions. In that portion which must be considered as potentially somatic, there is a slight proliferation which gives rise to a more or less well defined rod (*p. d.*) which soon becomes the pronephric duct.

Embryo About One Hundred and Five Hours After Fertilization. Embryo Surrounds 220°.—The embryo (Fig. 17) shows a marked advance beyond the condition represented in Figs. 15 and 16. The divisions of the brain are more distinct. The hind brain shows a decided thinning of its dorsal wall. In front of the anterior end of the fore brain there is a slight pocket followed by a projection or median knob. On either side of this knob, are the large adhesive organs which are now apparent in surface view. The large optic vesicles lie just behind, and now show the first beginnings of the lenses. On either side of the medulla the auditory vesicles are faintly shown. The pronephric ducts have extended both anteriorly and posteriorly. The anterior portion of an embryo about five hours older is shown in Fig. 18.

In this stage but few changes are noted beyond those described. The lateral walls of the medulla are more widely separated and the roof has become thinner. The visceral arches and clefts are more pronounced. There is no trace as yet of the olfactory organs. As a result of the uplifting of the embryo through growth, the adhesive organs have assumed an oblique position.

A sagittal section, passing slightly to one side of the median plane of an embryo in this stage, is represented in Fig. 48. The lumen of the brain is enlarged and its subdivisions more clearly marked. The dorsal wall of the fore brain now shows a slight evagination which is the beginning of the epiphysis. Just opposite in the floor is another evagination which is the beginning of the infundibulum. Anteriorly the cavity narrows down in conformity with its external contour. Just in front of

the anterior end of the fore brain, the continued invagination of the superficial epiblast gives rise to a deep pocket in which a lumen is sometimes plainly apparent, while at other times its walls are so closely apposed that no lumen is discernible. The fore gut is here well shown with its forward extension into the precerebral region where it ends in a dilated cavity. The walls of this cavity, except the ventral, are made up of elongated hypoblastic cells. Just beneath this median evagination there is a large chamber surrounded by a double wall. The lining wall is made up of elongated cells which strongly resemble those lining the body cavity. Outside this layer is a second wall made up of large yolk-laden entoblastic cells. This chamber represents the beginning of the heart. The discussion of its formation, however, may best be deferred until we have studied the series of transverse sections of the next stage.

Embryo One Hundred and Twenty-five Hours After Fertilization. Embryo Covers 260°.—The last stage of the embryo included in the present study is represented in Figs. 19 and 20, the anterior portion being shown in Fig. 19, while the posterior is represented by Fig. 20. The embryo has increased greatly in length and its body is more prominent above the surface of the yolk, while the tail is just becoming free from the yolk. The increase in the length of the head has caused further shifting in the position of the adhesive organs which, instead of having their surfaces directed above, have come to occupy such an oblique position that their surfaces are almost directed forward. The so-called "button" (Reighard) is likewise carried forward and is no longer visible when the embryo is viewed from the dorsal surface. Just behind the adhesive organs are the two nasal pits which are visible for the first time in surface views. In the eyes, the lenses are plainly shown in the surface views. The mid brain has extended backward, while the hind brain has pushed forward in such a manner that its anterior portion envelops the posterior portion of the mid brain. In this embryo, the roof of the hind brain has been removed and one can plainly see in its floor a number of neuromeres. These are variable in the different embryos of this age, ranging from six to eight. On either side of the hind brain, the auditory vesicle shows as a deep pit. Three visceral arches are now well defined, as are also the three visceral clefts which appear as darker portions between them. The cœlomic cavity shows as a darker circle around the margin of the embryo, although its boundaries are not as clearly defined as in some of the earlier stages. (Fig. 17). The protovertebræ have extended on either side until they now reach from the extreme posterior end nearly up to the auditory pits. The pronephric ducts have extended both anteriorly and posteriorly. At their anterior ends they curve outward, then inward, in the form of a shepherd's crook.

A transverse section of an embryo in this stage is represented in Fig. 49. This section is taken through the region just anterior to the adhesive organs. On either side the cœlomic cavities show plainly as they approach the median line. The layers of the splanchnopleure are thus brought in such close contact above that the gut (*g.*) is almost closed off. During the time these layers are approaching they become folded backward into the cœlom on either side. In the figure, the left side is considerably in advance of the right. Through this folding there is formed a second closed cavity (*ht.*) which is the beginning of the heart. There is present at this time a lining layer, but its origin is uncertain.

The section represented in Fig. 50 passes somewhat obliquely through another embryo in about the same stage of development. The anterior end of the fore brain (*f. b.*) appears as a solid mass of elongated cells. In connection with its ventral wall, the optic stalk passes obliquely outward and terminates in the optic vesicle. On the other side the section passes through the anterior portion of the adhesive organ which here shows its connection with the anterior end of the fore gut (*g.*). The fore gut is almost closed off ventrally through the approximation of the cœlomic cavities. Between the end of the brain and the optic vesicles, there is a slight invagination (*n.*) of the deep ectoblast to form the beginning of the nasal pits.

The section represented in Fig. 51 is from the same series as the preceding and three sections farther back. The section through some oversight is not magnified quite so highly. The chief point of interest, as compared with the preceding is the rapid separation of the cœlomic cavities so that the gut is here widely open upon the yolk. It should also be noted that the cavities of the optic stalk, the fore brain and the adhesive organs are becoming apparent.

A section of the same series is shown in Fig. 52 at the level of the auditory vesicles. The section shows the extension of the cavities of these vesicles (*a. v.*) In other respects the section shows nothing more than is shown in Fig. 46.

The section shown in Fig. 53 is taken in a horizontal plane and shows practically all the structures which have been described in the series of transverse sections. The divisions of the brain are very clearly shown. Just in front of the anterior end of the fore brain is the invagination of the superficial ectoblast which we have previously described. On either side are the nasal pits (*n.*) with well defined lumina. Just anterior to these are the adhesive organs (*a. o.*) made up of the coarsely granular hypoblastic cells. Behind these are the large optic vesicles (*o. v.*) in

which the lenses are present, but not shown. Behind these in turn are three gill clefts (*v. c.*) and between them the mesoblastic bases of the corresponding arches. Close to the medulla are the auditory vesicles (*a. v.*). Around the periphery the lines of darker cells represent the hypoblastic walls of the gut (*g.*) The section is cut so thick that the mesoblast shows above, making it almost appear as if the gut were filled with these cells.

SUMMARY AND GENERAL REMARKS.

The Segmentation Cavity.

Before considering the stages which properly belong to the present paper, we are obliged to say a few words concerning the segmentation cavity. Whitman and Eycleshymer, **96,** pointed out that there are to be found in the egg, even in the earliest cleavage stages, certain irregular cavities which sooner or later become continuous with the cleavage grooves and in many cases unite to form a common cavity. These cavities appear in eggs collected in different years and in different seasons of the same year and fixed and imbedded in various ways. Since it is from these spaces that the segmentation cavity later takes its origin they have been subjected to renewed study. As segmentation progresses the cleavage grooves, in many cases at least, expand into broad spaces as they approach the center of the egg, in which locality they become continuous with the earlier spaces described above. Often these cavities unite and give rise to a more spacious one as figured by Whitman and Eycleshymer. That the cavities should be regarded as artifacts seems highly improbable. In the first place no cellular fragments are to be found in the spaces which would indicate imperfections in cutting. Again these cavities often shade off by imperceptible degrees into veritable intercellular spaces which no one would consider as artifacts. As the later stages of the blastula approach, the cavities no longer show as large irregular spaces, but become more or less obliterated by being filled with the rapidly proliferating cells of the blastodisc and yolk. In view of these facts, we cannot agree with Sobotta, **97,** that these cavities are artifacts.

Periblast.

The periblast in ganoids was first discussed by Dean, **95, 96,** who pointed out the homology of the upper layer of yolk cells in Acipenser, Lepidosteus and Amia with the periblast of teleosts. Sumner, **00,** later gives two figures showing the periblast in Amia. In one of these (Fig. 16) he represents a well defined, clear zone lying on the large yolk masses

and covered by a cellular layer which is considered as periblast. The author states that the figure is slightly schematized, but to what part of the figure he refers is uncertain. If to the periblast we have no criticism to offer. If the author, however, intended to represent the periblast as it is actually found in Amia we must emphasize the fact that our material shows nothing of the sort. There is no layer of cells between the periblast and the gastral cavity. The floor of the gastral cavity is, to our minds, the homologue of the periblast in teleosts. We find in the ganoids a complete series of gradations from the teleostean to the amphibian conditions. In Lepidosteus, as described and figured by the senior author, 03, we find the closest approach to the teleostean periblast. Amia comes next with its homologous layer in the floor of the gastral cavity; this floor is made up in part of detached cells and in part by the projecting ends of the large yolk masses. From this condition we can readily pass to the homologue of the periblast in Acipenser which is the layer of cells forming the floor of the gastral cavity. We are thus prepared to support the statement of the Zieglers, 92, that the floor of the gastral cavity in the amphibia is the homologue of the periblast in fishes.

The Mesoderm.

The origin of the mesoderm in Amia has been previously studied by both Dean and Sobotta. Their descriptions, however, are quite unlike.

Dean, 96, in describing a stage in which the blastopore has nearly closed, says: "The mesoblast is found to arise peristomal; on the dorsal side it arises from the undifferentiated tissue (of the tail mass), thence extending forward as a separate cell layer, and finally appears to be blended with the loosely associated cells of the entoblast; ventrally the mesoderm, although distinctly to be recognized, is not to be separated from the cellular elements of the entoderm. In its early growth it extends forward as a wide and flattened cell mass, thinning distally and becoming confluent with the inner germ layer. As in the teleosts, gastral mesoderm is absent, and the division of the middle layer into its somatic and splanchnic layers is not apparent until a comparatively late stage of development."

Sobotta, 96, describes the origin of the mesoderm in a much earlier stage in the following words: "Nun tritt, noch ehe es zur Urdarmbildung, also zur eigentlichen Gastrulation kommt, eine Differenzirung der Furchungszellen zu Keimblättern auf, indem sich eine compacte mehrschichtige Zellage an der Oberfläche des Eies durch einen feinen Spalt von den darunten gelegenen, mit grösseren Dotterkörnern beladenen Zellen sondert (Fig. 3). Diese Erscheinung trennt bereits jetzt das Ek-

toderm von der später zu Mesoderm und Entoderm werdenden Zellage— Sehr bald beginnt nun am Aequator des Eeies die Urdarm-Bildung, und zwar zuerst an der Stelle der spateren Embryonalanlage. Es entsteht dadurch die dorsale Urmundlippe (Fig. 4). Letztere ist zur Zeit, wo der Urdarm als feiner Spalt sichtbar wird, sofort deutlich dreiblättrig, nicht zweiblättrig wie Dean angiebt. Die verschiedene Gehalt der Zellen an Dotterkörnern, resp. die verschiedene Grösse derselben in den Zellen, ermöglicht die Unterscheidung drier Keimblätter sehr leicht." The writer then points out the differences in the sizes of the granules in the different layers, stating that those in the cells of the ectoderm are all fine, those in the cells of the mesoderm are considerably larger, while those in the cells of the entoderm are coarse. The writer further states that when the gastral cavity has extended beneath the dorsal lip of the blastopore, the dorsal and ventral mesoderm are united.

It is evident from our studies that we agree in general with Sobotta in that we find the ectoderm early separated from the underlying layer of cells by the slit-like remains of the segmentation cavity. This underlying layer represents, according to Sobotta, the mesoderm. We do not agree, however, that in the early gastrula the size of granules or their staining capacity will enable one, as Sobotta claims, to distinguish mesoderm from entoderm. It is not until the time when the blastopore is nearly closed that a differentiation of cells is apparent. Even then we are not certain that these cells represent the mesoderm since the marked contrast in the staining capacity later disappears. To know precisely when and how the mesoderm arises and how it extends in Amia will involve better methods of staining than we now possess.

The Archenteron. Kupffer's Vesicle and Adhesive Organs.

As the archenteric cavity extends the innermost layer of mes-entoderm early is differentiated into a well defined layer which we have called hypoblast. This layer, together with the invaginated dorsal ectoblast, forms the dorsal wall of the archenteron. At the same time there is differentiated a superficial yolk layer which forms the ventral wall of the archenteron. The extent of this primitive gut, however, does not correspond to the extent of the embryo. There is formed both an appendicular (Salensky) or post-annal gut and a precephalic gut. Since the changes in the posterior portion precede those in the anterior they will be considered first.

It should be remarked here that the closure of the blastopore is complete, no portion persisting to form the anus. Its line of closure is in-

dicated for some time by the large coarsely granular cells which lined it. The anus arises through an invagination just behind the line of closure of the blastopore and soon becomes continuous with the appendicular gut. The portion posterior to this or the post-anal gut proper soon shows retrogressive changes. The walls lose their distinctive hypoblastic character, the lumen becomes obliterated and the entire structure later disappears, playing no part in the formation of later embryo.

Kupffer's vesicle has been studied previously in Amia by Dean, 96, and Sumner, '00. Dean writes as follows: "The cœlenteron, now a deep cavity beneath the dorsal lip, extends forward below the entire head; its hinder dilation immediately below the dorsal lip is to be interpreted as representing Kupffer's vesicle." Regarding Dean's interpretation Sumner says: "Dean maintained that this cavity simply represented the angle formed by the blastoderm's margin, as it was mechanically turned in upon itself during its circumcrescence of the yolk. This simple mechanical explanation I cannot accept for the teleosts because (among other reasons) the vesicle in some fishes is not formed until the blastoderm has nearly or quite finished its journey over the yolk and thus the supposed mechanical cause no longer exists." In considering the function of this structure in fishes Sumner further says: "It has for some time been my view that this vesicle contains a more fluid yolk, partly assimilated through the activity of the periblast and intended for the nourishment of the growing embryo. I have also expressed the view that Kupffer's vesicle represents an embryonic digestive organ (more properly an organ of absorption)."

In considering this structure it is necessary to recall what has been said regarding the periblast in the teleosts, ganoids, and amphibia. While the roof of the vesicle in all these forms is the same, the floor in the teleosts is sometimes of periblast and again there is a cellular floor lying upon the periblast. These facts are at first difficult to interpret, yet if as H. V. Wilson, 91, suggests the latter condition is to be regarded as secondary, the difficulties are in a measure overcome. If it be accepted that the floor of Kupfer's vesicle is periblast in the teleost and that the periblast of Lepidosteus is the homologue of that of the teleost Eycleshymer, 03, we are placed in position to say that the ventral wall of the vesicle in Amia, Acipenser and the amphibia is represented by nothing more or less than the gastral floor. The vesicle then represents the posterior portion of the primitive digestive tract. This being the case in Amia no one need hesitate to accept Sumner's view that the vesicle may have had a digestive function.

The first description of the growth of the adhesive organs is given by

Dean, 96, who says: "The mode of origin of the sucking disc gives the most interesting evidence of how precociously embryonic and larval structures may be developed. As far as histological evidence goes there is certainly no difference between the enlarged thick-walled, cup-shaped organs which arise on the snout of the late embryo of Amia or of Lepidosteus, and the typical pit organs, or sense buds, which later occur on other integumental regions. It is found, in fact, that a gradation in size exists which connects the huge sucking organs of the snout with the inconspicuous pit organs of the trunk."

These organs were later studied in Reighard's laboratory by Miss Phelps, 99, who found that "the organ is formed in a very early stage as a diverticulum of the fore gut. This diverticulum subsequently divides into two, each of which continues to communicate for a time with the cavity of the fore gut." The author further observed that the organs open to the exterior, but become cut off from the fore gut and degenerate leaving no trace behind.

Our studies show that these organs arise from paired diverticula of the fore gut and not from a single diverticulum which later divides. While it is undoubtedly correct to state that the paired gut diverticula are derived from an unpaired condition, there is not the slightest evidence that the anlagen of these structures appear before the gut diverticula are well established. The beginnings are first visible as slight thickenings of the hypoblast, forming the antero-dorsal walls of these diverticula. As development progresses these thickened areas evaginate and the cells begin to elongate. Soon a longitudinal constriction forms which divides each of these structures, giving rise to four. Meantime the lumen of each is reduced, the walls of the gut become apposed and the organs are cut off from further communication with the gut. After losing their connection with the gut they continue to divide until eight or more are formed. They then come in contact with the ectoblast whose cells undergo cytolysis, leaving the hypoblastic cells of the organs projecting to the free surface. We have not followed the later changes in these organs. Miss Phelps states that after being functional for a time the organs are pushed beneath the surface of the thickened ectoblast, become filtrated with leucocytes and finally disappear.

As to the meaning of these remarkable organs we are in the dark. Their function may be only to hold the larva in position for a certain period. Again they may serve to convey some sort of nutriment to the digestive tract. That they are modified sense buds, as Dean suggests, seems highly improbable. Their interpretation from a phylogenetic standpoint is certainly most difficult. About all that can be said is that

structures such as these give rise to some of the most perplexing problems with which embryology has to deal.

In the surface views (Figs. 17, 18, 19) there is a peculiar median knob which appears soon after the adhesive discs are differentiated. This structure lies between and somewhat anterior to the discs. It has been observed, as earlier stated, by Reighard (see Keibel, 03). Sagittal sections through embryos of this stage (Fig. 48) show that in addition to the lateral evaginations of the fore gut there is a less marked evagination of the median wall. The hypoblast in this region is thickened and becomes continuous on either side with that of the adhesive discs. The epiblastic pocket behind separates this structure from the anterior end of the fore brain and together with the surrounding mesoblast gives it considerable prominence in surface views. We conclude that the so-called "button" is nothing more than the strongly evaginated median portion of the adhesive organs.

The Chorda and Hypochorda.

Dean, 96, has described the formation of the chorda in Amia as follows: "The notochord arises as in the sturgeon or gar-pike: it separates directly (i. e., delaminates) from the entoderm." We have studied the origin of the notochord in many series of embryos, but are unable to add much to Dean's description. In the great majority of cases examined the sections show conditions similar to that observed in Fig. 40; often the mesoblast has not yet separated from the axial rod as shown in Fig. 41. In but one instance have we found anything which would lead us to regard the chorda as formed by an evagination of the dorsal wall of the archenteron, as is known to be the case in many amphibia. We therefore conclude that the chorda is derived by delamination from the layers of cells which we have called mes-entoderm. The controversies that have been waged over this structure and the failure to homologize it in the various groups, especially amphibia and fishes, hold out little promise that a definite solution of the problem in harmony with the germ layer theory is near at hand.

The hypochorda, so far as we know, has not been previously seen in Amia. It arises from the hypoblast forming the dorsal wall of the gut in a stage just prior to the appearance of the pronephric ducts. It extends the length of the notochord and presents in sagittal sections appearances which lead us to regard it as irregularly segmented. This peculiar structure has been observed in many vertebrates and numerous suggestions offered as to its significance. By some it has been considered

as the anlage of a ligament or a blood vessel. By others it is regarded as the remains of a blood vessel or of a blood-forming organ. Still others think it entirely disappears. We incline toward the last view, but our later stages are not complete enough to settle the question definitely.

The Heart.

In considering the origin of the heart, it is necessary to recall that toward the anterior end of the embryo the cœlomic cavities on either side approach the middle line. This approximation proceeds anteriorly until the two halves of the cœlomic cavities are brought closely together. Just before they meet each becomes folded back at the edge. Through this folding back of the splanchnopleure there are formed two grooves; the edges of these two grooves unite across the median line to form a single oval sac which is open both anteriorly and posteriorly. This sac is lined by entoderm and surrounded by the splanchnic mesoblast. While these changes have been going on there has appeared within the heart cavity thus formed a layer of cells which have the appearance of mesoblast cells, but apparently they are derived from the hypoblast. Whether they are to be considered as mesoblast, that at this relatively late period has differentiated from the hypoblast, or whether they are to be considered as hypoblast we are unable to say. Only by knowing the fate of these layers could one hazard an interpretation.

The Central Nervous System and Sense Organs.

The central nervous system, as observed by Dean, 96, is first formed as a solid rod or keel from the deeper ectoblast. Soon after the appearance of the optic vesicles a lumen is formed, but whether through cytolysis or delamination or both is uncertain. There are indications which lead us to regard cytolysis as the most probable.

Towards the caudal end of the embryo the superficial ectoblast folds downward into the neural keel forming the neural trench, which at the posterior end passes over into the blastopore. The question whether or not this is to be interpreted as a neurenteric canal depends upon the significance of the neural trench. If this trench is to be considered as homologous with the extreme lower part of the medullary grooves in Amniota, as Kupffer regards it in the trout, we should certainly consider its continuation over into the blastopore as a reminiscence of the neurenteric canal. However, both Wilson's and Kupffer's views are questioned by Minot and others, and since the interpretation rests upon funda-

mentally different conceptions which are at present beyond proof or disproof, we may dismiss the question without further comment.

Concerning the neuromeres which are so well shown in Fig. 19, we can only say that at present we are unable to interpret these structures. They have not been found in the preceding stages and have not been followed in the succeeding stages. Why they should appear at this time and be wanting in the stage shown in Fig. 18, is at present unexplainable. It is impossible to state whether they are secondary foldings due to the formation of protovertebræ or whether they are formed independently in the floor of the hind brain and are the first definite expression of segments. If the latter be true, as many embryologists hold, then we should find in the hind brain of Amia indications of seven or eight primitive segments.

The median pit, which first appears in Fig. 42, has been followed in both the earlier and later stages. After a careful study much doubt lingers in our minds as to whether or not it takes any part in the formation of the hypophysis. Kupffer maintains that in Petromyzon and Acipenser this structure forms the hypophysis. It seems to us possible that the invagination of the gut to form the median portion of the adhesive organs, as shown in the figure, would carry the epiblast outward in such a manner that it results in an increase in this invagination. In other words, the mechanical factors operating could cause just the appearance observed. We should hesitate to regard this structure in Amia as of great value in phylogenetic interpretation.

The previous observations on the development of the optic vesicles in Amia are embodied in the following sentence by Dean, 96: "The mode of development of the eye and of the nasal and auditory capsules differs but little from that typical in the lower vertebrates generally." Our studies show that the eyes first appear as solid outgrowths which shortly after become hollow.

Concerning the early development of the auditory vesicle there is nothing beyond the sentence quoted above. According to our observations, the ear likewise begins as a solid thickening of the deep epiblast over which the superficial layers pass unmodified. This thickening continues until there is an oval mass lying on either side of the anterior portion of the medulla. When the embryo reaches the stage shown in Fig. 18, a cavity is present.

The olfactory organs first appear as proliferations of the deep ectoblast in the stage represented in Fig. 19. In this mass an invagination soon appears forming well defined pits.

The Pronephric Duct.

The pronephric duct is preceded by a solid rod of cells which arises through a proliferation of the cells of the somatopleuric portion of the mesoderm, but before the appearance of a well defined cœlom. We do not agree with the observations of Felix and Bühler, 04, who state that it arises as an evagination of the somatopleure. The further study does not come within the scope of the present paper.

Systematic Position of Amia.

Dean concludes his second paper, 96, on Amia with the statement that "at the base of a gradational series stands Lepidosteus, near it and in some ways even below it is Acipenser; next is Amia; next, and very closely related, is Amiurus; and, finally, are the many remaining forms of teleosts."

Previous studies by the senior author on Amia and Lepidosteus, the present study of Amia, together with the unpublished work on Ameiurus by the junior author, all indicate that such an arrangement, based upon early developmental characters, is not only premature but incorrect. The only conclusion which can be reached at the present time is that the evidence from œcology, anatomy, histology, embryology, is so fragmentary that it affords no secure basis for assigning the various ganoids their respective places within the group, nor the group its position in the vertebrate phylum.

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ABBREVIATIONS.

a. g., appendicular gut.
a. o., adhesive organ.
a. v., auditory vesicle.
bl., blastopore.
b. c., brain cavity.
c., coelom.
ch., chorda.
d. ec., deep ectoblast.
d. l., dorsal lip of blastopore.
env., envelope.
f. b., fore-brain.
f. g., fore-gut.
g., gut.
g. c., gastral cavity.
g. d., gut diverticulum.
g. hy., gut hypoblast.
h., anlage of head.
h. b., hind-brain.
hch., hypochorda.
ht., heart.
m. b., mid-brain.
m. en., mes-entoblast.
mes., mesoblast.
n., nasal pit.
n. c., neural canal.
n. t., neural trench.
op. t., optic thickenings.
o. v., optic vesicle.
p. cb., pre-cerebral mass.
p. d., pronephric duct.
s. c., segmentation cavity.
s. ec., superficial ectoblast.
v. a., visceral arch.
v. c., visceral cleft.
v. l., ventral lip of blastopore.
y. m., yolk mass.

EXPLANATION OF PLATES.

Plate I.

Fig. 1. Profile view of egg about nine hours after fertilization.

Fig. 2. Profile view of egg about twelve hours after fertilization.

Fig. 3. Profile view of egg about twenty hours after fertilization.

Fig. 4. Profile view of egg about forty hours after fertilization.

Fig. 5. Profile view of egg about fifty-three hours after fertilization, anlage of embryo faintly outlined.

Fig. 6. Profile view of egg about fifty-five hours after fertilization, outline of embryo discernible.

Fig. 7. Same egg viewed from the lower pole, showing infolded blastodisc.

Fig. 8. Embryo about sixty hours after fertilization, viewed from above, embryo well defined posteriorly, neural (?) trench visible.

Fig. 9 . Embryo about sixty-five hours after fertilization, viewed from above, embryo narrower, better defined outline, blastopore nearly closed.

Fig. 10. Embryo about seventy hours after fertilization, embryo longer, neural (?) trench extended, blastopore closing.

Figs. 11, 12. Anterior and posterior portions of embryo about seventy-five hours after fertilization, viewed from above, optic thickenings visible, two or three protovertebrae differentiated.

Figs. 13, 14. Anterior and posterior portions of embryo, eighty hours after fertilization, viewed from above, divisions of brain and precerebral process apparent, seven to nine protovertebrae, beginnings of pronephric ducts, extent of mesoblast indicated by dark area around head.

Figs. 15, 16. Anterior and posterior portions of embryo about ninety hours after fertilization, viewed from above, showing pre-cerebral process, optic thickenings, first gill arch and cleft, about sixteen protovertebrae, dark shading around embryo indicates extent of coelom.

Fig. 17. Anterior portion of embryo about one hundred and five hours after fertilization, viewed from above, showing divisions of brain, optic vesicles, adhesive organs, median knob, two gill clefts, beginnings of auditory vesicles.

Fig. 18. Anterior portion of embryo about one hundred and ten hours after fertilization, showing slight advance beyond condition represented in Fig. 17.

Figs. 19, 20. Anterior and posterior portions of embryo about one hundred and twenty-five hours after fertilization, viewed from above, showing, in addition to structures previously described, the anlage of the heart, the beginnings of the olfactory organs, neuromeres, three gill arches and their corresponding clefts.

Plate II.

Fig. 21. Meridional section of an egg about nine hours after fertilization. Showing early condition of blastodisc segmentation cavity and yolk.

Fig. 22. Meridional section of egg about twelve hours after fertilization. Showing the yolk masses actively budding off entoblastic cells, and obscuring more or less the segmentation cavity.

Fig. 23.. Meridional section of egg about forty hours after fertilization showing proliferation of superficial ectoblast at the point where invagination begins.

Fig. 24. Meridional section of egg. about fifty hours after fertilization showing beginning of invagination and maximal thickness of blastodisc.

Fig. 25. Portion of meridional section of egg of about same age as above through region of blastopore showing first formation of mes-entoblast.

Fig. 26. Meridional section of egg slightly older than preceding stage showing further extension of gastral cavity.

Fig. 27. Meridional section of an egg about fifty-three hours after fertilization, showing reduction in number of entoblastic cells, also thinning of blastodisc.

Fig. 28. Portion of meridional section of egg in same stage as above more highly magnified, showing region of blastopore.

Fig. 29. Meridional section of an egg in stage somewhat later than above showing further thickening of margin of blastodisc.

Fig. 30. Horizontal section of egg just above level of equator, showing lateral extent of embryonic anlage, also beginning of notochord.

PLATE III.

FIG. 31. Meridional section of egg about fifty-five hours after fertilization, showing thickening of ectoblast to form head of embryo.

FIG. 32. Sagittal section of an embryo some sixty hours after fertilization showing further differentiation of embryo.

FIG. 33. Sagittal section of an embryo about sixty-three hours after fertilization, showing further growth of embryo, the obliteration of the segmentation cavity, the extension of the gastral cavity, the reduction of yolk plug.

FIG. 34. Sagittal section of posterior end of embryo about seventy hours after fertilization, showing the closure of the blastopore.

FIG. 35. Transverse section through the blastopore of an embryo in same stage as above.

FIGS. 36, 37. Sagittal section of embryo about seventy-two hours after fertilization. The anterior portion of the embryo is shown in Fig. 36, while the posterior portion is shown in Fig. 37.

FIG. 38. Oblique section of an embryo seventy-five hours after fertilization showing the thickenings which later form the optic vesicles.

FIG. 39. Transverse section of an embryo of same age passing through the posterior portion of the hind brain.

FIG. 40. Transverse section of same embryo at the level of Kupffer's vesicle.

FIG. 41. Transverse section of an embryo of same age, showing deep neural trench and posterior end of notochord.

FIG. 42. Sagittal section of head end of embryo about ninety-five hours after fertilization, showing divisions of brain, notochord, gut, adhesive organs, and the peculiar invagination of the ectoblast just anterior to the fore-brain.

PLATE IV.

FIG. 43. Transverse section through embryo of same age at the level of the adhesive organs.

FIG. 44. Transverse sections of same embryo at level of optic vesicles.

FIG. 45. Transverse section of same embryo at level of anterior margin of medulla..

FIG. 46. Transverse section of same embryo through the region of the auditory vesicles, showing the first appearance of pronephric duct and hypochorda.

FIG. 47. Transverse section of same embryo at the level of last protovertebra.

FIG. 48. Sagittal section of embryo one hundred and ten hours after fertilization, showing the beginning of the epiphysis and infundibulum, also the median portion of the adhesive organs and the heart.

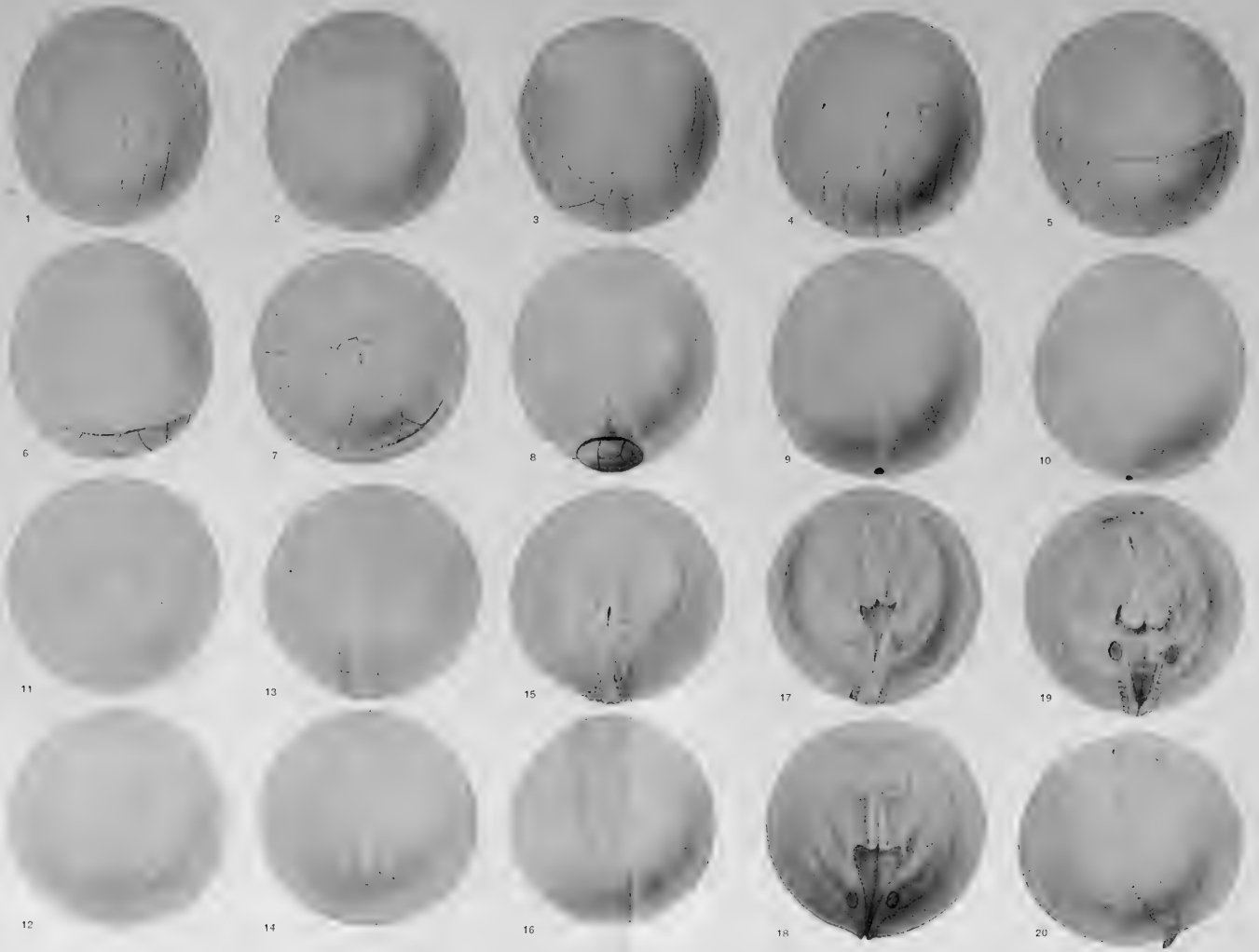
FIG. 49. Transverse section of an embryo one hundred and twenty-five hours after fertilization taken just in front of the region of adhesive organs showing heart, gut and coelom.

FIG. 50.. Obliquely transverse section of an embryo of same stage passing through the anterior margin of the adhesive organs on the one side and the optic stalk and vesicle on the other, showing the approximation of the layers of the splachnopleure to form the heart.

Fig. 51. Obliquely transverse section of same embryo taken a few sections behind that shown in Fig. 50, showing same structures as above.

Fig. 52. Transverse section of embryo in same stage showing the extension of the cavity of the auditory vesicles also the gut and coelomic cavities.

Fig. 53. Horizontal section through the head region of embryo in same stage showing various structures.



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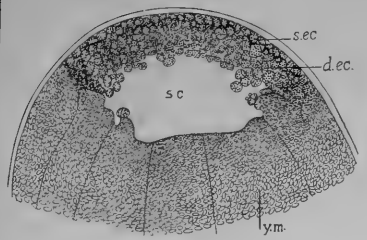

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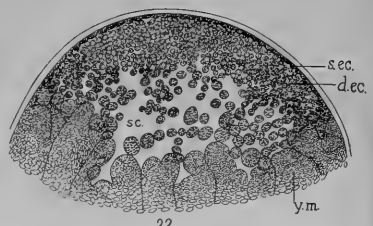

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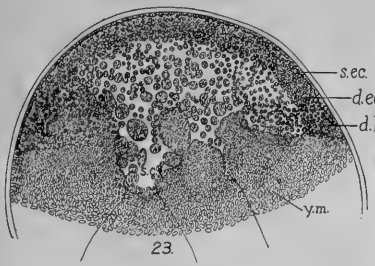

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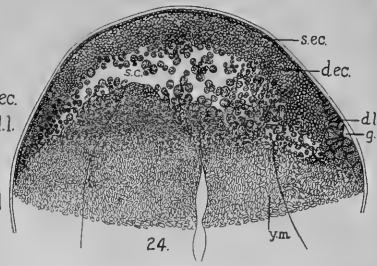

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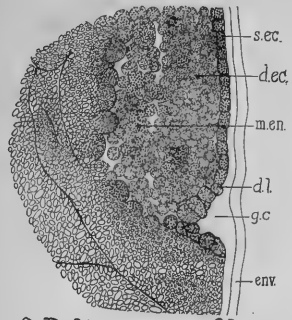

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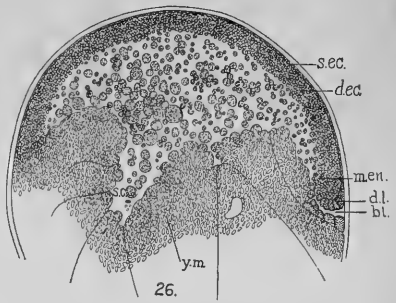

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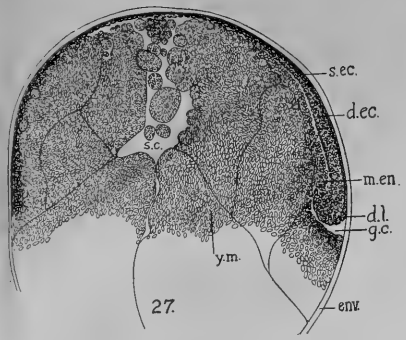

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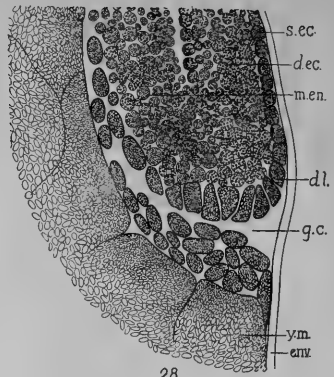

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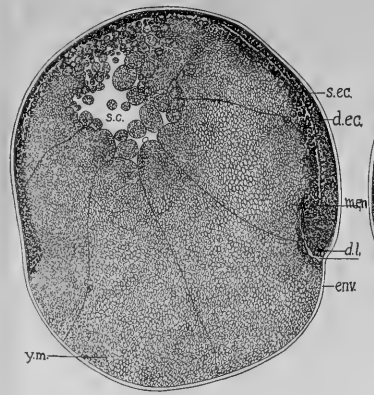

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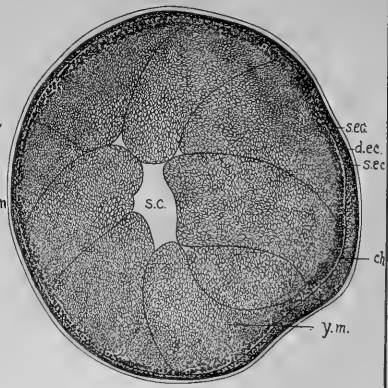

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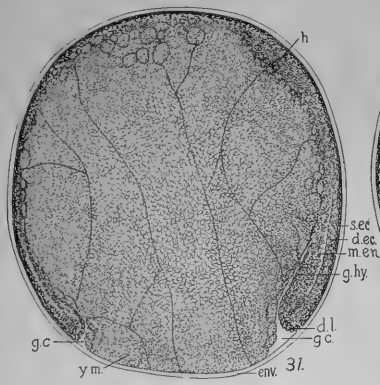

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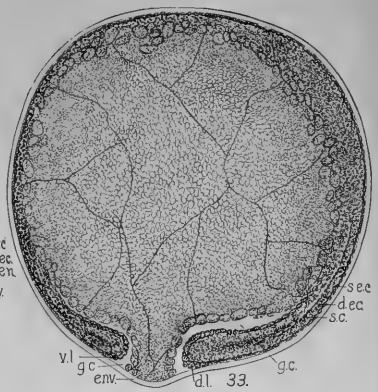

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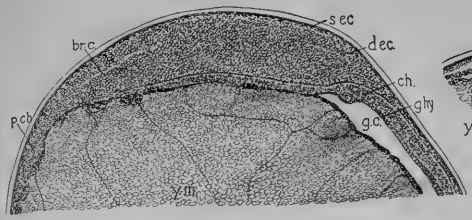

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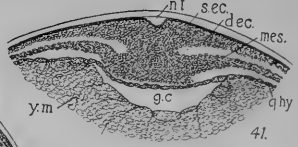

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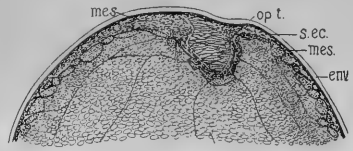

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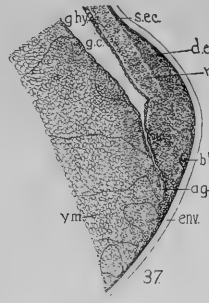

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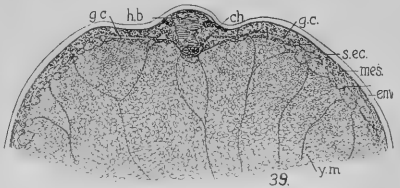

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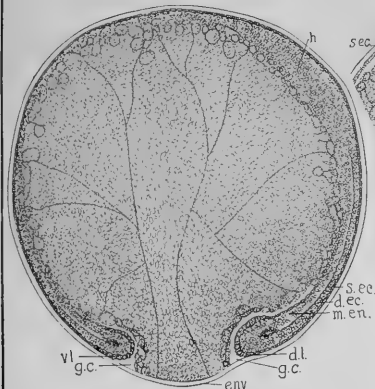

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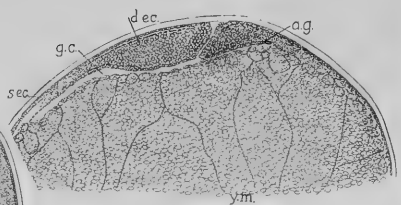

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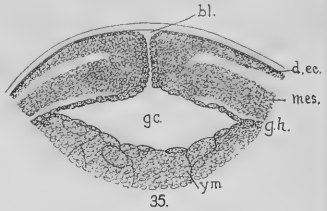

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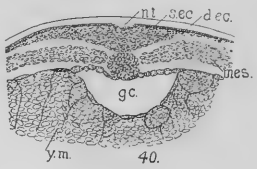

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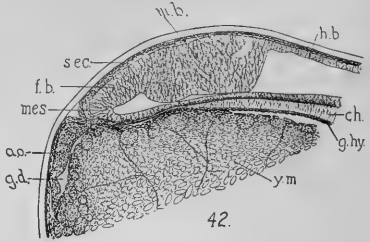

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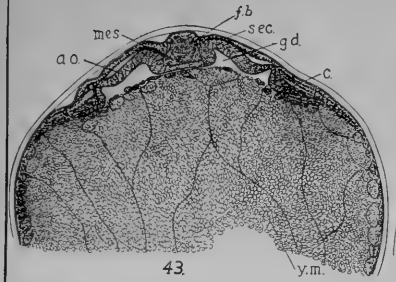

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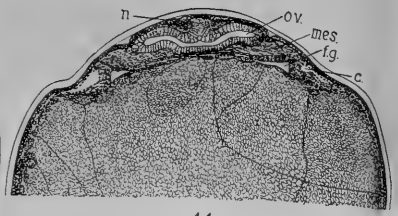

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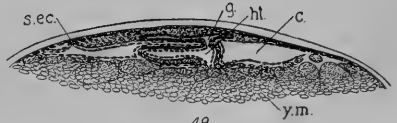

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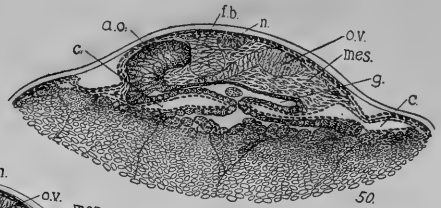

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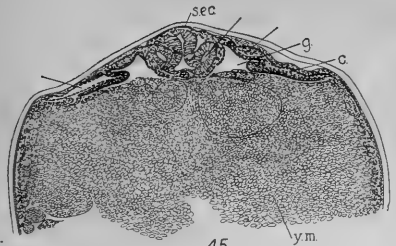

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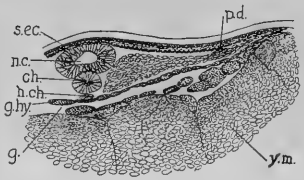

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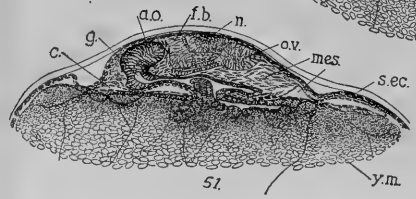

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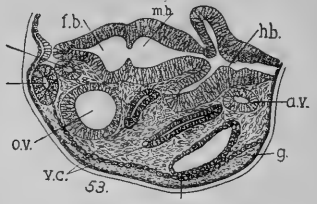

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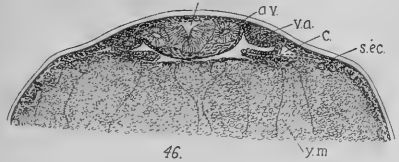

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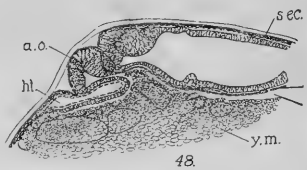

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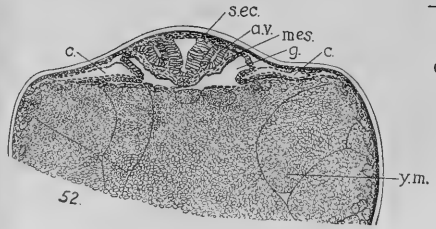

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A CONTRIBUTION TO THE ANATOMY AND DEVELOPMENT OF THE VENOUS SYSTEM OF DIDELPHYS MARSUPIALIS (L).[1]

Part II, Development.

BY

CHARLES F. W. McCLURE.

Professor of Comparative Anatomy, Princeton University.

With 5 Double Plates and 27 Text Figures.

A number of publications have appeared, especially the monographs of Selenka, 86-7 and 91, and Semon, 94, in which have been described the arrangement of the blood vessels in the extra-embryonic vascular area of marsupials. So far as known to the writer, however, the only actual contributions that have been made to the development of the intra-embryonic venous system of this group of mammals are one by Broom, 98, and a preliminary notice by the writer, 02.

The publication of the present paper (Part II) has been unavoidably delayed, owing to the writer's inability to obtain embryos sufficiently young to show the earliest stages in the development of the postcaval vein. These early stages have, unfortunately, not yet been obtained, and were it not for the circumstance that so little has been published upon the development of the veins of marsupials, an apology would be due for presenting what must necessarily be an incomplete account.

In writing this paper the writer has fully borne in mind the danger involved of drawing conclusions from an incomplete series, and, in the case of Didelphys, the danger is especially great on account of the variable character of its venous system. A complete account of the development of the veins of Didelphys, especially of the variations of the postcava, necessitates not only a complete series of embryos and pouch young, but a number of examples from each stage as well. Such an

[1] The publication of this paper in two parts, one dealing with the Anatomy (Part I) and the other, or present paper (Part II), with the Development of the venous system, was unavoidable and it is, therefore, to be hoped that the frequent references made in the following pages to Part I will not prove too great a source of confusion or inconvenience to the reader.

Part I of this paper was published in The American Journal of Anatomy, Vol. II, No. 3, 1903.

abundance of material the writer has been unable to obtain, and, therefore, appreciates that many modifications, as well as additions, to his account may possibly be necessary before a complete history of the veins is at hand.

In the following pages an attempt has been made, on the basis of the material at hand, to present an account of the development of the postcava from a time soon after its first appearance until the adult stage is reached; also, an account of the development of the azygos veins, as well as the transformations which the umbilical and omphalomesenteric veins undergo during the different stages of development.

An attempt to breed opossums in captivity proved only partially successful, the failure being due, I am convinced, to the unsuitable conditions which necessarily prevail in my laboratory. Most of my Didelphys material was therefore obtained outside of the laboratory, and, for this reason, it is impossible to give the exact age in days and hours of any of the embryos or pouch young studied. It has been possible, however, by means of Selenka's, **86** and **87**, figures and descriptions to approximate their ages in a few cases and to establish the fact that the embryo of Dasyurus which Dr. J. P. Hill, of the University of Sydney, kindly sent me is, in point of structure, relatively younger than my youngest Didelphys embryo.

According to Selenka, the interval between copulation and birth in Didelphys virginiana is about thirteen days (twelve days and twenty hours), while that between copulation and the beginning of cleavage is five days. In the following list where ages are mentioned the age has been reckoned from the beginning of cleavage.

List of Material Studied.

1. One Dasyurus embryo measuring about 6 mm. in length (crown-rump measurement).[2] From a comparison of the structure and external characters of this embryo with that of Selenka's, **86** (Fig. 3, Taf. XXVI), six days old Didelphys embryo, it is evident that the latter is slightly more advanced than the Dasyurus embryo, which, in point of structure, corresponds to a Didelphys embryo of about five and one-half days.

2. Eleven Didelphys embryos averaging 8 mm. in length. From a comparison of their measurements these embryos may be a few hours older than Selenka's, **86**, (Fig. 3, Taf. XXVI) six days old embryo, although in their external characters the two appear to be identical.

[2] All measurements were made in this manner. Embryos and pouch young were measured by the writer after fixation.

3. Three embryos of Didelphys averaging 11.5-12 mm. in length. These embryos were kindly presented to me by Dr. Bremer, of Harvard University, to whom my thanks are due.

It is a curious fact that these embryos measure more than certain of the pouch young studied by the writer; a circumstance which shows, as suggested by Professor Minot, that opossums may vary considerably as to the degree of development attained before they enter the pouch.

4. One pouch young of Didelphys measuring 10.5 mm. in length. Harvard Embryological Collection, No. 614.

5. One pouch young of Didelphys measuring 11.5 mm. in length. Harvard Embryological Collection, No. 617. This measurement corresponds to that of Selenka's, **87** (Fig. 2, Taf. XXIX), newly-born pouch young of eight days (eight days after beginning of cleavage), but is apparently not constant for young of this age, since the Harvard specimen, No. 614, which is undoubtedly the younger of the two, measures only 10.5 mm.

6. Five pouch young of Didelphys averaging about 14 mm. in length.

7. Eight pouch young of Didelphys averaging about 15 mm. in length. According to measurements these correspond approximately to Selenka's (**87**, Fig. 8, Taf. XXX) four days old pouch young (twelve days from the beginning of cleavage).

8. One pouch young of Didelphys measuring 17 mm. in length.

Preparation of Material.[3]

The writer has experienced no difficulties as regards the fixation of the embryonic material. Any one of the ordinary fixing agents, such as picro-sublimate, Perenyi's fluid, or a 10% solution of formaldehyde,[4] will produce good results. The fixation of the pouch young, however, is a very difficult matter. In these the epitrichium is so impervious to the penetration of fluids that the writer has been unable to find any fixing

[3] I take much pleasure in expressing my thanks and appreciations to the following gentlemen for the unusual courtesies they have extended to me in connection with the preparation of this paper: To Professor Charles S. Minot of Harvard University for the loan of the opossum material in the Harvard Embryological Collection; to Dr. Bremer of Harvard University for three opossum embryos and three pouch young; to Dr. J. P. Hill of the University of Sydney for an embryo of Dasyurus; to Professor Bashford Dean of Columbia University for a number of kangaroo pouch young; to Mr. Stephen S. Palmer of New York for funds necessary to cover the cost of several of the figures and plates in this paper, and to Professor Macloskie and Mr. Silvester of Princeton University for many helpful suggestions.

[4] A 10 per cent solution of the 40 per cent commercial formaldehyde.

agent that, with the usual after-treatment, will fix the tissues of the older pouch young without, at the same time, producing a considerable shrinkage. Although I have not yet had the opportunity of trying this method, I am inclined to believe that shrinkage can only be avoided by removing the epitrichium before the pouch young are placed in the fixing agent.

Two methods of staining which proved to be most satisfactory for the study of blood vessels were a combination of Delafield's hæmatoxylin and picric acid and one of bleu de Lyon and safranin.

It is evident from the recent investigations of Lewis, 02, that the development of the postcaval vein in mammals cannot be adequately considered without taking into account the rôle played by the subcardinal veins, since he has shown that a portion of the right subcardinal in the rabbit enters into its formation. Lewis' description of the subcardinal veins and his conclusions regarding the origin of the postcava in the rabbit, are given in the following quotation from his paper (page 241):

"Small vessels from the mesentery pass into the cardinals. They anastomose in front of the aorta with vessels of the other side. They form a longitudinal anastomosis parallel with the cardinal vein, with which it is connected by numerous short veins, and from which it is separated by a line of mesonephric arteries. This longitudinal vessel connected with the cardinal vein at both ends, and bilaterally symmetrical in its early stages is the subcardinal vein."

"The cross connections between the subcardinal veins give place to a single large cross anastomosis caudad to the origin of the superior mesenteric artery. Above this anastomosis the right subcardinal connects with the liver and rapidly enlarges; the left subcardinal becomes very small—Hochstetter says that it forms the left suprarenal of the adult. Below the anastomosis the subcardinals cease to exist as veins; they may persist as lymph spaces."

"The vena cava inferior is a compound vessel composed of parts of the heart, the vena hepatica communis, the hepatic sinusoids, the upper part of the right subcardinal, and the lower part of the right cardinal vein."

Miller, 03, under the direction of the writer, has followed the development of the postcaval vein in birds and has likewise noted and described a system of veins in the embryo which corresponds exactly to that described by Lewis in the rabbit as the subcardinal system of veins. He also found in birds that a portion of the right subcardinal vein, as in the rabbit, enters into the formation of the adult postcava and that, in

addition to this, the subcardinal veins persist in the adult as the left suprarenal and genital veins.

The veins which Lewis and Miller have described under the name of "subcardinals" were, so far as known to the writer, first described in the embryos of birds and mammals by Hochstetter, **88** and **93**, who regarded them as the homologues of the revehent veins of the Wolffian bodies in reptiles. In tracing their subsequent development, however, Hochstetter found that they disappeared for the most part, and were represented in the adult by only the left suprarenal and possibly the genital veins in the chick, and by the left suprarenal vein in the rabbit.

In addition to the mammals, Lewis, **04**, has also recently described the subcardinal veins as met with in the selachians (Torpedo and Acanthias), amphibians (Necturus) and reptiles (Lacerta) and, in the writer's opinion, has correctly interpreted the rôle which these veins play in the formation of the adult renal portal system. He states that in Torpedo and Acanthias, after fusing to form the genital sinus, the subcardinals make connections anteriorly with the cranial ends of the postcardinals and with the latter form the revehent veins of the adult renal portal system. In Necturus the subcardinals fuse to form an unpaired vessel, which, after making connections with the hepatic circulation, constitutes the greater portion of the postcava. In Lacerta the subcardinals also form a large part of the postcava, although the fusion between the two veins is less complete here than in Necturus (Lewis).

Lewis, so far as known to the writer, was the first investigator to interpret the development of the venous system of the selachians and amphibians in the terms of the subcardinal veins, and, although I feel confident his interpretations are correct, at the same time a more thorough investigation of the amphibia is to be desired before any definite conclusion can be established. In reptiles, however, Hochstetter, and, more recently, my pupil Stromsten, **05**, have conclusively shown that the veins which form a large portion of the postcava are the homologues of the so-called subcardinal veins of birds and mammals. To what extent the subcardinal veins may be developed in the embryos of vertebrates other than those mentioned above, it is impossible to state without further investigation. From our present knowledge of the subcardinals, however, it is evident that they possess so great a morphological significance in certain vertebrates, that any interpretation of the vertebrate venous system must necessarily be incomplete without, at least, taking into consideration the presence or absence of these veins.

There can be no doubt as to the morphological significance of the subcardinal veins; that their development is primarily correlated with the

presence of a renal portal system as is the case in selachians, (amphibians), reptiles and the embryos of birds. Their presence, therefore, in the embryos of mammals in which a renal portal system is usually wanting[5] is most suggestive, and indicative of a "ground-type" of venous system of which the subcardinal veins form a constituent element. It is evident, therefore, from what we at present know of the subcardinal veins that they can no longer be regarded as transitory structures of little importance, since they form an essential and important element of the embryonic venous system in a number of vertebrates, and are retained in the adult, to a greater or lesser degree, in accordance with the presence or absence there of a renal portal system.

Since the subcardinal veins play such an important rôle in the development of the mammalian postcava (rabbit) it may be well, in order to better appreciate the conditions in the marsupials, to first give a comparative sketch of the transformations which these veins undergo in reptiles, birds and the rabbit.

The figures recently published by Miller and Lewis show more clearly than has hitherto been observed the striking parallelism that exists, up to a certain period, between the development of the subcardinal system in reptiles, birds and the rabbit. In the latter stages, however, this parallelism ceases to exist, owing to the divergence from the common ground-plan which occurs in birds and the rabbit in connection with a partial degeneration of the subcardinal veins. For convenience of description, therefore, the transformations which the subcardinal veins undergo will be considered as they occur under the following periods: I. Period of parallelism—(a) before postcava is formed, (b) after postcava is formed; II. Period of divergence.

I. Period of Parallelism.

(a) *Before Postcava is Formed.*—According to Hochstetter, **92**, upon whose investigations the following account of the development of the reptilian venous system is based (Lacerta), the veins which subsequently become the Vv. revehentes anteriores and posteriores of the mesonephroi at first convey blood to these organs. These veins are branches of the caudal vein in Lacerta, but in Tropidonotus arise independently of this vein at the caudal end of the mesonephroi. They consist at first of two bilaterally symmetrical vessels (Text Fig. 1) which lie on the ventromedial side of the mesonephroi (see Hochstetter, **92**,

[5] See reference to Perameles under Résumé and General Considerations (page 223).

Fig. 5, Plate XV), anastomose with the postcardinals, give off branches to the mesonephroi and receive tributaries from the tissue ventral to the aorta.

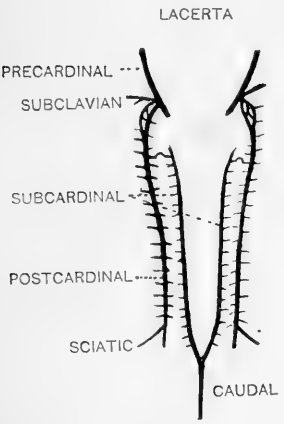

Fig. 1.

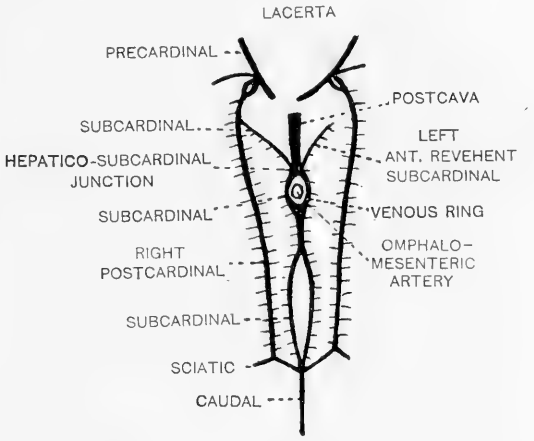

Fig. 2.

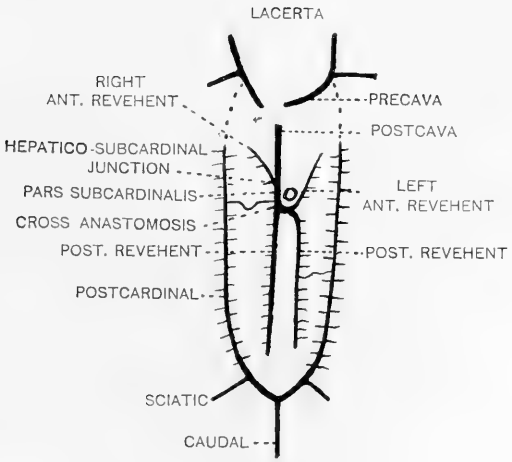

Fig. 3.

Figs. 1, 2 and 3. Diagrams illustrating the development of the veins in Lacerta. After Hochstetter.

At a certain period of development the subcardinal veins of birds (chick, ninety hours) and the rabbit (twelve days and twelve hours) have recently been shown by Miller (03, Figs. 1 and 4) and Lewis (02,

Text Fig. 7 and Figs. 1 and 2, Plate 1), respectively, to attain a high degree of development and to consist, as in reptiles, of two bilaterally symmetrical vessels which hold the same relation to the mesonephroi and postcardinal veins as the subcardinals do in reptiles.

(b) *After Postcava is Formed.*—In connection with the development of the postcava in reptiles the ground-plan of the venous system, as represented by Text Fig. 1, undergoes considerable modification. The proximal or hepatic portion of the unpaired postcava in Lacerta grows caudad from the V. hepatica revehens dextra and, at a point slightly craniad of the origin of the omphalomesenteric artery, anastomoses with both subcardinal veins at a point which, for convenience of description, may be designated as the hepatico-subcardinal junction. The subcardinal veins also anastomose with each other caudad of this artery so that a complete venous ring, ventral to the aorta, is formed about the origin of the omphalomesenteric artery (Text Fig. 2). This condition is only temporary, however, since the anastomosis craniad of the omphalomesenteric artery between the subcardinal of the left side and the hepatic portion of the postcava is not long retained, with the result that the right side of the venous ring (a portion of the right subcardinal) enters into the formation of a portion of the unpaired postcava (pars subcardinalis, Text Fig. 3). Correlated with the above changes the caudal vein (Lacerta) gives up its connections with the subcardinals (Text Fig. 3) and joins the postcardinals so that the latter, after giving up their connections with the ducts of Cuvier, function as the advehent veins of the mesonephroi. The subcardinal veins, on the other hand, through their connection with the unpaired portion of the postcava, function as the anterior and posterior revehent veins of the mesonephroi. The posterior and left anterior revehent veins open into the cross anastomosis between the subcardinals behind the omphalomesenteric artery; while the right anterior revehent vein opens into the unpaired portion of the postcava, somewhat craniad of the anastomosis at the hepatico-subcardinal junction (Text Fig. 3).

A ground-plan of the venous system similar to that last described for reptiles (Text Fig 3) is also met with in the embryos of birds (chick, five days incubation) and the rabbit (thirteen days) as described and figured by Miller (**03**, Fig. 6) and Lewis (**02**, Figs. 3 and 4, Plate 1 and Figs. 5 and 6, Plate 2), respectively. In the case of both the birds (Text Fig. 4) and the rabbit (Text Fig. 6) the subcardinal veins have anastomosed with each other caudad of the origin of the omphalomesenteric artery and the right subcardinal has been "tapped" by the hepatic circulation at the hepatico-subcardinal junction. The subcardinal sys-

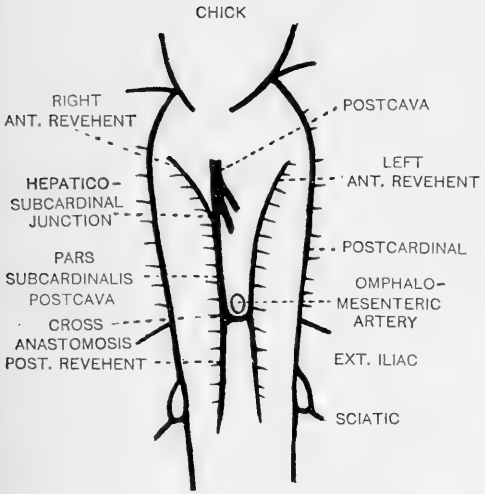

FIG. 4.

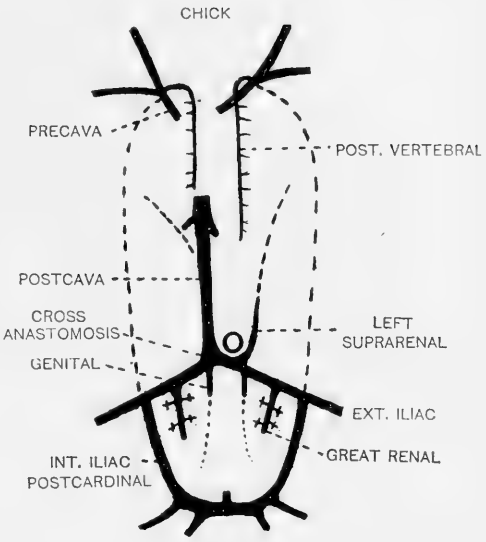

FIG. 5.

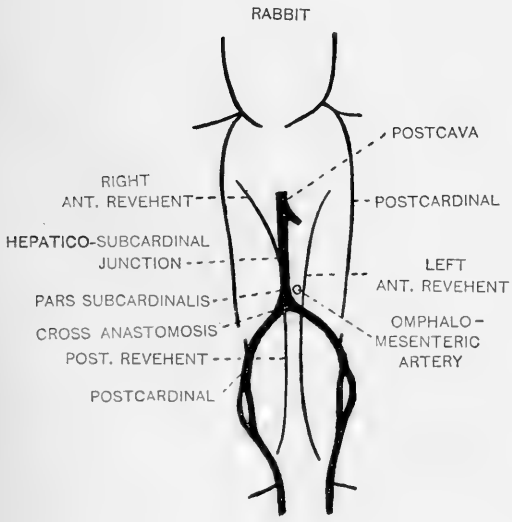

FIG. 6.

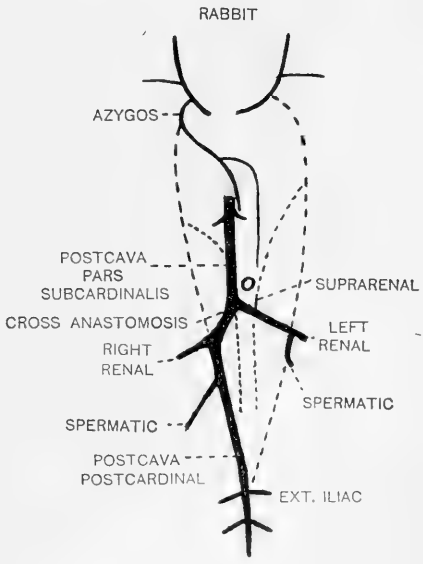

FIG. 7.

FIGS. 4 and 5. Diagrams illustrating the development of the veins in birds. After Hochstetter and Miller.

FIGS. 6 and 7. Diagrams illustrating the development of the veins in the rabbit. After Hochstetter and Lewis.

tem is now represented in birds and the rabbit, as in reptiles, (1) by an anterior and posterior pair of revehent veins which hold the same relations to the unpaired postcava and cross anastomosis between the subcardinals, as the anterior and posterior revehent veins in reptiles; and (2) by a portion of the unpaired postcava (pars subcardinalis) which consists, approximately, of that portion of the right subcardinal vein which is included between the hepatico-subcardinal junction and the cross anastomosis.

It is evident from Hochstetter's figures and description of the veins in Lacerta that the right side of the venous ring which is formed around the origin of the omphalomesenteric artery, and which enters into the formation of the unpaired postcava is derived from the right subcardinal vein. Such being the case, we then have in Lacerta a portion of the unpaired postcava which corresponds in its relations to the subcardinal portion of the postcava described above for birds and the rabbit, since the right side of the venous ring in Lacerta is composed of that portion of the right subcardinal, which is included between the hepatico-subcardinal junction and the original cross anastomosis between the two subcardinals.

Miller, 03, has shown that in chick embryos the subcardinal veins may occasionally, as in reptiles, form a venous ring around the origin of the omphalomesenteric artery and has kindly permitted the writer to publish his reconstruction of the same (Text Fig. 8). Although Miller did not publish this figure in his paper, he described it as follows on page 291: "At about the stage from which Fig. 6 was taken (fifth day of incubation) the writer found a most interesting exception to the general plan of development of the subcardinal system in birds, which exception shows a striking combination of the conditions described by Hochstetter in reptiles and Echidna. Anterior to the origin of the A. omphalomesenterica and ventral to the aorta there is present a large anastomosis between the right and left subcardinals, just caudal to the point where the postcava joins the right subcardinal. Such a remarkable similarity to the conditions found in the earlier stages of reptilian development is certainly unusual." The reference to Echidna mentioned in the above quotation does not refer to the formation of a venous ring about the artery, but to the secondary anastomosis between the subcardinals, as shown in the reconstruction.

There can be no doubt as to the subcardinal character of the right side of the venous ring in Miller's figure of the chick and, also, that it corresponds, in all essential details, to the right side of the ring in Lacerta.

At the stages of development represented by Text Figs. 3, 4 and 6, it is, therefore, seen that the unpaired postcava, as thus far developed, con-

sists in reptiles, birds and the rabbit of two principal subdivisions which are genetically independent of each other; one of which is formed between the sinus venosus and the hepatico-subcardinal junction and the other between the latter and the cross anastomosis between the subcardinals. The latter subdivision is formed in all three cases from a portion of the right subcardinal vein; while the former has a somewhat different

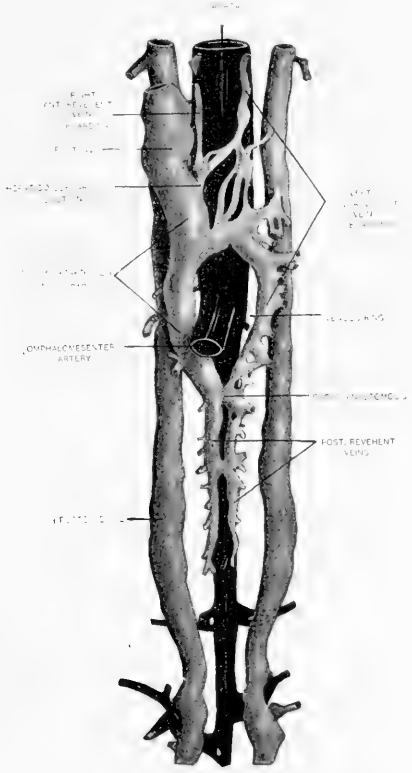

Fig. 8.

Fig. 8. Reconstruction of the venous system of a chick embryo of five days incubation in which the subcardinals form a venous ring about the origin of the omphalomesenteric artery as in Lacerta. After Miller. Ventral view.

origin in reptiles, birds and the rabbit which need not be mentioned here.

There is one feature at this period of development, however, in which the venous system of the rabbit differs from that of reptiles and birds. In the rabbit (Text Fig. 6) the unpaired postcava is connected at its caudal end with each postcardinal vein by means of large anastomoses so that a continuous and uninterrupted channel is established between the

hinder end of the body and the postcava. By this means the blood may flow directly to the heart without passing through the mesonephric circulation, as is the case in reptiles (Text Fig. 3) and the embryos of birds (Text Fig. 4 and 8) in which a renal portal system is present (see Miller, 03, Fig. 6).

II. Period of Divergence.

The final changes which take place in connection with the development of the venous system, subsequent to those represented by Text Figs. 3, 4 and 6, and which lead up to the adult condition are in reptiles, birds and the rabbit somewhat divergent.

In reptiles the fundamental plan of the venous system as represented by Text Fig. 3, is, with slight modifications, retained in the adult. The posterior revehent veins which remain, for the most part, separate in Lacerta and snakes and fuse to form a single vein in turtles (Stromsten, 05) function as the revehent veins of the permanent kidneys. The advehent veins, on the other hand, are formed from the caudal divisions of the postcardinals which, after giving up their connections with the ducts of Cuvier, return blood from the hinder end of the body to the permanent kidneys.

In order to attain the adult condition in birds (Text Fig. 5), in which a renal portal system is absent,[6] the Vv. renales magnæ (the revehent veins of the permanent kidneys) grow caudad from the caudal end of the postcava (pars subcardinalis) and, at the level of the external iliac veins, anastomose with the postcardinals. A continuous channel is thereby established, on each side, between the hinder end of the body and the postcava through which the blood may flow without previously passing through the mesonephric circulation (see Miller, 03, Fig. 7).

The postcardinals which lie craniad of their anastomosis with the great renal veins atrophy, while those which lie caudad of the same fuse at their caudal ends and persist in the adult as the so-called internal iliac veins (Miller). Unless the unpaired portion of the postcava together with the internal iliac veins (postcardinals) may be regarded as representing a type of bifurcated or double postcava, it is evident that the postcardinal veins or any part of the same do not, in birds, as in the rabbit, enter into the formation of the adult postcava, since the latter

[6] Parker and Haswell, 97 (page 375), figure and describe the presence of a partial renal portal system in adult birds (pigeon). This system in the adult, however, differs fundamentally from that in the embryo in that the revehent veins (Vv. renales magnae) are independent formations and are not formed from the subcardinal veins.

terminates in birds at the caudal end of the pars subcardinalis which, in addition to the portion which is developed between the heart and the hepatico-subcardinal junction, constitutes the postcava in the adult.

The subcardinal system is represented in the adult bird by the following veins: The left anterior revehent vein forms the left suprarenal; the right anterior revehent probably atrophies; the section of the right subcardinal included between the hepatico-subcardinal junction and the original anastomosis between the two subcardinals forms the pars subcardinalis of the postcava, and the two posterior revehent veins enter into the formation of the genital veins.

In birds the azygos veins, as met with in the rabbit, are not developed; their place being taken, for the most part, by the newly formed posterior vertebral veins (Text Fig. 5) which open, on each side (chick) into the precava in common with the internal jugular and the subclavian veins (see McClure, 03, page 381).

In the rabbit the fundamental plan of the venous system, as represented by Text Fig. 6, undergoes a number of changes before the adult stage is reached.

The section of the right postcardinal vein which lies caudad of its anastomosis with the pars subcardinalis of the postcava, after forming a collateral channel on the medial side of the ureter (Text Fig. 6), constitutes in the adult that portion of the postcava which lies caudad of the renal veins (Text Fig. 7). The corresponding section of the left postcardinal atrophies with the exception of a small portion at its proximal end which usually persists as the left spermatic vein, and of a portion at its caudal end which fuses with the postcardinal of the opposite side to form the common internal iliac vein. The postcava of the adult rabbit is thus seen to be a compound vessel which is formed from four distinct sets of veins: The vena hepatica communis, the hepatic sinusoids and portions of the right subcardinal and right postcardinal veins.

Correlated with the completion of the adult postcava in the rabbit a number of changes, also take place in connection with the remaining portions of the postcardinal and subcardinal veins. The postcardinals which lie craniad of the level of the renal veins entirely disappear with the exception of the proximal end of the vein of the right side which persists as the common trunk of the newly formed azygos veins. Also, with the exception of a section of the right subcardinal which enters into the formation of the adult postcava, a portion of the left subcardinal which forms the left suprarenal, and possibly a portion of the right which forms the right suprarenal vein (Hochstetter, 03), the subcardinal veins are completely lost at the time the adult stage is reached.

The Development of the Venous System in Marsupials.

All of my marsupial embryos, as stated above, are too advanced to definitely determine the earliest stages in the development of the postcaval vein, as well as the condition presented by the subcardinal veins at a

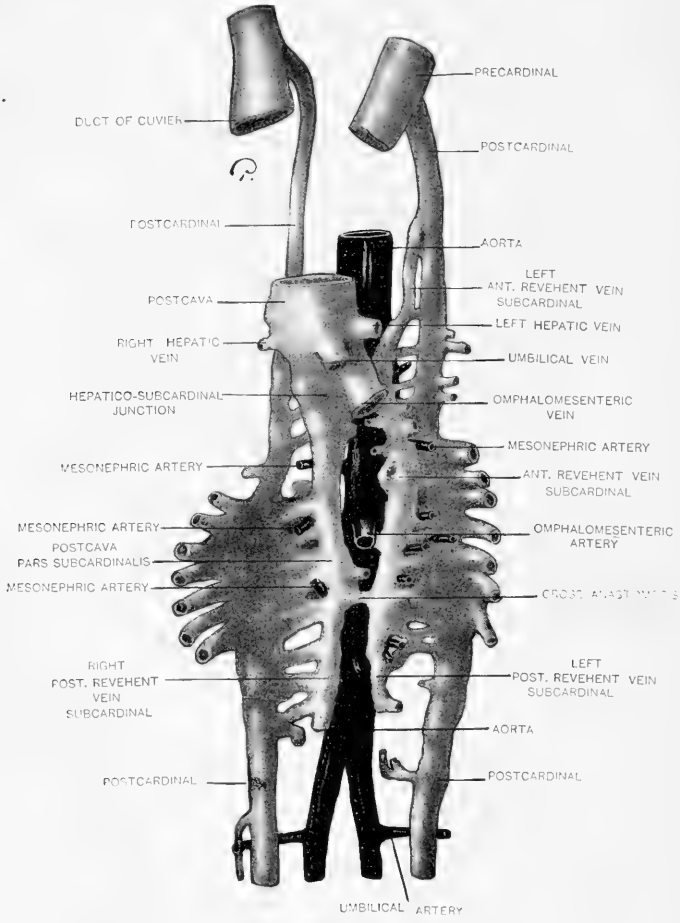

Fig. 9.

Fig. 9. Reconstruction of the venous system of a 6 mm. embryo of Dasyurus. Ventral view.

time before the postcava is formed. There can be no doubt, however, so far as the subcardinal system is concerned, that it plays the same rôle in the marsupials, as thus far examined, as in the rabbit (Text Fig. 6), in which it enters into the formation of a portion of the postcava, as well as

into that of the anterior and posterior revehent veins. This is clearly shown to be the case by the reconstructions of the venous system of the 6 mm. Dasyurus (Text Fig. 9) and 8 mm. Didelphys embryos (Text Fig. 10) in which the ground-plan is fundamentally the same as that described by Lewis, 02 (Plate I, Figs. 3 and 4), for a rabbit embryo of thirteen days, where the right and left subcardinals have anatomosed in the median line, caudad of the origin of the omphalomesenteric artery, and the right subcardinal has been "tapped" by the hepatic circulation. The point at which the right subcardinal vein makes connection with the hepatic circulation is designated by the writer in the following pages as the *hepatico-subcardinal junction.*

On account of the bilateral symmetry of its subcardinal veins the 6 mm. embryo of Dasyurus undoubtedly represents a stage of development which is relatively earlier than that of the 8 mm. embryo of Didelphys, and, for purposes of comparison with the latter, a reconstruction of its venous system has been added to the text (Text Fig. 9).

The Venous System of the 8 mm. Embryos of Didelphys.

The Postcardinal Veins.—In the majority of the 8 mm. embryos of Didelphys (Text Fig. 10) the postcardinal veins can be traced as continuous vessels between the ducts of Cuvier, into which they open dorsally, and the caudal end of the body where they are formed, on each side, through the union of the internal and external iliac veins. Caudal to the origin of the omphalomesenteric artery each postcardinal vein joins the root of the postcava by means of a single large anastomosis. The postcardinals which lie caudad of this anastomosis with the postcava are vessels of large size and constitute its principal, though not direct, caudal continuation; the latter being formed by the right posterior revehent vein (subcardinal). The relation of the postcardinal veins to the umbilical arteries is most complex and will be treated more fully in connection with another topic. It may be mentioned here, however, that the umbilical artery of each side, instead of lying ventral to the postcardinal vein as in most mammalian embryos or dorsal to the same as in Echidna (Hochstetter) and the 6 mm. embryo of Dasyurus (Text Fig. 9), is encircled by a circumarterial venous ring.

Craniad of the anastomosis with the postcava the postcardinals are much reduced in size and slightly caudad of their union with the ducts of Cuvier each postcardinal receives a tributary which can be traced caudad for only a short distance as a continuous vessel. These two tributaries (Fig. 31, Plate II and Text Fig. 10) which lie lateral or dorso-

lateral to the aorta and ventral to its segmental branches, appear to be formed through a longitudinal anastomosis between branches of the postcardinals and undoubtedly represent a portion of the future azygos system of veins.

The Postcava.—The postcava of the 8 mm. embryos of Didelphys

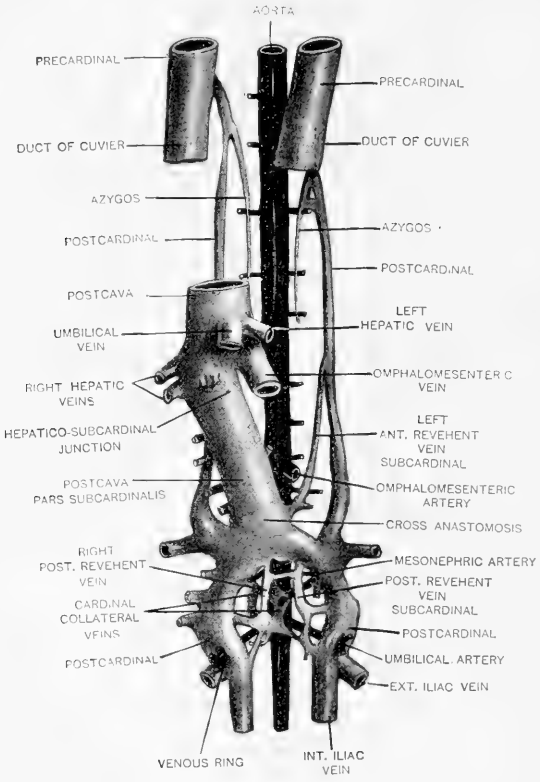

Fig. 10.

Fig. 10. **Reconstruction of the venous system of an 8 mm. embryo of Didelphys. Ventral view.**

(Text Figs. 10 and 11), which represents only a portion of this vein as met with in the adult, is a vessel of relatively greater size than that found in the Dasyurus embryo. It extends as an unpaired vessel between the sinus venosus and a point slightly caudad of the origin of the omphalomesenteric artery where it anastomoses with the right and left postcardinal veins which form its principal caudal continuation, and where it also receives the left anterior and the two posterior revehent veins.

Between the sinus venosus and the point where it receives the right anterior revehent vein (hepatico-subcardinal junction, Text. Fig. 11) the postcava, at its cranial end, occupies a position ventral to the right lung (Fig. 31, Plate II), and further caudad is embedded in the liver. Here it receives the following tributaries (Text Fig. 10): (a) One or two

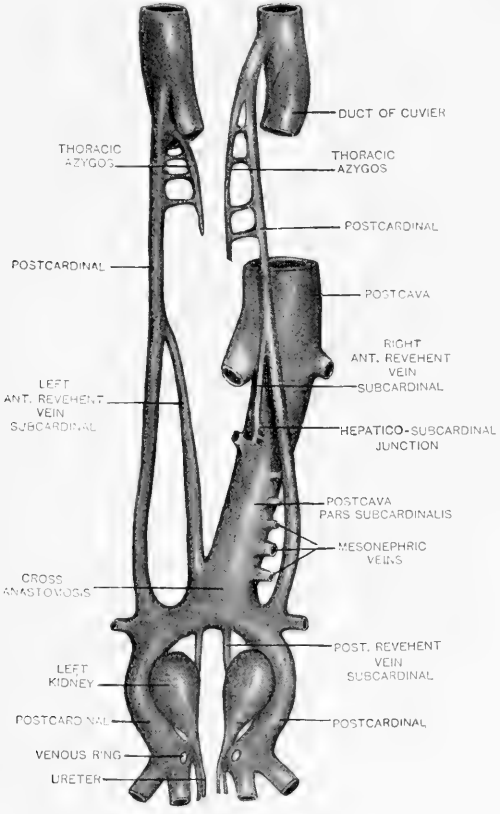

FIG. 11.

FIG. 11. Dorsal view of the venous system of an 8 mm. embryo of Didelphys. Semi-diagrammatic.

hepatic veins from the right side of the liver (V. hepatica revehens dextra); (b) a large hepatic vein from the left side of the liver (V. hepatica revehens sinistra) which opens into the postcava in common with the hepatic continuation of the umbilical veins; (c) the continuation of the omphalomesenteric vein which, after tunnelling the liver, opens into the postcava independently of the umbilical veins and finally, (d) a number of small hepatic veins which open at irregular intervals. Be-

tween the sinus venosus and the hepatico-subcardinal junction (Text Fig. 11) the postcava is without doubt formed, as in the rabbit, independently of the right subcardinal vein. The remaining portion of the unpaired postcava, that section of the vein which lies caudad of the hepatico-subcardinal junction, is formed from the right subcardinal vein with the exception of a portion near the hepatico-subcardinal junction which is partially embedded in the parenchyma of the liver and which is formed from the hepatic sinusoids in conjunction with the right subcardinal vein. The term hepatico-subcardinal junction refers to the most cranial of the anastomoses that may exist between the right subcardinal and the hepatic circulation (Text Fig. 11) since in some cases the section of the postcava which is formed from the hepatic sinusoids and which lies partially embedded in the liver does not fuse along its entire extent, but only at intervals, with the right subcardinal vein. Fig. 34, Plate II, represents the section preceding and Fig. 35, Plate II, a section taken through the hepatico-subcardinal junction in which it is seen that ventrally the postcava is formed by hepatic sinusoids and dorsally by the right subcardinal vein. Slightly caudad of the hepatico-subcardinal junction the postcava lies upon the dorsal surface of the liver (Fig. 36, Plate II), where, as well as caudad of the liver itself (Figs. 37 and 38, Plate III), it occupies the same relative position with respect to the mesonephros and suprarenal body and, with the exception of those from the liver, receives the same class of tributaries as the anterior revehent vein of the left side (left subcardinal). These tributaries are veins from the suprarenal body, the mesonephros, the genital anlage and from the tissue ventral to the aorta. Finally, the pars subcardinalis of the postcava does not, as in Dasyurus, anastomose at intervals along its course with the right postcardinal vein; the absence of such connections being probably correlated with the degeneration of the postcardinal vein.

The Anterior Revehent Veins.—The right anterior revehent vein (Text Fig. 11 and Fig. 34, Plate II) is derived from that portion of the right subcardinal which lies craniad of the hepatico-subcardinal junction. The left anterior vein (Figs. 10 and 11 and Figs. 34, 35 and 36, Plate II, and Fig. 37, Plate III) consists of that portion of the left subcardinal which lies craniad of the anastomosis (Fig. 38, Plate III) between the two subcardinals. In the 8 mm. embryos of Didelphys this anastomosis between the two subcardinals (cross anastomosis) is, as a rule, more extensive and complete than in the 6 mm. embryo of Dasyurus so that the left anterior revehent vein usually has the appearance of opening into the postcava rather than being directly continuous caudad, as in the Dasyurus embryo (Text Fig. 9), with the left posterior revehent vein. In one

of the 8 mm. embryos, however, the fusion between the two subcardinals was not as complete as in some of the others so that the left anterior revehent vein could be traced directly craniad from the left posterior revehent vein (See Text Fig. 12). Both of the anterior revehent veins occupy the same relative position in the embryo with respect to the suprarenal bodies and the mesonephroi and, with the exception of the direct anastomoses with the postcardinals which are wanting, receive the same class of subcardinal tributaries as the corresponding veins in the 6 mm. embryo of Dasyurus.

The Posterior Revehent Veins.—The right and left posterior revehent veins (subcardinals) which lie caudad of the cross anastomosis can, in

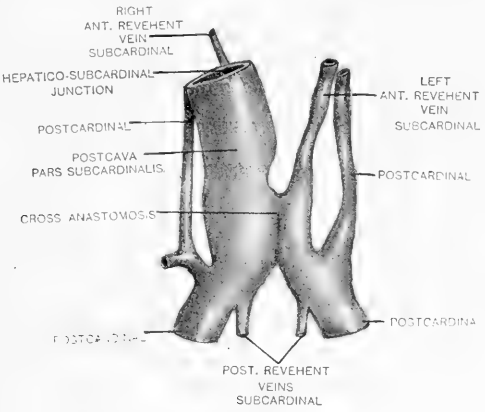

Fig. 12.

Fig. 12. Reconstruction of the venous system of an 8 mm. embryo of Didelphys in the region of the original cross anastomosis between the subcardinals. Ventral view.

most of the 8 mm. embryos, be traced as continuous vessels between the cross anastomosis, into which they open cranially, and the hinder end of the body where they sometimes aid in the formation of the venous rings which encircle the umbilical arteries. Each posterior revehent vein lies ventral to the mesonephric arteries on the medial side of the mesonephros and receives tributaries from the latter as well as from the genital anlage and tissue ventral to the aorta (Fig. 39, Plate III). Each vein also anastomoses at intervals along its course with the postcardinal vein of the same side as well as with a complicated system of vessels which, for the most part, lies dorsal or dorsolateral to it and which I shall describe under the name of the *cardinal collateral* system of veins (Vv. cardinales collaterales).

Correlated with the atrophy of the mesonephroi, the cardinal collateral veins, or veins which are derived from them, assume the function of the postcardinals in returning the blood from the hind limbs and pelvic region to the root of the postcava (pars subcardinalis); and, after fusing ventral to the aorta, constitute the greater portion of the stem of the postcava which is developed caudad of the original cross anastomosis between the subcardinals. From a physiological standpoint the cardinal collateral veins of Didelphys may be said to correspond to that portion of the postcardinal in the cat and rabbit which is formed on the medial side of the permanent kidney and ureter, respectively.

The Cardinal Collateral Veins.—The cardinal collateral veins, as represented in the reconstructions (Text Figs. 10 and 13) and in section (Figs. 40 and 41, Plate III) constitute an extremely complicated system of vessels which, in the 8 mm. embryo, are so irregular in character that it is difficult, at this stage, to assign to them any definite ground-plan arrangement which may be regarded as characteristic of these veins in general.

In some of the 8 mm. embryos examined the cardinal collateral veins appear to be present, for the most part on one side (Text Fig. 10), while in others they approach a bilateral arrangement as represented by Text Fig. 13. They may anastomose in front with the postcardinals (Text Fig. 10, left side) or, as is usually the case, with the root of the postcava as in Text Fig. 13. They may also extend caudad, on each side, parallel to the postcardinals either as single vessels or as a network of vessels which spread out in the space ventral to the aorta as in Text Fig. 13. The cardinal collateral veins often anastomose with each other in the median line ventral to the aorta (Text Fig. 10); they may also form frequent anastomoses with the postcardinal and posterior revehent (subcardinal) veins and, on being traced caudad, appear, in some cases to be directly continuous with that portion of the circumarterial venous ring which encircles the umbilical artery ventrally (Text Fig. 13, left side). In a few cases the cardinal collateral veins could be traced for a short distance caudad of the circumarterial venous rings where they appeared to terminate in capillary vessels (Text Fig. 13).

The question as to origin of the cardinal collateral veins is difficult of solution and with the material at hand impossible to determine definitely. They do not, however, appear to be formed through a longitudinal anastomosis between the dorsal somatic branches of the postcardinals, but rather through a longitudinal anastomosis between the cross connections which exist between the post and subcardinal veins.

Having considered the postcardinal, cardinal collateral and posterior

revehent (subcardinal) veins of the 8 mm. embryo of Didelphys, we are now in a position to consider the circumarterial venous rings or loops which encircle the umbilical arteries.

The Circumarterial Venous Rings.—It has been stated above, as well as in a preceding paper (McClure, 02), that in the 8 mm. embryos of Didelphys the umbilical arteries, instead of lying ventral to the postcardinal veins, as in most mammals, or dorsal to the same, as in Echidna

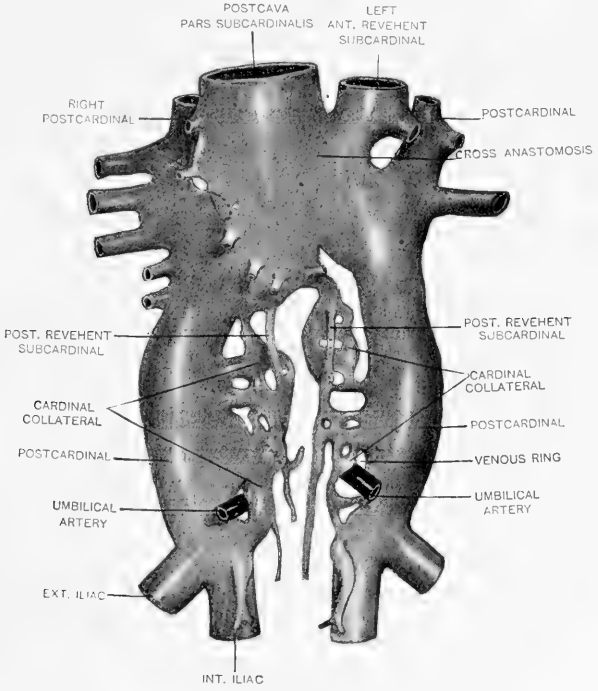

Fig. 13.

Fig. 13. **Partial reconstruction of the venous system of an 8 mm. embryo of Didelphys showing the cardinal collateral and posterior revehent veins and the venous rings which encircle the umbilical arteries. Ventral view.**

(Hochstetter) and Dasyurus (Text Fig. 9), are encircled near their origin by complete circumarterial venous rings. These venous rings are situated slightly craniad of the point of junction of the external and internal iliac veins, and, so far as their general make-up is concerned, are extremely variable in character, not only in the different embryos, but even upon opposite sides of the same individual. Two main types of venous rings may be distinguished:—One in which the portion of the ring which

encircles the umbilical artery ventrally possesses a caliber either subequal with or greater than that which encircles it dorsally, as in Text Fig. 10 and Fig. 42, Plate III; the other, in which the portion of the venous ring which encircles the umbilical artery ventrally possesses a smaller caliber than that which encircles it dorsally as in Text Fig. 13 and Fig. 41, Plate III. The latter type of ring is by far the more common of the two since, with one exception, it was characteristic of all the 8 mm. embryos examined.

As regards the veins which enter into the formation of these venous rings there is also considerable variation and, in some cases, it is quite impossible to determine definitely how these rings are formed. In all of the rings the portion which encircles the umbilical artery dorsally is formed by the postcardinal vein. The portion of the ring which encircles the umbilical artery ventrally, however, may be formed exclusively by the cardinal collateral vein as in Text Fig. 13 (left side), or by a vein which appears to be formed as the result of a fusion between the cardinal collateral and posterior revehent (subcardinal) veins. In addition to the above, in one embryo (Text Fig. 10) the circumarterial venous rings appear to be formed exclusively by the postcardinal veins, although it is impossible to determine in this case to what extent the cardinal collateral veins may have also entered into their formation.

The variable character of these circumarterial venous rings foreshadows the unusual variations recently described by the writer in Part I of this paper as regularly occurring in the adult, and the relationship which exists between the two will be considered in connection with another topic.

The presence of circumarterial venous rings about the origin of the umbilical artery is not so uncommon as is generally supposed to be the case. The writer has recently observed these rings in the embryos of a lizard (Sceloporus undulatus). Hochstetter, **88**, and Miller, **03**, have observed them in the embryos of the chick and the English sparrow (Passer domesticus), respectively, and Lewis, (**02**, Figs. 7 and 8, Plate 2) has recently figured them as occurring in a rabbit embryo of 14.5 mm. in length. In Sceloporus the portion of the ring which encircles the umbilical artery dorsally disappears before the adult condition is reached, while in birds, as well as in the rabbit, it is the ventral portion of the ring that atrophies. In Sceloporus and birds the portion of the ring which is not formed from the postcardinal vein appears to be formed through a longitudinal anastomosis of the somatic branches of the postcardinals, while in the rabbit it appears from Lewis' figures as if it might be formed from the subcardinal vein. Whatever the case may be, I am

inclined to believe that some of the abnormalities which are met with in adult mammals, in which the internal iliac artery passes through a foramen in the common iliac vein, as described by Treadwell, 96, McClure, 00, (1) and Weysse, 03, may be accounted for on the ground that they represent instances in which these embryonic circumarterial venous rings have persisted in the adult.

Up to this point we have considered more or less in detail the general plan of the venous system as met with in the 8 mm. embryo of Didelphys and, since the latter represents the youngest stage of Didelphys possessed by the writer, its venous system may be taken as the starting point from which may be traced the subsequent transformations that lead up to the adult condition. From now on, therefore, and beginning with the 8 mm. embryo of Didelphys, we will trace in a connected manner through the different stages of embryos and pouch young possessed by the writer the transformations which the different portions of the venous system undergo before arriving at the adult stage.

The Azygos Veins.

In the adult of Didelphys there is, as a rule, but one azygos vein present and that is situated on the left side (Fig. 28, Plate I). At its cranial end it opens into the left precava about opposite the head of the third rib, while at its caudal end it invariably joins the postcava caudad of the renal veins and about opposite the second lumbar vertebra. Between its point of union with the postcava and about the middle of the tenth thoracic vertebra, the left azygos vein lies dorsal to the segmental branches of the aorta; between the tenth thoracic vertebra and its connection with the precava, however, it lies ventral to these branches (see McClure, 03, pp. 381-2 and Fig. 28, Plate I, at the end of this paper).

The right azygos vein, when present in the adult, opens into the precava about opposite the head of the second rib. It is always a small and insignificant vessel, and its tributaries are confined to the first five intercostal spaces of the right side.

In the 8 mm. embryo of Didelphys, as stated above, each postcardinal receives a tributary slightly caudad of its junction with the duct of Cuvier (Text Figs. 10 and 11). Each tributary, which can be traced caudad for only a short distance, lies lateral or dorsolateral to the aorta (Fig. 31, Plate II) and ventral to the latter's segmental branches. These two tributaries, as stated above, which appear to be formed through a longitudinal anastomosis between the somatic branches of the postcardinals, together with the proximal ends of the two postcardinals, undoubtedly constitute the anlages of the right and left azygos veins.

It is a curious fact that the azygos veins are more advanced in development in the 11.5-12 mm. embryos than in the youngest of the pouch young studied by the writer (10.5 mm.). This circumstance clearly proves that the opossums are not born, in all cases, at a corresponding period of development, but that some are born at a more advanced stage than others. In order, therefore, to give a connected account of the development of the azygos system it will be necessary to describe the conditions as met with in the 11.5-12 mm. embryos after those of the youngest pouch young have been considered.

In the 10.5 mm. pouch young two azygos veins are present in the thoracic region which, as in the 8 mm. embryo, open dorsally into the ducts of Cuvier, the opening of the right vein being somewhat craniad of that of the left. These two veins, as stated above, are formed from the cranial ends of the two postcardinals as well as from veins which have united with the latter and which have probably been formed through a longitudinal anastomosis between the somatic branches of the postcardinals.

The right azygos can be traced caudad from its connection with the duct of Cuvier for about 89 sections where it appears to terminate as a small capillary vessel which lies on the ventral surface of the vertebral column. The left azygos is, however, of much greater extent and can be traced caudad as a continuous vessel for about 156 sections where it then appears to terminate in the region slightly caudad of the point where the omphalomesenteric vein enters the liver. Each azygos vein, along its entire extent, lies dorsolateral to the aorta and *ventral* to the segmental arteries and, at intervals along its course, receives tributaries from the body walls contiguous to the vertebral column (Fig. 43, Plate III). Somewhat caudad of the apparent termination of the left azygos vein (37 sections) small capillary vessels are met with which lie in the tissue dorsal and dorsolateral to the aorta and dorsal to the segmental arteries, which become more prominent near the origin of the omphalomesenteric artery and especially so, further caudad, in the neighborhood of the permanent kidneys (Fig. 46, Plate IV). These vessels can be traced without difficulty caudad of the anastomosis between the pars subcardinalis and the postcardinal veins where they form frequent anastomoses with vessels which lie in the tissue ventral to the aorta (Figs. 47 and 48, Plate IV). These latter or ventral vessels with which they anastomose (Figs. 47 and 48, Plate IV) are, in my estimation, representatives of the cardinal collateral veins which have been described above in connection with the 8 mm. embryos. They can be traced caudad almost as far as the origin of the umbilical arteries, but whether they join the postcardinal veins at their caudal ends I am unable to determine definitely.

The separation of the azygos system into two subdivisions (thoracic and lumbar) is a marked feature at this stage of its development. The separation may, however, be apparent rather than actual since a capillary anastomosis may exist between the two which cannot be determined in section. In later stages the two subdivisions do become connected so that the blood from the lumbar azygos tributaries is returned to the heart, for the most part, by the left thoracic azygos vein. In the 10.5 mm. pouch young, however, the large size of the azygos veins in the lumbar region precludes the possibility of any such route for all of the blood collected by them; and I am, therefore, inclined to believe that it is returned, for the most part, through capillaries directly to the postcava, which is the course pursued at a subsequent stage of development in which large and frequent anastomoses are formed between this vessel and the lumbar azygos veins.

In the 8 mm. embryo the azygos veins of the lumbar region have not as yet been formed and this region is drained by the dorsal somatic tributaries of the postcardinal veins. This circumstance leads one to infer that the lumbar azygos veins as met with in the 10.5 mm. pouch young may also be formed from branches of the postcardinals and in the same manner as a portion of the azygos veins in the thoracic region, although, on account of the lack of intermediate stages, it is impossible to determine this question.

The azygos veins in the 11.5 mm. pouch young appear to present the same arrangement as in the preceding stage, although on account of the circumstance that the specimen was cut along the frontal plane it is difficult to determine the exact extent of the thoracic azygos veins, as well as whether a direct anastomosis exists between them and the azygos veins of the lumbar region. There can be little doubt, however, if such an anastomosis exists that it is still of minor importance as compared with that at a later stage, and that the thoracic and the lumbar azygos veins are, as in the 10.5 mm. pouch young, practically independent of each other.

At the caudal end of the body the sections are cut almost at right angles to the long axis of the body so that in this region the lumbar azygos veins are not difficult to follow.

In the region of the permanent kidneys and craniad of the point where the left anterior revehent vein joins the postcava (Fig. 49, Plate IV) the lumbar azygos veins are extremely prominent and lie, for the most part, dorsal to the segmental branches of the aorta. Between its junction with the left anterior revehent vein and that with the two postcardinals, the postcava gradually approaches the aorta and in the region

dorsal to this section of the postcava the lumbar azygos veins send tributaries into the tissue ventral to the aorta. These tributaries, if they do not already anastomose by means of capillaries with the postcava are at least preparing to do so, since direct anastomoses between these two veins are of constant occurrence in this region in more advanced stages.

Slightly caudad of the junction of the two postcardinals with the postcava the lumbar azygos system anastomoses with the left postcardinal vein(Fig. 50, Plate IV) and further caudad becomes continuous with small vessels (cardinal collaterals, Fig. 51, Plate IV) which lie, one on each side, ventrolateral to the aorta between the aorta and the ureter, and which frequently anastomose with each other ventral to the aorta (Fig. 52, Plate IV). As in the case of the 10.5 mm. pouch young, I have been unable to establish a connection between the caudal ends of these vessels and the postcardinal veins.

In the 11.5-12 mm. embryos the permanent kidneys have not migrated as far forward as in the 11.5 mm. pouch young. The postcardinal veins present the same arrangement as in the 10.5 and 11.5 mm. pouch young and still form the principal route by means of which the blood reaches the root of the postcava from the mesonephroi, the hind limbs and pelvic region. The azygos system, however, appears to be more highly developed in these embryos than in either the 10.5 or 11.5 mm. pouch young, a circumstance which has already been mentioned.

The right and left azygos veins in the thoracic region open, as in the preceding cases, into the ducts of Cuvier. Instead, however, of terminating blindly at their caudal ends, as in the 10.5 mm. pouch young, they, or at least the vein of the left side, are now directly continuous with the lumbar azygos veins so that a continuous chain of veins can be traced from the ducts of Cuvier to the hinder end of the body. The junction of the left anterior revehent vein with the postcava still forms a prominent landmark at this stage of development, and the section of the unpaired postcava which lies caudad of this junction has become somewhat elongated and anastomoses freely with the lumbar azygos veins (Fig. 54, Plate V). Also, slightly caudad of the root of the postcava, the lumbar azygos veins, by means of ventral prolongations, anastomose with the postcardinals (Fig. 55, Plate V), and then become directly continuous with the cardinal collateral veins which lie ventrolateral and ventral (Fig. 56, Plate V) to the aorta between the two postcardinals.

The azygos veins of the thoracic region still occupy a somewhat different position from that occupied by the azygos veins of the lumbar region. In the thoracic region they lie ventral to the segmental branches of the aorta, while in the lumbar region they lie, for the most part, dorsal to these branches.

The cardinal collateral veins in the 11.5-12, as in the 8 mm. embryos, constitute an extremely complicated system of vessels which lie in the tissue ventral and ventrolateral to the aorta between the postcardinal veins (Fig. 56, Plate V). Here they form frequent anastomoses with the postcardinals and with veins which occupy the position of the posterior revehent veins (subcardinals). They, also, at their caudal ends, join the postcardinals and appear, as in the 8 mm. embryos, to form the ventral portions of circumarterial venous rings which encircle the origins of the umbilical arteries.

In the 14 and 15 mm. pouch young of Didelphys the azygos veins show a marked advance in their development over that met with in the preceding stages; an advance which is undoubtedly correlated with the degeneration of the mesonephroi and the mesonephric divisions of the postcardinal veins.

A right and a left azygos vein are present in the thoracic region. The vein of the right side is small in caliber and, on being traced caudad, appears to terminate in the thoracic region. The vein of the left side possesses a large caliber at its cranial end, but gradually diminishes in size from before backward where, as a rule, it becomes directly continuous with a single azygos vein of the lumbar region. Although a direct anastomosis is established between the azygos veins of the lumbar and thoracic regions the character of the connection is such that the two systems, even at this stage of development, are, as in the preceding stages, practically independent of each other.

The right thoracic azygos along its entire extent lies ventral to the segmental branches of the aorta. The left thoracic azygos, for a portion of its course, occupies the same relative position, but, at the caudal end of the thoracic cavity, where it becomes continuous with the lumbar azygos vein, it lies dorsal instead of ventral to these arteries.

The lumbar azygos system is represented by a single vein which lies dorsal to the segmental branches of the aorta. It increases in size from before backward and in places frequently possesses a caliber as large as that of the postcava (Fig. 57, Plate V). The renal veins have both been formed; the vein of the left side taps the left anterior revehent vein near its point of junction with the postcava, as will be described further on. Caudad of the renal veins and between the latter and the junction of the postcava with the two postcardinals, the lumbar azygos vein anastomoses in a number of places directly with the postcava, so that the blood collected by its tributaries is now returned to the heart by the postcava. The anastomoses which are formed between these two veins are extremely variable in their character, since they may be formed on

the right, the left, or on both sides of the aorta (Fig. 58, Plate V); a circumstance which, we will see later, accounts for the variations in connection with the lumbar veins, as well as the variable manner in which the caudal end of the azygos may join the postcava in the adult (McClure, 03, p. 382). One of these anastomoses invariably occurs slightly caudad of the renal veins and another at the junction of the postcava and the two postcardinal veins and between these two some other anastomoses are met with which are apparently more or less variable in character.

Remarkable changes, as will be more fully described later on, have also taken place in connection with the cardinal collateral veins. These veins, at their cranial ends, anastomose with the root of the postcava (pars subcardinalis) and at their caudal ends with the postcardinals; and, in correlation with the atrophy of the mesonephric divisions of the postcardinals (Urnierennabschnitt) have so increased in size that in most of these pouch young they now return to the postcava practically all of the blood collected by the tributaries of the external and internal iliac veins. The original mesonephric divisions of the postcardinals now function chiefly as mesonephric veins (Urnierenvenen) which return blood from the mesonephroi to the pars subcardinalis of the postcava.

In the 17 mm. pouch young the arrangement of the venous system is essentially the same as in the adult, so far as the completion of the postcava and the formation of the azygos system are concerned.

The cardinal collateral veins now receive all of the blood collected by the tributaries of the external and internal iliac veins and constitute that portion of the unpaired postcava which lies in the adult caudad of the spermatic veins. The mesonephroi, although more atrophied than in the 14 and 15 mm. pouch young, are still functional and are connected with the body walls by means of extremely narrow mesenteries. The mesonephric divisions of the postcardinals have entirely ceased to be continuous vessels and are now represented by small veins which return the blood from the mesonephroi to the postcava.

The changes, about to be described, that have taken place in connection with the azygos veins are without doubt correlated with the atrophy of the mesonephric divisions of the postcardinal veins and the consequent completion of the postcava.

In the 17 mm. pouch young the lumbar azygos vein now forms with the thoracic azygos of the left side a single, continuous vessel of considerable size which extends between the left precava and a point slightly caudad of the renal veins where, in the pouch young at hand, it joins the postcava on the *right* side of the aorta. Caudad of this point of junction with the postcava the lumbar azygos now ceases to be a continu-

ous vessel, and the blood from this section of the lumbar region is returned, as in the adult, directly to the postcava, by means of lumbar veins.[7] These lumbar veins are undoubtedly formed through the persistence of the anastomoses that were formed at an earlier stage between the continuous lumbar azygos vein and the postcava. As these anastomoses may occur on either side or on both sides of the aorta, we find in them an explanation of the variations which were described in Part I of this paper in connection with the lumbar veins of the adult (p. 387), as well as those concerning the manner in which the caudal end of the azygos may join the postcava (p. 382).[8]

The left azygos vein of the 17 mm. pouch young occupies exactly the same relative position with respect to the segmental branches of the aorta as in the adult (see Plate I, Fig. 28), in which along its cranial half it lies ventral and along its caudal half it lies dorsal to the segmental arteries of the left side. The transition from the ventral to the dorsal position takes place at the caudal end of the thoracic cavity (about opposite the 10th thoracic vertebra in the adult) at a level which probably marks the embryonic point of union of the two originally separate components of the left azygos channel. It is, therefore, evident that the original positions occupied in the pouch young by the azygos veins of the thoracic and lumbar regions are retained in the adult.

A right azygos vein which opens into the right precava is also present in the 17 mm. pouch young. It is a small and insignificant vessel which lies ventral to the segmental branches of the aorta and which is confined to the anterior portion of the thoracic cavity.

It is an interesting fact that the right azygos vein is apparently a con-

[7] In four of the five adult specimens of Petrogale penicillata recently examined by the writer two essentially independent subdivisions of the azygos system were met with similar to those described above for the pouch young of Didelphys. The thoracic region was drained chiefly by a right thoracic azygos vein, while the lumbar region was drained, for the most part, by a single continuous vein which extended forward, dorsal to the aorta, and which opened into the postcava near the opening into the latter of the renal veins. The lumbar azygos vein, by means of slight connections, anastomosed with the postcava at intervals along its course and also received the lumbar veins.

[8] The lumbar veins of the adult may open into the postcava either in pairs or, on either side of the aorta, by means of a common trunk. The caudal end of the azygos vein of the adult may join the postcava either to the left, which is the usual method, or to the right of the aorta; or it may bifurcate into two branches on the ventral circumference of the aorta which join the postcava on the right and left side of the aorta, respectively. In this case the aorta is encircled by a venous ring formed by the azygos and the postcava.

stant character up to a late period of development, while in the adult its presence is an exception. Its constancy during the developmental period may account, however, for the fairly large percentage (30 per cent) of cases observed by the writer in which the vein was present in the adult, but in which it was extremely variable in character and confined to the first few intercostal spaces (McClure, **03**, p. 383).

A connection between the caudal end of the azygos vein and the postcava is apparently not of constant occurrence among all adult marsupials. In Phalangista, Beddard, **95**, found such a connection in only one of several individuals examined, and in this case, on account of its large size, it practically took the place of the postcava.

The writer's interpretation of the azygos and cardinal collateral veins seems the one best fitted to the conditions met with in the pouch young at hand, although it is possible that it might be slightly modified if some of the material studied were in a better state of preservation. The cardinal collateral veins of Didelphys may possibly be regarded by some as corresponding to the derivative of the postcardinal veins which is formed in the embryos of some of the higher mammals on the dorsomedial side of the ureters (rabbit) and permanent kidneys (cat). I cannot accept this view, however, for the reasons that the cardinal collaterals occupy an entirely different position with respect to the aorta (Fig. 56, Plate V), and also appear to have a different mode of origin than the derivative of the postcardinal veins in question.

The Completion of the Postcava.

Up to and including the stages of development represented by the 11.5-12 mm. embryos and the 11.5 mm. pouch young the unpaired postcava as met with in the adult is as yet incomplete, since the portion which forms the caudal continuation of the pars subcardinalis has not been fully established. In the 8 and 11.55-12 mm. embryos, as well as in the 10.5 and 11.5 mm. pouch young, the postcava consists of an unpaired vessel which extends between the sinus venosus and a point in the lumbar region where it anastomoses with the two postcardinal veins which still form its principal caudal continuation. This unpaired portion of the postcava consists embryologically of two independent divisions: One formed between the sinus venosus and the hepatico-subcardinal junction, in a manner yet to be determined; the other between the hepatico-subcardinal junction and the junction between the postcava and the postcardinals which is formed in part by the hepatic sinusoids, but chiefly by the right subcardinal vein. The unpaired postcava, as thus formed, receives most of the blood collected by the tributaries of the external and

internal iliac veins, as in the 8 mm. embryo, through the mesonephric divisions of the postcardinal veins.

From a functional standpoint the mesonephroi may be said to be at the height of their development as long as the mesonephric divisions of the postcardinals retain the function of returning most of the blood to the pars subcardinalis which is collected by the tributaries of the external and internal iliac veins.

In the 14 and 15 mm. pouch young there is a noticeable degeneration of the mesonephroi, and correlated with this also a degeneration of the

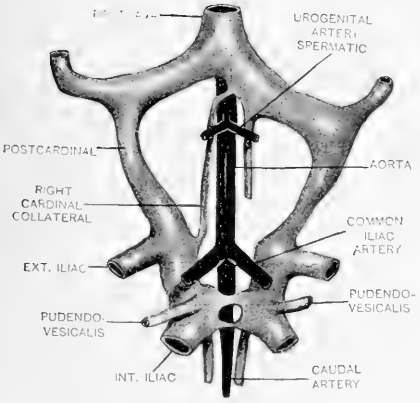

Fig. 14.

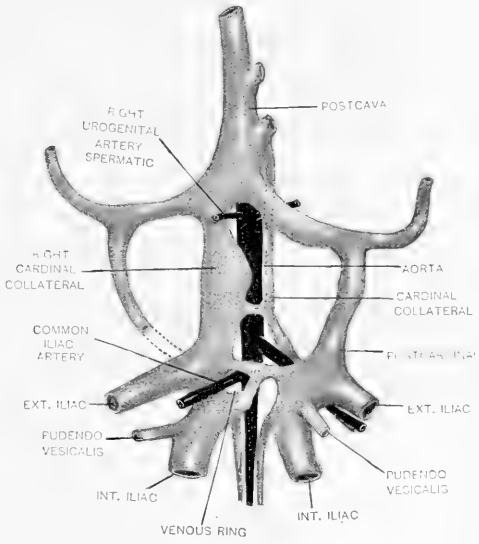

Fig. 15.

Fig. 14. Reconstruction (slightly schematic) of the venous system of a 14 mm. pouch young of Didelphys in which a postcava of Type II is already established. Ventral view.

Fig. 15. Reconstruction (slightly schematic) of the venous system of a 14 mm. pouch young of Didelphys in which a postcava of Type III, *A* is already established. Ventral view.

mesonephric divisions of the postcardinals. As a result of this degeneration the blood from the hind limbs and the pelvic region is directed toward the pars subcardinalis of the postcava through the cardinal collateral veins; while the mesonephric divisions of the postcardinals (Urnierenabschnitt) retain the function of returning the blood from the mesonephroi and constitute the mesonephric veins (Urnierenvenen).

The correlation which exists between the atrophy of the mesonephric

divisions of the postcardinal veins and the establishment of this new section of the postcava (cardinal collateral) is clearly illustrated by the following transverse sections (Figs. 59, 60 and 61, Plate V) and reconstructions of the venous system (Text Figs. 14, 15, 16, 17 and 18) of the 14 and 15 mm. pouch young.

In the case represented by Text Fig. 14, the mesonephric divisions of the postcardinals are still, as in the 11.5 mm. pouch young, the chief channels through which the blood reaches the pars subcardinalis from the hind limbs and hinder end of the body. This condition is unusual for this period of development and represents the only instance met with among the 14 and 15 mm. pouch young, in which both of the postcardinal veins function in such a manner. An advance in development over that in the preceding stages is evident, however, since the cardinal collateral of the right side has made a connection at its caudal end with the postcardinal vein and the postcardinals have anastomosed with each other ventral to the caudal artery. In all probability the cardinal collateral of the left side also joins its corresponding postcardinal, although I am unable to establish definitely such a connection, on account of the vessel not being filled with blood at its caudal end.

Text Figs. 15, 16 and 17 represent examples of unilateral atrophy in which, in one case the left (Text Figs. 16 and 17), and in the other the right (Text Fig. 15) mesonephric division of the postcardinal has atrophied, and in which there is an hypertrophy of the corresponding cardinal collateral vein. In each case the atrophied postcardinal now functions as a vein which returns blood to the pars subcardinalis solely from the mesonephros (Urnierenvenen) and not, as hitherto, from the hind limb and hinder end of the body.

Finally, Text Fig. 18 represents a case in which the postcardinals have atrophied on both sides, with the result that both of the cardinal collateral veins collect all of the blood from the tributaries of the external and internal iliac veins and constitute the caudal end of the postcava, while both of the postcardinals now function solely as veins of the mesonephroi (Urnierenvenen).

It has been stated in Part I of this paper (p. 398) that in 42 of the 101 adult opossums examined by the writer the postcava was either bifurcated as far craniad as the level of the internal spermatic veins, or otherwise presented some indication of an incomplete fusion between the two vessels (cardinal collateral) which form the postcava caudal to the level of the internal spermatic veins; also, that in some of the adults, either one or both of the posterior internal spermatic arteries were found to pass between the two divisions of the postcava or through a foramen

in the same (McClure, 03, Plates I, II and IV). The presence of a bifurcated postcava in the adult is easily explained on embryological grounds as the result of a non-fusion of the two veins (cardinal collateral) which normally form the postcava caudal to the spermatic veins. Text Figs. 14, 15, 16, 17 and 18 clearly show how variable the character

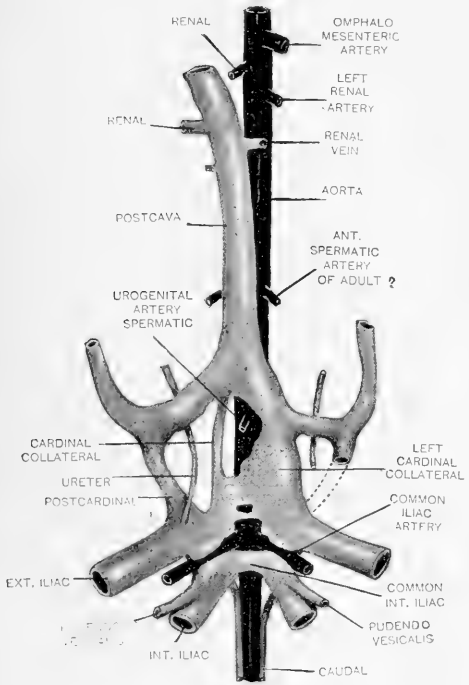

Fig. 16.

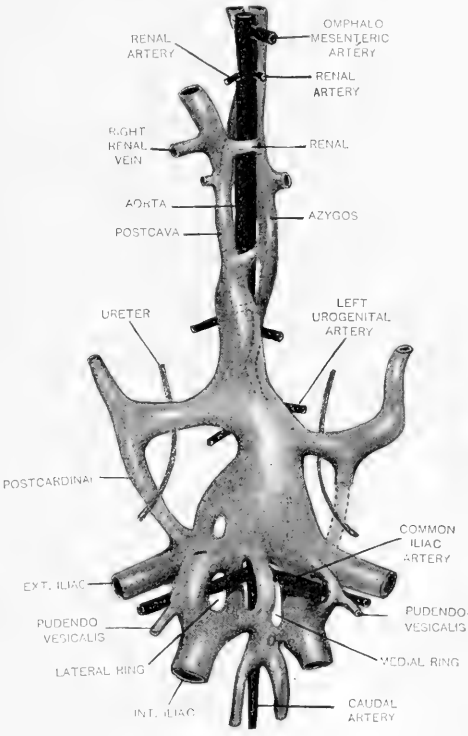

Fig. 17.

Fig. 16. Reconstruction (slightly schematic) of the venous system of a 15 mm. pouch young of Didelphys in which a postcava of Type II is already established. Ventral view.

Fig. 17. Reconstruction (slightly schematic) of the venous system of a 15 mm. pouch young of Didelphys in which a postcava of Type III, *B* is already established. Ventral view. This figure also shows the ventral portions of the lateral rings into which the Vv. pudendovesicales open.

of the fusion may be between the two cardinal collateral veins even before the adult stage is reached, as well as the formation of the foramina, through which the posterior internal spermatic arteries pass.

In the 17 mm. pouch young the cardinal collateral division of the

postcava is fully established and the postcardinals now function as veins which return blood to the postcava from the still functional mesonephroi and the anlages of the genital glands.

The Migration of the Permanent Kidneys and the Development of the Renal Veins.

After the permanent kidneys have completed their forward migration in the embryos of the rabbit and cat, renal veins are developed which open into the postcava, approximately, at the level at which the latter joins the two postcardinal veins (cross anastomosis, Text Fig. 7). When

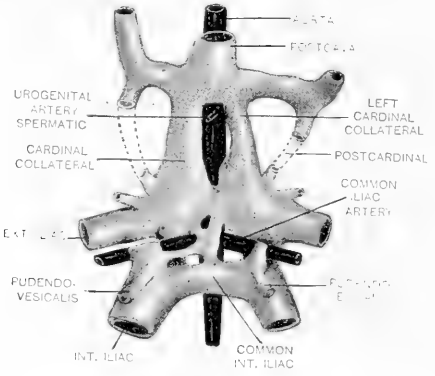

Fig. 18.

Fig. 18. Reconstruction (slightly schematic) of the venous system of a 15 mm. pouch young of Didelphys in which a postcava of Type III, *B* is already established. Ventral view.

a double or bifurcated postcava is met with in an adult rabbit or cat the bifurcation reaches, approximately, as far forward as the level at which the renal veins open into the postcava.

In Didelphys, on the other hand, after the permanent kidneys have completed their forward migration, renal veins are developed which open into the postcava some distance craniad of the latter's junction with the two postcardinal veins (Text Fig. 17). This circumstance now explains why the postcava of the adult opossum was never found by the writer (Part I, page 398) to be bifurcated as far forward as the level at which the renal veins opened into the postcava. The position of the renal veins in Didelphys with respect to the junction of the postcardinal veins and the postcava, does not appear to indicate that the permanent kidneys in Didelphys have undergone, relatively, a more extensive

migration than in the rabbit and cat, since the renal veins in Didelphys, as in the embryos of the rabbit and cat, open into the postcava only slightly caudad of the origin of the omphalomesenteric artery and contiguous to the junction of the postcava and the left anterior revehent vein (subcardinal). The opening of the latter vein into the postcava, however, lies relatively much further craniad of the junction of the postcava and postcardinal veins in the pouch young, than is the case in the 8 mm. embryos of Didelphys and the embryos of the rabbit and cat. This circumstance I can only account for on the basis that the junction of the postcardinal veins with the postcava remains, more or less, as a fixed point, in front of which the vessels elongate more rapidly than those that lie behind. This growth in length, so far as the postcava is concerned, principally affects that portion of the vein which lies between its junction with the two postcardinals and that with the left anterior revehent vein. Strictly speaking, this portion of the postcava corresponds to the original cross anastomosis between the two subcardinals[9] (pars subcardinalis of postcava and left anterior revehent vein; see Text Figs. 10 and 12).

A comparison of Text Figs. 11 and 17 gives a clear idea of the relative positions occupied by the permanent kidneys during their migration and after it is completed. The permanent kidneys are not represented in Fig. 17, but the renal veins sufficiently indicate their position.

In the 11.5 mm. pouch young a renal vein was met with for the first time. Here a left renal vein was present, which opened into the postcava in common with the left anterior revehent vein (Fig. 49, Plate IV). Both renal veins were present in the 14 and 15 mm. pouch young.

In the adult opossum the left suprarenal body lies in close contact with the left renal vein (Fig. 28, Plate I) so close, in fact, that it is impossible to speak of the existence of a left suprarenal vein. On account of the intimate relation, therefore, which exists betwen the left renal vein and suprarenal body, it appears probable that the slight venous connections between the two have been derived, as in the rabbit, from the left anterior revehent vein.

[9] Hochstetter (1893, Taf. XXIII, Fig. 25) suggests a somewhat similar explanation for the development of that portion of the unpaired postcava in Dasypus novemcinctus which lies, ventral to the aorta, between the renal veins and the origin of the posterior mesenteric artery. Referring to this portion of the postcava, he says (page 621): "—— das der zwischen Mündüng der Nierenvenen und der Theilung in die beiden hinteren Hohlvenen gelegene Abschnitt der V. cava posterior einem stärkeren Wachsthum der Lendenwirbelsäule seine Entstehung verdanke."

During the migration of the kidneys the ureters also undergo certain changes in position. In the 8 mm. embryo they lie dorsal to the cardinal collateral and subcardinal veins along their entire extent and are not, at this period, so far as the writer can observe, encircled by venous loops. In the 14 and 15 mm. pouch young, on the other hand, at least in those cases in which the mesonephric divisions of the postcardinals have not yet atrophied, each ureter passes through a venous loop (Text Figs. 16 and 17) which is formed laterally by the postcardinal and medially by the cardinal collateral vein. The relation of the ureters to the veins at this period of development thus resembles the conditions met with in some placental mammals (cat and rabbit) with the exception that among the latter the medial side of the loop lies dorsolateral instead of ventrolateral to the aorta, as is the case in the opossum.

The manner in which the ureters migrate from a position dorsal to the cardinal collateral veins to the position which they occupy in the 14 and 15 mm. pouch young, ventral to these veins, is not clearly shown in the stages studied. I am inclined to believe, however, that the apparent discontinuity, mentioned above, which exists caudally between the postcardinal and cardinal collateral veins in the 10.5 and 11.5 mm. pouch young has been brought about by a ventral migration of the ureters.

The Spermatic Veins.

In the 8 mm. embryos of Didelphys, the blood from the anlages of the genital glands is collected by a number of tributaries (genital veins, Figs. 38 and 39, Plate III), which open into the derivatives of the subcardinal veins (pars subcardinalis of the postcava, the left anterior and posterior revehent veins).

At a subsequent stage of development (14 and 15 mm. pouch young), with the retraction of the mesonephroi from the body walls, the genital veins return their blood to the postcava (pars subcardinalis) by means of a right and left mesonephric vein which opens into the postcava, caudad of the renal veins, at a point which corresponds to the junction of the postcava and the postcardinal veins (Figs. 59, Plate V). Since this junction corresponds to the level at which the spermatic veins open into the postcava in the adult, I am, therefore, convinced that these two mesonephric veins, together with their genital connections, are retained in the adult as the spermatic veins, although I have not been able to establish definitely that such is the case.

The spermatic veins in all of the adult opossums examined by the writer (101) were connected with the postcava caudad of a point mid-

way between the left renal and common iliac veins (Fig. 28, Plate I); and, when the postcava was bifurcated, the connection was *invariably* found at the level of the bifurcation (see Part I, Fig. 8, Plate II). In none of the adults examined did the spermatics open into the renals, although an anastomosis between the latter and the spermatics was *invariably* present, on each side, in the form of a small vein which followed the ureter (see Fig. 28, Plate I).

In a number of adult Australian marsupials, however, the spermatics do not open into the postcava, as in Didelphys, but open into it at the base of the renal veins, as in Phascolomys Mitchelli (McClure, 03, p. 388), or into the renal veins themselves, as in Notoryctes typhlops (Sweet, 04). Considering the position at which the spermatic veins are developed in Didelphys, the question arises, how can these differences be explained? At the present time but two possibilities suggest themselves to the writer: (1) Either the lumbar portion of the postcava may not elongate to such an extent in these two forms as in Didelphys, so that the renal veins are developed, as in the rabbit and cat, at the level of the root of the postcava (anastomosis between postcava and postcardinals, Text. Fig. 7); or, (2) if an elongation does take place, as in Didelphys, the connection of the spermatics with the renal veins in Notoryctes may be accounted for on the ground that the spermatics have given up their original connection with the root of the postcava in favor of the channel, mentioned above, which follows the ureter and which opens into the renal veins.

The Variations Presented by the Postcava in the Adult Didelphys.

In all mammals, hitherto examined, the postcava is formed in the adult through a union of its iliac tributaries which takes place in a definite and uniform manner so that when variations occur they are regarded as exceptions to the general rule. Such, however, is not the case in Didelphys marsupialis. Here, instead of occurring as exceptions, variations appear to be the rule, so that it is actually impossible in this mammal to assign any one mode of origin for the postcava that may be regarded as typical of the species. This opinion is based upon the examination of 101 individuals; and a full description of these variations, as well as figures of the same, have already been published in Part I of this paper, to which the reader is referred (page 390).

In all but two of the 101 adult opossums examined the variations of the postcava can be easily classed under three main types. In two in-

dividuals, however, neither in its position with respect to the aorta nor in its mode of formation through a union of the iliac veins does the postcava conform to the usual marsupial type, but rather to the type of postcava which is characteristic of most placental mammals and, for this reason, these two exceptions are regarded by the writer as the only cases of postcaval abnormalities met with among the 101 opossums examined (Part I, p. 395).

The three types under which the postcaval variations are classified are as follows:

Type I. Those cases in which the internal iliac veins unite with the external iliacs ventral to the common iliac arteries or ventral to the aorta to form the postcava.

This type of postcava is the one commonly met with among the Australian marsupials, and may be spoken of as the marsupial type (see Text Fig. VI, Part I). The writer at present knows of but three cases among the Australian marsupials, thus far examined, in which the postcava is formed in any other manner; two in which it is formed as in placentals and in a manner similar to that in the cat (Petaurus taguanoides[10] and Phalanger ursinus[11]) and one, Trichosurus vulpecula,[12] in which it is formed as in Didelphys (Type II) and as figured on Plate II, Fig. 6, Part I).

Type II. Those cases in which the internal iliac veins unite with the external iliacs *dorsal* to the external iliac arteries, or dorsal to the aorta to form the postcava.

Type III. Those cases in which the internal iliac veins unite with the external iliacs both *dorsal* and *ventral* to the common iliac arteries or both *dorsal* and *ventral* to the aorta to form the postcava.

So many variations of this last type were met with that a further subdivision of Type III was found necessary, as follows:

Type III, A. Includes those cases in which the principal union between the internal and external iliac veins takes place *ventral* to the arteries in question.

Type III, B. Includes those cases in which the principal union between the internal and external iliac veins takes place *dorsal* to the arteries in question.

Type III, C. Includes those cases in which the above-mentioned dorsal and ventral unions are about *subequally* developed.

[10] Hochstetter, 93.

[11] Morphological Museum, Columbia University, No. 199.

[12] Morphological Museum, Columbia University, No. 234.

The following table shows the distribution of the above-mentioned types among 99 individuals; the postcava of the two remaining adult opossums, as mentioned above, not finding a place in the classification.

TYPE	♂	♀	TOTAL
Type I	11	18	29
Type II	9	18	27
Type III			
A	3	5	8
B	9	15	24
C	2	9	11
Total	34	65	99

The Development of the Three Types of Postcaval Variations Which Normally Occur in Didelphys marsupialis.

Considering the uniform manner in which the three types of postcaval variations occur in the adult there can be little doubt that their development in the embryo is also a normal procedure, and that they are not abnormalities in the strict sense of the word. Also, the circumstance that so many as 99 variations can be classed under so few as three types is certainly suggestive that there may be some common ground-type to which they can all be referred not only in the adult, but in the embryo as well.

In the 8 mm. embryo of Didelphys, as stated above, the umbilical artery of each side, near its origin from the aorta, is encircled by a complete circumarterial venous ring. These venous rings (see Text Figs. 10 and 13), as stated on a preceding page, are situated near the confluence of the external and internal iliac veins and are extremely variable in their character, not only in different embryos but even upon opposite sides of the same individual. For example, the portion of the venous ring which encircles the umbilical artery ventrally may possess a caliber subequal with or greater in size than that which encircles it dorsally (Text Fig. 10 and Fig. 42, Plate III); or, the portion of the ring which encircles the artery ventrally may possess a smaller caliber than that which encircles it dorsally (Text Fig. 13 and Fig. 41, Plate III).

This variation in caliber of the venous rings, as well as the relations the venous rings hold to the umbilical arteries in the 8 mm. embryos, is certainly suggestive of the conditions which characterize the three types of postcaval variations in the adult, in which a corresponding variation in caliber is met with as regards the veins which lie dorsal

and ventral to the common iliac arteries. On purely theoretical grounds, therefore, it is not difficult to explain the origin of all the adult variations on the basis that the formation of a particular type of postcava, depends upon the manner, as well as upon the extent, to which the circumarterial venous rings of the 8 mm. embryo might be affected by atrophy during the subsequent stages of development. On this basis the formation of Type III, in which the postcava is formed through a union of the external and internal iliac veins which takes place *both dorsal* and *ventral* to the *common iliac arteries,* might be explained on the grounds that portions of the venous rings which lie dorsal, as well as those which lie ventral to the umbilical arteries in the embryo, have been retained in the adult. See Text Figs. 19 and 20.

Type III, as represented by these figures, in which both embryonic venous rings retain their integrity and individuality might then be regarded as a ground-type[13] arrangement of the venous system, of which all of the postcaval variations which fall under Type III (Plates III, IV and V, Part I), are modifications, as well as are those variations which fall under Types I and II (Plates I and II, Part I). Thus Type III, A (see Plate III, Part I), in which the principal union between the internal and external iliac veins lies ventral to the common iliac arteries, might be formed as the result of the partial atrophy of the dorsal, and Type III, B (Plate IV, Part I), in which the principal union between the iliac veins lies dorsal to the arteries, might be formed as the result of the partial atrophy of the ventral portions of the circumarterial venous rings. Type III, C, in which there is practically no difference in the caliber of the vessels which lie dorsal and ventral to the common iliac arteries(see Plate V, Part I), might represent a case in which atrophy had affected the dorsal and ventral portions of the circumarterial venous rings in a like manner.

Type I (see Plate I, Part I), in which the internal iliac veins unite with the external iliacs *ventral* to the arteries to form the postcava, and Type II (see Plate II, Part I), in which the reverse is the case, might be formed as the result of the complete atrophy of the dorsal (Type I) and ventral (Type II) portions, respectively, of the circumarterial venous rings in the manner illustrated by Text Figs. 21 and 22.

[13] Among the 101 adult opossums examined by the writer one was met with in which the postcava was formed as in text Fig. 20, in which the common iliac vein unites with the external iliac of each side by means of two vessels which lie dorsal and ventral, respectively, to the common iliac artery. See Figs. 13 and 14 on Plate III, Part I, which are dorsal and ventral views, respectively, of the same preparation.

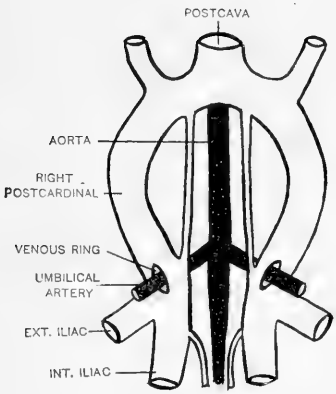

FIG. 19.

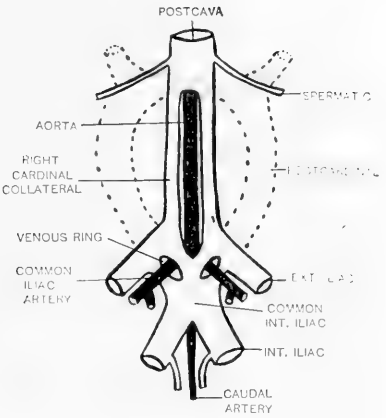

FIG. 20.

FIG. 19. Diagram of the venous system of an 8 mm. embryo of Didelphys showing the circumarterial venous rings. Ventral view.

FIG. 20. Diagram of the venous system of Didelphys in which the internal iliac veins have fused ventral to the caudal artery and in which a postcava of Type III has been established as the result of the persistence of the dorsal and ventral portions of both embryonic circumarterial venous rings. Ventral view.

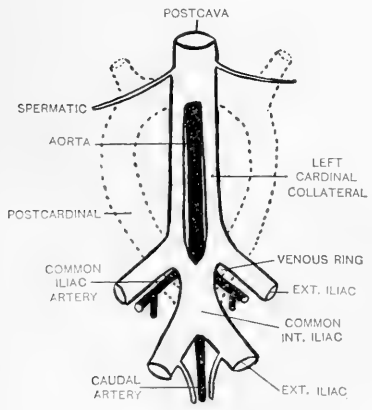

FIG. 21.

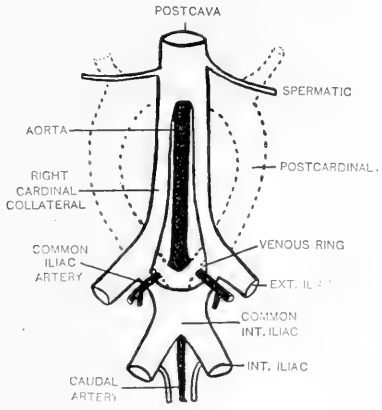

FIG. 22.

FIG. 21. Diagram of the venous system of Didelphys in which a postcava of Type I has been established as the result of the complete atrophy of the dorsal portions of the circumarterial venous rings. Ventral view.

FIG. 22. Diagram of the venous system of Didelphys in which a postcava of Type II has been established as the result of the complete atrophy of the ventral portions of the circumarterial venous rings. Ventral view.

The general principles, stated above, concerning the origin of the three main types of postcaval variations are, without doubt, fundamentally correct. The reconstructions of the pouch young clearly show that the formation of a particular type of postcava depends upon the manner as well as upon the extent to which certain veins that lie dorsal and ventral to the umbilical arteries (common iliacs in pouch young) are affected by atrophy. This general principle is well illustrated by the reconstruction of the venous system of a 14 mm. pouch young (Text Fig. 15) in which a postcava of Type III, A, in which the principal union between the internal and external iliac vein lies ventral to the common iliac arteries, is already established. In this particular case the internal iliac vein of the right side unites with the external iliac of the same side by means of two veins which lie dorsal and ventral, respectively, to the common iliac artery; while on the left side the union between these two veins takes place exclusively on the ventral aspect of the common iliac artery. Furthermore, it is evident, as the result of a complete atrophy of the ventral portion of the venous ring which encircles the right common iliac artery, that a postcava of Type III, C could be established. In the latter case, the internal iliacs would then unite with the external iliacs by means of two vessels, subequal in caliber, which lie dorsal and ventral, respectively, to the common iliac arteries. The important question to be determined in this case, as well as in connection with other reconstructions of the pouch young, is the extent to which the vessels that lie dorsal and ventral to the umbilical arteries in the 8 mm. embryos are involved in the formation of the vessels in the pouch young and adults which occupy corresponding positions with respect to the common iliac arteries. The only doubt that can exist as to their correspondence is the circumstance that the venous rings of the pouch young (Text Fig. 15) as well as those which occasionally persist in the adult (Fig. 17, right side, Plate IV, Part I) occupy a slightly different position with respect to the external and internal iliac veins than is the case in the 8 mm. embryos (Text Fig. 13). In the 8 mm. embryos (Text Fig. 13) the umbilical arteries, as well as the dorsal and ventral portions of the venous rings, lie somewhat craniad of the point of confluence of the internal and external iliac veins; while in the pouch young (Text Fig. 15) and adult (Fig. 17, right side, Plate IV, Part I) these structures occupy a more caudal position, so that the dorsal portion of the ring is now formed by the section of the postcardinal which constitutes the internal iliac vein, and the ventral portion of the ring by a vein which joins, at its caudal end, the internal iliac vein. Although a difference exists regarding the position of the rings and arteries with respect to the internal and ex-

ternal iliac veins it is seen, on comparing the figures, that the relations of the dorsal and ventral portions of the venous rings to the arteries which they encircle are the same in all cases in that the ventral portion of the venous ring lies nearer the median line than the dorsal portion. In view of this last-mentioned relation to the arteries, as well as the circumstance that the postcardinal vein (int. iliac) still forms the dorsal portion of the venous rings, I am convinced that the two cases cited above for the pouch young (Text Fig. 15) and the adult (Fig. 17, Plate IV, Part I), respectively, represent instances in which the ventral portion of the circumarterial venous rings of the 8 mm. embryo has been retained, and that the change in the position of the rings has been brought about secondarily as the result of a growth of the embryo.

It has already been stated that the left internal iliac vein of the 14 mm. pouch young in question (Text Fig. 15) joins the external iliac of the same side, exclusively on the ventral aspect of the left common iliac artery. It is plain from what we have learned from the reconstructions of the 8 mm. embryos that this ventral connection cannot have been formed by the postcardinal vein since the latter lies dorsal to the umbilical arteries of the embryo. This ventral union, therefore, can be accounted for only on the grounds that it has either been formed through the persistence of the same class of vessels as those that lie ventral to the umbilical arteries in the embryo; or, as will be described later on, through the persistence of a vein which has been secondarily developed in connection with the V. pudendovesicalis. Whatever the case may be, the large size of the ventral anastomosis between the internal and external iliac veins of the left side is undoubtedly correlated with the complete atrophy of the vessel which, in the embryo, formed a union between these two veins dorsal to the artery.

Text Fig. 17, which is a reconstruction of the venous system of a 15 mm. pouch young, presents a somewhat different arrangement of the veins which unite to form the postcava, from that just described for the 14 mm. pouch young (Text Fig. 15). In this case (Text Fig. 17) the internal iliac veins unite with the external iliacs to form the postcava by means of five vessels, three of which lie ventral and two dorsal to the common iliac arteries. It is further seen that these dorsal and ventral connections between the iliac veins form, on each side, two complete circumarterial venous rings, both of which encircle the common iliac artery. The ventral portion of the more medially situated rings is formed by a common vessel which is situated in the mid-ventral line and which is continuous caudad with the caudal veins; while the dorsal portion of the medial rings is formed by the postcardinal veins which also form

the dorsal portion of the more laterally situated rings (lateral rings). The ventral portion of the more laterally situated ring presents a marked difference in caliber on opposite sides and receives a vein which the writer regards as the V. pudendovesicalis (McClure, 00, 2, p. 457).

There can be no doubt as to the postcardinal origin of the dorsal portion of both sets of rings. There is some doubt, however, regarding the origin of the median ventral vein which forms the ventral portion of the two medial rings, as well as that of the ventral portion of the two lateral rings into which the V. pudendovesicalis opens.

The median ventral vein presents the same relative position with respect to the dorsal portion of each of the two medial rings, as is the case with the ventral portion of the circumarterial venous rings of the 8 mm. embryo (Text Fig. 13), the 14 mm. pouch young (Text Fig. 15), and the case of the adult (Fig. 17, right side, Plate IV, Part I), and, for this reason, has been most likely derived from the same class of vessels as those which form the ventral portions of the venous rings in the 8 mm. embryos. Whether, however, it corresponds to the ventral portion of one ring or has been formed as the result of a fusion between the ventral portion of two rings, as represented in the diagram, Fig. 20, it is impossible to state. Whatever its mode of origin may be, its presence in the pouch young undoubtedly accounts for the presence of a similarly situated vessel which is frequently met with among the adult variations (see Fig. 19, Plate IV, Part I).

The ventral portion of each of the lateral rings, on the other hand, occupies an entirely different position with respect to the dorsal portion of the ring from what is the case with the ventral portion of the medial rings, since it lies lateral instead of medial to the dorsal portion of the ring. I am, therefore, inclined to conclude, for this reason as well as others given below, that the ventral portions of the lateral rings are secondary formations which are first met with in the pouch young and which are developed here in connection with the V. pudendovesicalis. In addition to the reason already mentioned, my reasons for so thinking are as follows: (1) On account of the presence of these veins in the 15 mm. pouch young (Text Fig. 17) in addition to the median ventral vein; as well as the occasional persistence in the adult of the ventral portion of a right lateral ring in addition to a vein whose origin cannot be accounted for unless it has been derived from the ventral portion of an embryonic circumarterial venous ring of the same side (see Fig. 4, right side, Plate I, Part I, and compare with Fig. 10, right side, on Plate II, Part I) and, (2) because the presence of these veins in the pouch young explains, for the most part, the variable character of the V. pudendovesicalis in the adult.

The writer has already described in a previous paper (McClure, 00, 2, p. 457) the variable manner in which the Vv. pudendovesicales of the adult may open into the iliac veins. They may open in the adult into the angle of union of two veins which join the external and internal iliac veins, respectively, ventral to the iliac arteries; or, they may open into the angle of union of three veins, two of which lie ventral to the arteries and join the iliac veins as above, while the third lies dorsal to the arteries and joins the external iliac vein (see McClure, 00, 2, Figs. 20 and 21). Also, they may open as single vessels on each side either into the external or internal iliac veins (Fig. 16, Plate IV, Part I); or, into the external iliac vein on one side and into the internal iliac on the other (Fig. 9, Plate II, Part I).

Cases in the adult in which the V. pudendovesicalis opens into the angle of union of two veins which join the external and internal iliacs, respectively, ventral to the arteries (as in Fig. 4, right side, Plate I, Part I, and in Fig. 10, right side, Plate II, Part I) can be explained on the ground of the persistence of the ventral portion of the lateral circumarterial venous ring. Cases in the adult in which the V. pudendovesicalis opens as a single vessel into either the external or internal iliac vein (Figs. 8 and 9, Plate II, Part I) can also be explained on the ground that the ventral portion of the lateral venous ring gives up its connection with one or the other of the iliac veins so that the V. pudendovesicalis will necessarily open only into the iliac vein with which the connection has been retained.

Although those cases in which the V. pudendovesicalis opens into the external and internal iliac veins, ventral to the arteries, appear to find an explanation, it is not so clear how this vein, as is frequently the case in the adult, makes connections with the iliac veins dorsal to the iliac arteries. It is possible that a considerable number of reconstructions of the pouch young would show that the V. pudendovesicalis does not always open into the iliac veins as represented in Text Fig. 17, but that it may also, in some instances, open into the angle of union of two veins which, as in the case of an adult, join the external iliac vein dorsal and ventral, respectively, to the external iliac artery (see Fig. 4, left side, Plate I, Part I). It is evident, if this case of the adult represents the persistence of a condition which sometimes prevails in the pouch young we then have an explanation of those peculiar cases in which the V. pudendovesicalis opens into the iliac veins dorsal to the iliac arteries.

Finally, I think it may be stated without fear of refutation, that the variable character of the V. pudendovesicalis in the adult is correlated with the variable manner in which the iliac veins unite to form the post-

cava, although, at the present writing, it is quite impossible to establish a relationship between a particular type of postcava and the manner in which the Vv. pudendovesicales join the iliac veins.

From what has already been stated above concerning the evanescent character of the ventral portion of the lateral venous rings it is clear that the type of postcava which at present characterizes the 15 mm. pouch young (Text Fig. 17) and which would most likely prevail in the adult, is Type III, B, and possibly of the variety represented by Fig. 19 on Plate IV of Part I. It is also possible that the ventral median vessel in Text Fig. 17 (ventral portion of medial rings) might become completely atrophied before the adult state was reached so that a postcava of Type II would result, possibly of the variety represented by Fig. 7 on Plate II of Part I, in which the connections between the internal and iliac veins present a marked difference in caliber. In case of either of the two possibilities the ventral portions of the lateral rings could persist in the adult as in Fig. 10, right side (Plate II, Part I); or, they could give up their connections with the external iliacs so that each V. pudendovesicalis would open into an internal iliac vein as in Fig. 7 (Plate II, Part I). It is also evident that the condition represented in Fig. 9 (Plate II, Part I) might result, in which the V. pudendovesicalis opens on one side into the external and on the other into the internal iliac vein.

We are now in a position to further consider the character of the anastomosis, ventral to the common iliac artery, which exists between the *left* external and internal iliac veins in the 14 mm. pouch young (Text Fig. 15). It has already been stated that this anastomosis, ventral to the artery, has probably been brought about either as the result of the persistence of a vein which corresponds to the ventral portion of an embryonic circumarterial venous ring, or as the result of the persistence of a vein which has been developed secondarily in the pouch young in connection with the V. pudendovesicalis. It is not improbable that the ventral portion of one of the lateral rings might, in some cases, become so enormously hypertrophied, that it would function in the adult as the sole channel through which the blood reached the postcava from the pelvic region. It is impossible, however, to state definitely what is actually the case in the 14 mm. pouch young, although I am inclined to believe that in this particular case (Text Fig. 15, left side) it is the ventral portion of a lateral ring which has persisted. My reason for holding this view is based on the relations of the V. pudendovesicalis to the ventral anastomosis in question.

Text Fig. 18 represents another reconstruction of the venous system

of a 15 mm. pouch young in which a postcava of Type III, B, has already been established; that is, the type in which the principal union between the internal and external iliac veins lies dorsal to the common iliac arteries. In this case, however, the connection between the internal and external iliac veins which lies ventral to the arteries does not consist of a single median vessel as in Text Fig. 17, but has the appearance of being formed as the result of a fusion between two medianly situated vessels, one of which, the right, follows a somewhat curved course. These median ventral vessels in Text Fig. 18 undoubtedly have the same origin as that of the single median ventral vessel in Text Fig. 17, although it is a difficult matter to determine in either case whether the ventral portions of one or of both of the embryonic circumarterial venous rings are involved in their make-up. The anastomosis between the ventrally situated veins and the right internal iliac in Text Fig. 18 is most interesting, however, since it appears to explain the presence in the adult variations of a similarly situated vessel which lies ventral to the arteries and extends between the internal iliac vein and the external iliac of the opposite side. See Figs. 15, 16 and 18 on Plate IV, Part I, for examples of this type of variation. There is one marked difference to be noted in comparing the venous systems of these two 15 mm. pouch young as represented by Text Figs. 17 and 18. In Fig. 17 the ventral portions of the lateral rings anastomose with the external iliac veins, while in Fig. 18 this anastomosis is wanting. Whether this latter condition indicates that the ventral portions of the lateral rings are undergoing atrophy or that they are merely in the process of formation, it is impossible to state, although I believe the former view to be correct, on account of the large size and prominence of the vessels which unite the iliacs dorsal to the arteries. These dorsal vessels in Text Fig. 18, as well as the similarly situated vessels in all of the other pouch young, are derived from the postcardinal veins.

Text Fig. 16 represents still another reconstruction of the venous system of a 15 mm. pouch young in which a postcava of Type II is already established. In this case the connections between the internal and external iliac veins lie exclusively dorsal to the common iliac arteries; there being no indication of any vessels which unite these veins ventral to the arteries, except the ventral portions of the lateral rings which, as in Text Fig. 18, are either in the process of formation, or, as is more likely, are undergoing atrophy.

Finally, Text Fig. 14 represents the reconstruction of a 14 mm. pouch young in which a postcava of Type II is also already established. In this case there is no anastomosis formed ventral to the common iliac

arteries between the external and internal iliac veins, either in the form of vessels which lie in the median line, or of those which are more laterally situated and which have been described above as the ventral portions of the lateral rings. Numerous examples were met with by the writer in which the postcava was formed in the adult in exactly the same manner as that represented by Fig. 14 (see Figs. 6, 7 and 8, Plate II, Part I); while the two variations represented by Figs. 9 and 10 on the same plate, are easily explained on the ground that the internal iliac veins have given up one of their connections with the external iliacs.

From what has already been said regarding the pouch young there can be no doubt that the establishment of a particular type of postcava in the adult depends upon the manner as well as the extent to which certain vessels which lie dorsal and ventral to the iliac arteries are affected by atrophy. There can also be no doubt but that, in all cases, the vessels which lie dorsal to these arteries are derived from the postcardinal veins. There is, however, some doubt in the case of certain vessels which extend between the iliac veins, ventral to the arteries. A doubt may exist whether all of these ventrally situated vessels have been derived from the ventral portions of embryonic circumarterial venous rings; or, whether they may not, in some cases, be new formations which have been developed in the pouch young, independently of these rings.

It is probable that in such a variable venous system as that of Didelphys any venous channel which might be established in the embryo or pouch young between the internal and external iliac veins could, in certain circumstances, be retained in the adult as a functional channel. I am, therefore, inclined to believe that in addition to the embryonic circumarterial venous rings, other venous elements may occasionally enter into the formation of the vessels which form an anastomosis between the iliac veins ventral to the common iliac arteries.

In all probability, the median ventral vessel in certain cases under Type I, as in Figs. 1 and 2 (Plate I, Part I) is formed as the result of the persistence of a single median vessel similar to that met with in the 15 mm. embryo (Text Fig. 17). It is a difficult matter to determine, however, whether both of the anastomoses between the iliac veins as in Fig. 3 (Plate I, Part I) have been derived from the ventral portions of the lateral rings, or, whether such is the case only with the anastomosis on the left side. It is also a question, on account of their connection with the Vv. pudendovesicales, whether the two veins which unite the iliac veins ventral to the arteries in Fig. 14 (Plate III, Part I) are not derived from the ventral portions of the lateral rings rather than from the ventral portions of the embryonic circumarterial venous rings. This and

similar questions cannot be definitely decided without examining a large number of embryos and pouch young; and, furthermore, without, at the same time being fortunate enough to meet with a similar type of variation. Whatever the case may be regarding the origin of these ventrally situated vessels, the venous channels which can be retained in the adult are all well defined in the embryos and pouch young. And, although certain difficulties are apparent in determining, in all cases, which of these embryonic venous channels have actually been retained in the adult, it is not impossible to interpret the adult variations (three types of postcaval veins) on the basis that they represent the possible combinations which could ensue as the result of the persistence or atrophy of certain of these embryonic vessels.

Explanation of the Two Abnormalities of the Postcava Which Cannot be Classed under Types I, II and III (see Text Figs. VII and VIII, Part I).

As stated on page 395, Part I of this paper, the main features which characterize these two abnormalities and distinguish them from the variations described under the three types are twofold: (1) All of the posterior tributaries of the postcava, including the external or common iliac veins, as the case may be, unite dorsal to the arteries to form the postcava, as is usually the case in placental mammals; (2) the postcava lies to the left of, instead of upon the ventral surface of, the aorta, and resembles in this respect the conditions met with in placentals when the left instead of the right postcardinal vein persists as the caudal end of the postcava.

Both of these variations are figured on page 396, Part I of this paper (Figs. VII and VIII) to which the reader is referred.

It appears to the writer that these two abnormalities in question may be explained on the ground that the caudal portion of the unpaired postcava has been formed, in each case, from one (the left) instead of from both of the cardinal collateral veins, as is usually the case in Didelphys. The persistence of the left vein explains the position of the postcava on the left side of the aorta; while the persistence of a single vein instead of two to form the caudal section of the postcava possibly duplicates the same physiological conditions that prevail in most placental mammals, and necessitates a union between the postcava and the iliac tributaries of the opposite side *dorsal* to the arteries.

The position of the ureters was normal in both cases since they were situated lateral to the postcava along their entire extent. For this reason

it is evident that the cardinal collateral vein must have entered into the formation of the postcava rather than the postcardinal, otherwise the left ureter would occupy the same relative position that it does in Text Fig. 17, and pass caudad between the postcava and the aorta.

Text Fig. 23 is a diagram illustrating the probable modifications which the venous system has undergone in establishing the abnormality represented by Text Fig. VII in Part I of this paper. The shaded portions indicate the veins which have atrophied and the crosses (+) the new formation by means of which the postcava anastomoses with the iliac tributary of the right side, *dorsal* to the aorta.

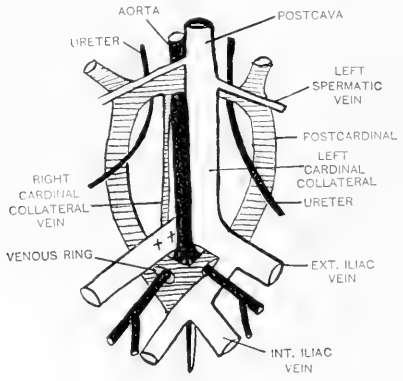

Fig. 23.

Fig. 23. Diagram illustrating the probable modifications which the venous system has undergone in establishing the abnormality represented by text Fig. VII in Part I of this paper. Ventral view.

The Umbilical, Abdominal and Omphalomesenteric Veins.

The Umbilical Veins.—In the youngest embryos of Didelphys (8 mm.) studied by the writer, the right and left umbilical veins, usually after fusing at the umbilicus to form an umbilical sinus, can be traced forward as independent vessels (Figs. 38, 37, Plate III, and Figs. 36, 34, 33, Plate II) to the ventral surface of the liver. Here they again fuse to form a sinus (Fig. 32, Plate II) from which they are continued dorsad through the parenchyma of the liver in a channel common to both which opens into the postcava in common with the left hepatic vein (Text Figs. 10 and 24).

The hepatic continuation of the umbilical veins opens into the postcava slightly craniad of that of the omphalomesenteric vein (Text Fig. 10) and, so far as the writer can determine, the umbilical and omphalo-

mesenteric veins do not anastomose with each other at any point within (except by sinusoids) or without the liver. Near their entrance at the umbilicus considerable variation was met with in the 8 mm. embryos as regards the size of the umbilical veins. In some cases the right (Fig. 38, Plate III) and in others the left umbilical vein was the larger of the two, so it can be said that at this stage of development the umbilical vein of a particular side does not invariably predominate as the principal channel between the allantois and the liver. Although a marked difference in size may characterize the umbilical veins in the region of the umbilicus, the smaller of the two veins invariably increases in size as the liver is ap-

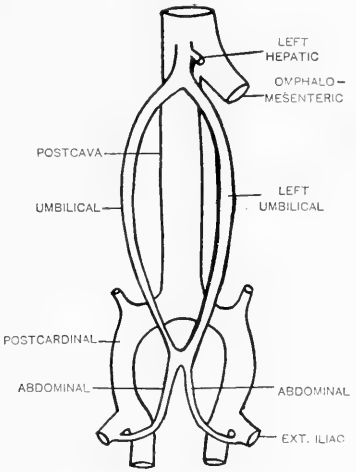

FIG. 24.

FIG. 24. **Diagram of the venous system of an 8 mm. embryo of Didelphys showing the umbilical and abdominal veins. Ventral view.**

proached, so it can be said that both umbilical veins are highly developed up to a relatively late stage of development; a circumstance which, as shown by Broom, 98, for Trichosurus, appears to be characteristic of the marsupials as thus far examined.

In the 11.5-12 mm. embryos of Didelphys one large umbilical vein now forms the principal channel between the allantois and the liver; a vein which I regard as the left umbilical vein. This large vein (Fig. 53, Plate V) lies in the ventral body-wall slightly to the left of the mid-ventral line, and to its right is situated a much smaller vessel which is difficult to follow in consecutive sections, but which is probably the remains of the right umbilical vein. The two umbilical veins appear to anastomose in places with each other, so that one might almost regard the larger of

the two veins as being formed, in places, through a fusion of the two umbilical veins. On reaching the ventral surface of the liver, the large umbilical vein enters the latter through which it continues as a single channel which at first turns caudad, and then dorsad before opening into the postcava as in the 8 mm. embryo, in common with the left hepatic revehent vein (Text Fig. 25).

In the 10.5 mm. pouch young both umbilical veins can be followed for only a short distance in front of the umbilicus which is now closed. Further forward, however, only one large umbilical vein can be clearly distinguished, which now returns blood solely from the body-walls, but

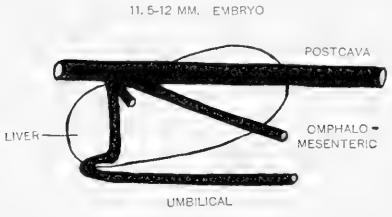

FIG. 25.

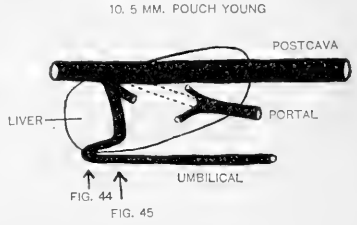

FIG. 26.

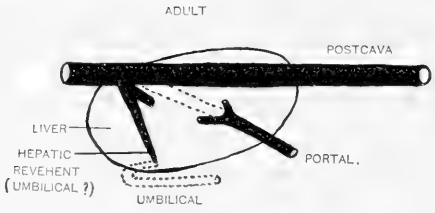

FIG. 27.

FIGS. 25, 26 and 27. Diagrams illustrating the transformations which the umbilical and omphalomesenteric veins undergo in the embryos and pouch young. Lateral views.

which is still continued through the liver where it opens into the postcava, as in the preceding stages, without anastomosing directly with the omphalomesenteric vein (Fig. 44, Plate III, and Fig. 45, Plate IV, and Text Fig. 26).

In the older pouch young the abdominal portion of the left umbilical vein ceases to be of prominence, and, so far as the writer can determine, entirely disappears before the pouch young have attained a length of about 17 mm. Its presence was noted, however, in the 14 and 15 mm. pouch young, although its connection with the hepatic circulation could not be determined.

The hepatic continuation of the left umbilical vein, after it has given

up its connection with the abdominal portion, continues to function in the pouch young as a revehent vein of the liver, and I am inclined to believe that it is also retained in the adult (Text Fig. 27) as the hepatic vein (revehent) which opens into the postcava in common with or in close proximity to the large left hepatic vein (see Text Fig. V, Part I, which represents a corrosion of the hepatic veins of the adult).

The arrangement of the umbilical veins in the 6 mm. embryo of Dasyurus is essentially the same as that described above for the 8 mm. embryos of Didelphys; the only exceptions being that the two in Dasyurus are of about the same size and that their continuation within the liver opens into the postcava independently of the left hepatic vein (Text Fig. 9, and Figs. 29 and 30, Plate II).

So far as known to the writer, Broom, 98, is the only investigator who has hitherto described the umbilical veins of the marsupials and, although his description is somewhat fragmentary, it conclusively shows, when compared with the above observations of the writer, that the plan of the umbilical circulation in marsupials not only differs from that in the higher mammals, but that there is also a difference even among the marsupials themselves.

Broom states that in an 8.5 mm. embryo of Trichosurus a single moderate-sized vein brings back the blood from the allantois and on reaching the umbilicus opens into a rather large sinus which lies round the margin of the umbilicus. From the umbilical sinus two large umbilical veins pass up to the liver on either side of the large umbilicus. The vein of the left side opens into the liver at a point which corresponds to that at which the left umbilical opens in the higher mammals; the right umbilical vein opens into the liver on the right side of the quadrate lobe and differs from the left in receiving a number of tributaries from the abdominal walls.

Regarding the course of the umbilical veins within the liver, Broom states as follows: "Each vein, on entering the liver through a small opening in its wall, falls into a comparatively large venous space. The tracing of the veins in the liver at this stage is a matter of considerable difficulty; but there is little doubt that each intra-hepatic venous sac gives off a small branch inwards and slightly downwards to the portal vein, and divides above into a large number of branches, which spread over the periphery of the upper part of the liver, and then pass inwards to fall into the inferior vena cava."

Broom further states that "In a 10.5 mm. Trichosurus embryo the umbilical sinus, though very much reduced, can still be detected. The development of the sides of the abdominal wall has brought both the

right and left veins much nearer to the middle line, and a further interesting change has taken place in that the right vein has become much reduced and no longer opens into the liver. It is now merely a small vein which brings some blood from the anterior abdominal wall into the umbilical sinus. The left vein which now carries all of the allantoic blood to the liver, runs only a little to the left of the middle, though the recti muscles are still widely apart."

"A little later all trace of the right vein disappears, and the left vein, though comparatively small, follows a course very similar to that of the umbilical vein in the higher mammals. At birth, of course, the circulation through the umbilical vein ceases. It will be observed that this doubling of the umbilical vein is very dissimilar to the condition found in the higher mammal, and very similar to that found in the early lacertilian embryo."

The Abdominal Veins.—The abdominal veins are most prominently developed in the 8 mm. embryo of Didelphys and consist of two small vessels which lie in the ventral body wall caudad of the umbilicus (Figs. 40 and 41, Plate III). These two veins resemble in all their relations the posterior division of the abdominal veins in the embryos of reptiles, since they receive tributaries from the body walls, open cranially into the umbilical veins and connect caudally with external iliac veins (Text Fig. 24). Veins occupying the same relative positions as the abdominal veins were also met with in the 11.5-12 mm. embryos of Didelphys, although they were much less prominent here than in the 8 mm. embryos. The abdominal veins are undoubtedly transitory in character and confined to the embryo during its uterine existence since no traces of them were met with in any of the pouch young examined.

The Omphalomesenteric Veins.—In the 8 mm. embryo of Didelphys the omphalomesenteric veins (Figs. 34, 35, 36, Plate II, and Figs. 37 and 38, Plate III) are represented by a single large vein whose earlier transformations I have been unable to follow. There can be little doubt, however, that a venous ring is formed about the intestine at a previous stage, as in the rabbit, since such a ring is actually present in the 6 mm. embryo of Dasyurus (Fig. 29, Plate II). In the 8 mm. embryo the large omphalomesenteric vein enters at the umbilicus on the left side of the intestine and then curves dorsad until it lies dorsal to the same. In this position it enters the liver through which it passes, without at any time anastomosing directly with the umbilical veins, and opens into the postcava slightly caudad of the opening of the umbilical veins (Text Fig. 10 and Fig. 33, Plate II). In the 11.5-12 mm. embryos of Didelphys the omphalomesenteric vein, except for minor changes due to the elongation

of the embryos, maintains essentially the same relations as in the preceding stage and, after tunnelling the liver, also opens into the postcava independently of the umbilical veins. In the 10.5 mm., as well as in all of the older pouch young, the hepatic continuation of the omphalomesenteric vein, instead of forming a continuous channel through the liver to the postcava, is broken up by the hepatic sinusoids so that the omphalomesenteric vein now functions as an advehent vein of the liver, or, in other words, has become transformed into the portal vein (Text Fig. 26).

From the above description of the hepatic circulation in Didelphys it is evident that it differs widely from that met with in monotremes or in any of the higher mammals thus far described. Although in monotremes the ductus venosus Aranzii differs from that of the higher mammals in that it does not form the direct continuation of the left umbilical vein, it is nevertheless formed through a union of the portal and umbilical veins (Hochstetter, 96). In none of the embryos of Didelphys studied by the writer was a ductus venosus Aranzii formed as in monotremes or placental mammals, since in every instance noted both the umbilical and omphalomesenteric veins passed through the liver without at any time, except by sinusoids, anastomosing with each other. It, therefore, appears questionable to the writer whether one can rightly speak of a ductus venosus in Didelphys that would correspond to that in monotremes and placental mammals, and for this reason this term has been omitted in the preceding description of the hepatic circulation.

The nearest approach to the conditions described above for the omphalomesenteric and umbilical veins of Didelphys appear to be present in birds as described by Hochstetter (03, Fig. 156, page 136). Here the omphalomesenteric and left umbilical veins open into the proximal end of the postcava without previously anastomosing with each other within the liver, except by sinusoids.

Résumé and General Considerations.

For convenience of description, the postcava of the adult Didelphys was described in Part I (page 386) as consisting of the following subdivisions:

A *prehepatic* division which includes that portion of the vein which extends between the right auricle and the most cranial of the hepatic veins; an *hepatic* division which is embedded in the liver and which includes that portion of the postcava into which the hepatic veins open; a *renal* division which includes that portion of the postcava which lies between the most caudal of the hepatic veins and a point just behind the most caudal of the two renal veins, and a *postrenal* division which con-

sits of that portion of the posteava which lies caudal to the renal veins.

The following table shows the approximate relations which exist between the veins in the embryo and the above-mentioned sub-divisions of the posteava in the adult:

ADULT	RABBIT EMBRYO [14]	DIDELPHYS EMBRYO
Prehepatic	V. hepatica communis	?
Hepatic	Hepatic sinusoids	Hepatic sinusoids and a small portion of the right subcardinal vein.
Renal	Right subcardinal	Right subcardinal.
Postrenal	Right postcardinal	Cross anastomosis, (formed by both subcardinals), and right and left cardinal collateral veins.

1. After the permanent kidneys have completed their migration in the embryos of the rabbit and cat, renal veins are developed which open into the posteava, approximately, at the level at which the latter joins the two postcardinal veins.

In Didelphys, on the other hand, after the permanent kidneys have completed their forward migration, renal veins are developed which open into the posteava some distance craniad of the latter's junction with the two postcardinals; a circumstance which now explains why the postcava of the adult opossum was never found by the writer to be bifurcated as far forward as the level at which the renal veins opened into the postcava.

The position of the renal veins in Didelphys, with respect to the junction of the postcardinal veins and the posteava, does not appear to indicate that the permanent kidneys in Didelphys have undergone, relatively, a more extensive migration than in the rabbit and cat, since the renal veins, as in the embryos of the rabbit and cat, open into the posteava only slightly caudad of the origin of the omphalomesenteric artery, and contiguous to the junction of the posteava and the left anterior revehent vein. The opening of the latter vein into the posteava, however, lies relatively, much further craniad of the junction of the posteava and postcardinal veins in the pouch young than is the case in the 8 mm. embryos of Didelphys and the embryos of the rabbit and cat. This circumstance I can only account for on the basis that in Didelphys the junction of the posteava and the postcardinal veins remains, more or less, as a fixed point, in front of which the vessels elongate more rapidly than those that lie behind. This growth in length, so far as the posteava

[14] After Lewis (02, page 242).

is concerned, principally affects that portion of the vein (cross anastomosis) which lies between its junction with the two postcardinals and that with the left anterior revehent vein.

The left renal vein of Didelphys is developed in connection with the left anterior revehent vein at the point where the latter opens into the postcava. The right renal vein is developed, as a rule, slightly craniad of the left and, possibly, from one of the urogenital tributaries of the pars subcardinalis of the postcava. Both renal veins were met with for the first time in the 14 mm. pouch young.

2. In the rabbit, the portion of the right subcardinal which, in part, forms the stem of the postcava terminates, approximately, at the level at which the renal veins will be developed; while the stem of the postcava caudad of the renal veins is developed from the right postcardinal, together with a derivative of the latter which is formed on the medial side of the ureter. In Didelphys, however, the portion of the subcardinal which, in part, forms the stem of the postcava does not terminate at the renal level, but at the level at which the internal spermatic veins subsequently open into the postcava; while caudad of the spermatic level the postcava is formed by two veins which usually fuse in the median line *ventral* to the aorta. This last feature, so far as I know, is distinctively a marsupial character. These two veins have been described in the preceding pages under the name of the cardinal collateral veins. In both the embryos and pouch young the cardinal collateral veins occupy a position ventrolateral or ventral to the aorta and, in this respect, differ from that of the postcardinal derivative which is developed on the medial side of the ureter, lateral to the aorta, and which forms, in part, the stem of the postcava in the rabbit. They are also to be distinguished from the posterior revehent veins (subcardinals) with which, however, they form frequent anastomoses. In correlation with the degeneration of the postcardinal veins the cardinal collaterals increase in size and subsequently function in place of the postcardinals in returning the blood to the root of the postcava from the hind limbs and pelvic region.

3. In the adult rabbit the postrenal division of the postcava lies to the right of the aorta and is usually formed through a union of its iliac tributaries which takes place dorsal to the arteries. In the adult Didelphys, on the other hand, the postrenal division of the postcava lies ventral to the aorta and its iliac tributaries normally unite in such a variable manner to form the postcava that it is actually impossible to assign any one mode of union for the iliac veins that may be regarded as typical of the species. In consequence of this variability, the writer has classified the different modes of union which characterize the external and internal

iliac veins of the adult under three types. Type I, in which the internal iliac veins unite with the external iliacs ventral to the arteries to form the postcava (marsupial type); Type II, in which the internal iliac veins unite with the external iliacs dorsal to the arteries to form the postcava; Type III, in which the internal iliac veins unite with the external iliacs, both dorsal and ventral to the arteries, to form the postcava.

From an embryological standpoint it is not a difficult matter to determine that the establishment of a particular type of postcava in the adult Didelphys depends upon the manner, as well as upon the extent to which certain well defined vessels in the embryos and pouch young, which lie dorsal and ventral to the umbilical or common iliac arteries, are affected by atrophy during the subsequent stages of development. These dorsally and ventrally situated veins are usually met with in the embryos and pouch young in the form of circumarterial venous rings which encircle the origin of the arteries in question. In establishing the three types of postcaval veins this embryonic ground-plan undergoes the following modifications:

Type I is established as the result of the complete atrophy of the vessels which lie dorsal, and Type II as the result of the complete atrophy of the vessels which lie ventral to umbilical or common iliac arteries. Type III, which is a combination of Types I and II, is established as the result of the persistence of vessels which lie both dorsal and ventral to the arteries in question.

So far as known to the writer, no adult vertebrate has hitherto been described which presents such a series of normally occurring variations of the venous system as those described by the writer for Didelphys. Also, as far as I am aware, a fixity of type normally characterizes the main stem of the venous system of all other adult vertebrates so that variations, when they occur, are exceptions rather than the rule.

It is an intereseting fact that an essentially similar ground-plan of the venous system as that described above for Didelphys, in which a circumarterial venous ring encircles the origin of the umbilical arteries, is also characteristic of the embryos of a number of other vertebrates. These vertebrates, however, differ from Didelphys in that the modifications which the embryonic ground-plan undergoes take place in a definite direction, so that a characteristic or fixed type of venous system normally results in the adult. For example: A circumarterial venous ring occasionally encircles the origin of the umbilical arteries in reptilian embryos (Sceloporus), although only the ventral or postcardinal portion of the ring normally persists in the adult.

Circumarterial venous rings which encircle the origin of the umbilical

arteries are invariably present in the embryos of birds, as thus far examined (Hochstetter and Miller), although in the adult only the dorsal portion of the embryonic ring persists normally as a functional vessel.

In the embryos of Echidna (Hochstetter) the internal iliac veins (postcardinals) at first lie ventral to the iliac arteries, but subsequently anastomose with the external iliacs by means of two vessels, one on each side, which lie dorsal to these arteries so, that the postcava is at one time formed through a union between its iliac tributaries which takes place both dorsal and ventral to the iliac arteries. The adult condition is reached as the result of the complete atrophy of the anastomosis between the iliac veins which lies ventral to the iliac arteries (see Text Figs. X and XI, Part I). In this, as in the preceding cases, however, a fixity of type normally prevails in the adult regardless of the conditions which prevail in the embryo.

Among the Australian marsupials it is not as yet known whether circumarterial venous rings encircle the umbilical arteries as in the embryos of Didelphys. There is no question, however, as to the constancy with which, in most of these animals, certain ventrally situated vessels aid in forming the stem of the postcava in the neighborhood of the iliac arteries.

Circumarterial venous rings have recently been figured by Lewis (02, Plate 2, Figs. 7 and 8) as encircling the origin of the umbilical arteries of a 14.5 mm. rabbit embryo. In this case the ventral portions of the rings normally atrophy, while only the dorsal or postcardinal element of the rings persists in the adult as the functional channel. The persistence of the ventral as well as the dorsal portion of such a venous ring may possibly account for those interesting abnormalities in the adult mammal in which the common iliac artery passes through a foramen in the common iliac vein.

In establishing the adult conditions in Didelphys this embryonic ground-plan, as stated above, is *not modified in any one definite direction*, but in such a manner that the resulting condition may be represented by any one of the possible combinations which such a ground-plan is capable of producing. These possible combinations constitute the three types of postcaval variations which have been described in the preceding pages under Types I, II and III. The production of these variations is in every sense a normal procedure, and it is an interesting fact that the variations described under Type I (Fig. 1, Plate I, Part I) resemble the usual condition of the adult postcava in the Australian marsupials, while those under Types III, B (Fig. 18, Plate IV) and Type II (Figs. 6 and 7, Plate II, Part I) are identical, respectively, with the em-

bryonic and adult condition of the postcava in Echidna (see Text Figs. X and XI, Part I).

Furthermore, two cases were met with among the 101 adults examined in which the postcava occupied the same relative position with respect to the aorta and was formed through a union of its iliac tributaries in exactly the same manner as in placental mammals when the left instead of the right postcardinal vein forms the caudal end of the stem of the postcava. These two cases are regarded by the writer as the only actual cases of abnormalities met with among the 101 adults examined. They are figured and described in Part I (page 395) and further considered from the standpoint of their development in Part II (p. 211).

It is impossible to state what the causes may be which are responsible for such a series of normally occurring variations as those which characterize the postcava of the adult Didelphys. Whatever they may be, they are apparently inherent in the individual itself since there is as much variation among the individuals of the same litter as among the individual members which constitute the species in general.

It appears to the writer that the causes which account for this constant variation in Didelphys may be analogous to those which only sporadically act in other vertebrates, but which are responsible for the well-known series of venous abnormalities which one occasionally meets with in the adult. Why they should constantly act in Didelphys and only occasionally act in other vertebrates, however, is difficult of explanation unless it is indicative of an extreme plasticity which may be regarded as characteristic of the species in general. This view certainly coincides with the investigations of Allen, 01, upon the variable character of the skeleton of Didelphys, as well as with those of Oldfield Thomas, 88, who in referring to Didelphys marsupialis, var. typica, says (p. 327): "This widespread species, owing to its remarkable variability in color, has been made the basis of a very considerable number of nominal species, of which the most commonly recognized are the North American D. virginiana, the Brazilian D. cancrivora, and the striped-faced D. azaræ. I find, however, such a considerable amount of variability in the specimens from every locality and such an entire absence of constancy in any character or set of characters, that I am constrained to unite the whole of this group of opossums into a single species, to which the Linnean name D. marsupialis is of course applicable."

It is generally conceded that variations of the venous system occur with greater frequency among domesticated animals than among those living in the wild state; an idea, however, which is most certainly erroneous, as proved by the conditions met with in Didelphys.

4. In the rabbit embryo the portal vein (omphalomesenteric) on reaching the liver anastomoses directly with the left umbilical vein to form the ductus venosus Arantii, so that the latter, at a certain period of development, returns to the heart most of the blood that reaches the liver through the portal and left umbilical veins. In Didelphys, however, no such direct anastomosis takes place between these two veins, but each vein is continued through the parenchyma of the liver in a separate channel which opens into the proximal end of the postcava independently of the other; a condition which, in some respects, resembles that met with in birds.

So far as known to the writer, Broom is the only investigator who has hitherto described the umbilical and omphalomesenteric veins in the embryos of marsupials (Trichosurus) and, although his account is somewhat fragmentary it conclusively shows, when compared with the above observations of the writer, that the plan of the embryonic hepatic circulation in marsupials not only differs from that of the higher mammals, but that there is also a difference even among the marsupials themselves.

5. Abdominal veins are present in the 8 mm. embryos of Didelphys which resemble in all respects the abdominal veins of reptiles. They lie in the mid-ventral body-walls, connect cranially with the umbilical veins at the umbilicus and caudally with the external iliacs.

In conclusion, I may state that in 1902 I received a letter from Dr. J. P. Hill, of the University of Sydney, in which he was kind enough to send me a few rough sketches illustrating the development of the veins in Perameles. He specifically stated that the schemes were not drawn to scale and, as they represented only a series of preliminary observations, he did not care to vouch for their accuracy in detail. The general conclusions which he drew from his observations are most interesting, and I quote them in full. "From these rude schemes there can be no doubt as to the origin of the postcaval vein from an unpaired anterior portion and paired posterior portions which probably fuse as shown in the 17 mm. stage. The most interesting find to me was that of a definite renal portal circulation in connection with the mesonephros."

These observations coincide with mine on Didelphys so far as the development of the caudal portion of the stem of the postcava is concerned. The presence of a definite renal portal system was not observed by the writer either in the Dasyurus embryo nor in any of the embryos of Didelphys examined, but its presence in Perameles, however, further illustrates the unexpected as well as unusual characters which one occasionally meets with in the embryo, as well as in the adult of the marsupials in general. Of these characters, so far as the venous system is concerned,

the following may be mentioned: The presence of veins in the adult of Didelphys which undoubtedly correspond to the posterior vertebral veins of the sauropsida (Part I, pp. 380-1, these veins have not yet been observed in the Australian marsupials); the course pursued in the adult by the V. cordis magna which is similar to that in birds (Part I, p. 375); the presence of a type of postcava in the adult of Petaurus taguanoides and Phalanger ursinus which is similar to that met with in placentals in which the postcava lies to the right of the aorta and is formed through a union of its iliac tributaries which takes place *dorsal* to the aorta; the presence of posterior abdominal veins in the embryo of Didelphys; the absence of an anastomosis in Didelphys, between the left umbilical and omphalomesenteric veins, to form a ductus venosus Arantii, as in placental mammals.

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EXPLANATION OF FIGURES ON PLATE I.

Fig. 28. Arteries and veins of an adult female Opossum (Didelphys marsupialis, L. or, as more commonly called, D. virginiana, Kerr, L.). Ventral view. Heart reflected to the right and liver, for the most part, cut away. The postcava is formed as in Type II, in which the internal iliac veins unite with the external iliacs dorsal to the common iliac arteries. In addition to the Type of postcava mentioned above the following features should be noticed: The peculiar course pursued by the V. cordis magna; the position occupied by the azygos vein with respect to the segmental arteries; the absence of the anterior and posterior mesenteric arteries; the anastomosis between the renal and spermatic veins which lies on the medial side of the ureter, and the two pairs of internal spermatic arteries.

EXPLANATION OF FIGURES 29 to 61 ON PLATES II-V.

Plate II.

Figures 29-36.

Figs. 29 (section 164) and 30 (section 171). Transverse sections of a 6 mm. embryo of Dasyurus, Series I.

Figs. 31 (section 187), 32 (section 208), 33 (section 211), 34 (section 225), 35 (section 226) and 36 (section 238). Transverse sections of an 8 mm. embryo of Didelphys, Series VIII.

PLATE III.

Figures 37-44.

FIGS. 37 (section 244) and 38 (section 253). Transverse sections of an 8 mm. embryo of Didelphys, Series VIII.

FIG. 39. Transverse section of an 8 mm. embryo of Didelphys, Series X, incomplete.

FIGS. 40 (section 384) and 41 (section 389). Transverse sections of an 8 mm. embryo of Didelphys, Series IX.

FIG. 42. Transverse section of an 8 mm. embryo of Didelphys, Series III, incomplete.

FIGS. 43 (section 293) and 44 (section 350). Harvard Embryological Collection, Series No. 614. Transverse sections of a 10.5 mm. pouch young of Didelphys.

PLATE IV.

Figures 45-52.

FIGS. 45 (section 368), 46 (section 505), 47 (section 520) and 48 (section 521). Harvard Embryological Collection, Series No. 614. Transverse sections of a 10.5 mm. pouch young of Didelphys.

FIGS. 49 (section 244), 50 (section 214), 51 (section 199) and 52 (section 197). Harvard Embryological Collection, Series No. 617. Frontal sections of a 11.5 mm. pouch young of Didelphys.

PLATE V.

Figures 53-61.

FIGS. 53 (section 635), 54 (section 750), 55 (section 772) and 56 (section 789). Transverse sections of 11.5-12 mm. embryo of Didelphys, Series II.

FIG. 57. Transverse section of a 14 mm. pouch young of Didelphys, Series IV.

FIG. 58. Transverse section of a 15 mm. pouch young of Didelphys, Series XII.

FIG. 59 (section 442). Transverse section of a 15 mm. pouch young of Didelphys. Series VII.

FIG. 60. Transverse section of a 15 mm. pouch young of Didelphys, Series IX.

FIG. 61 (section 448). Transverse section of a 15 mm. pouch young of Didelphys, Series VII.

C. F. W. McCLURE

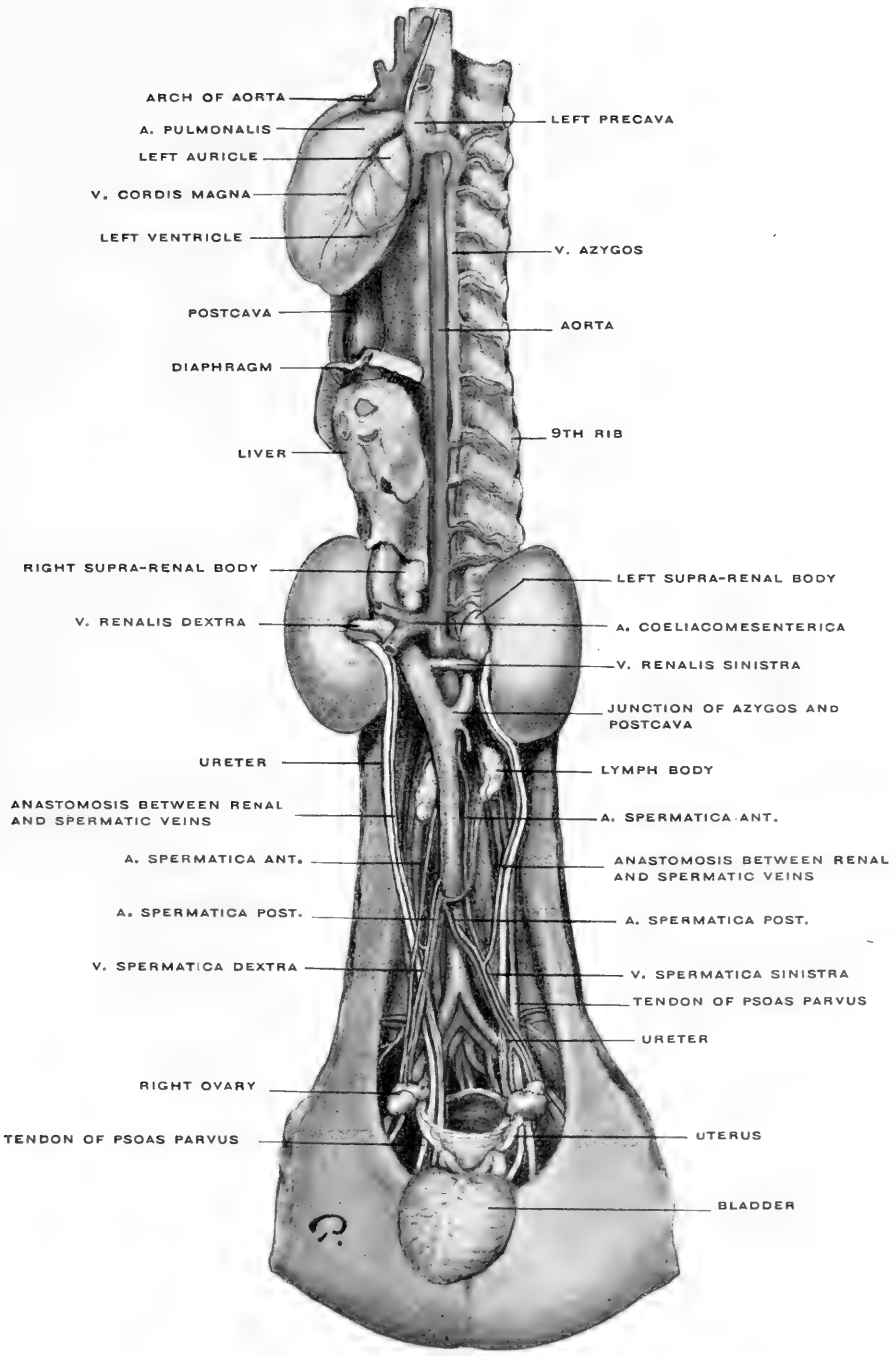

ARCH OF AORTA
A. PULMONALIS
LEFT AURICLE
V. CORDIS MAGNA
LEFT VENTRICLE
POSTCAVA
DIAPHRAGM
LIVER
RIGHT SUPRA-RENAL BODY
V. RENALIS DEXTRA
URETER
ANASTOMOSIS BETWEEN RENAL AND SPERMATIC VEINS
A. SPERMATICA ANT.
A. SPERMATICA POST.
V. SPERMATICA DEXTRA
RIGHT OVARY
TENDON OF PSOAS PARVUS
LEFT PRECAVA
V. AZYGOS
AORTA
9TH RIB
LEFT SUPRA-RENAL BODY
A. COELIACOMESENTERICA
V. RENALIS SINISTRA
JUNCTION OF AZYGOS AND POSTCAVA
LYMPH BODY
A. SPERMATICA ANT.
ANASTOMOSIS BETWEEN RENAL AND SPERMATIC VEINS
A. SPERMATICA POST.
V. SPERMATICA SINISTRA
TENDON OF PSOAS PARVUS
URETER
UTERUS
BLADDER

FIG. 30

FIG. 29

FIG. 32

FIG. 31

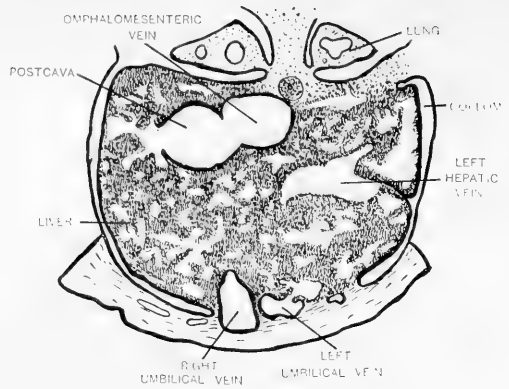


FIG. 33

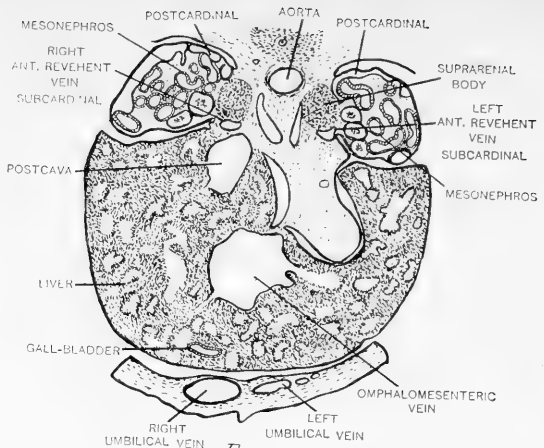


FIG. 34

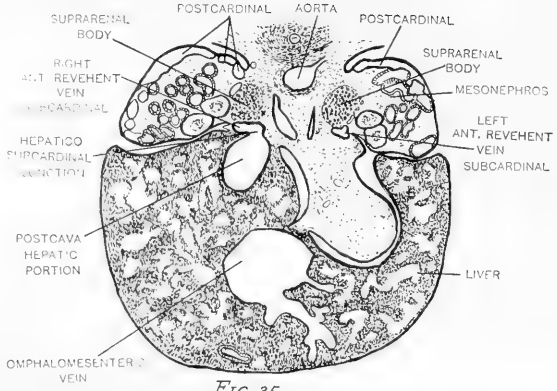


FIG. 35

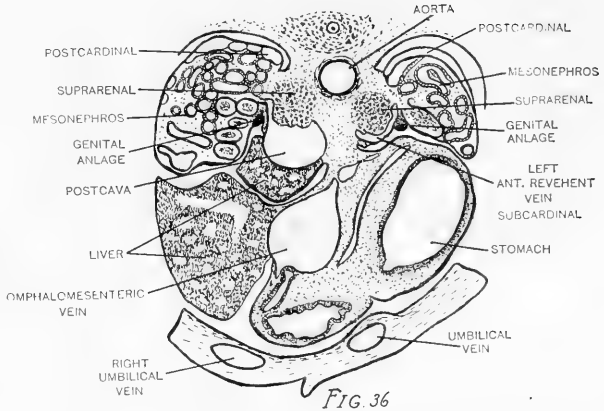


FIG. 36

PLATE II

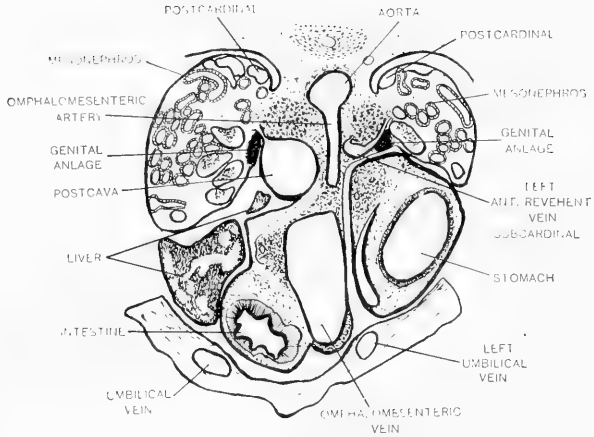


FIG. 37

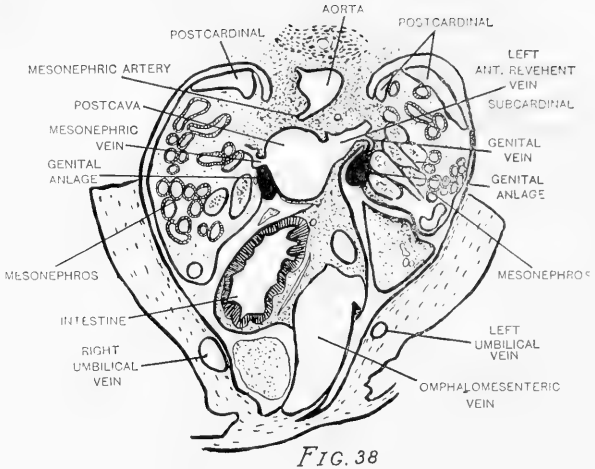


FIG. 38

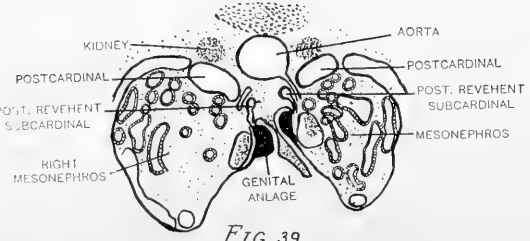


FIG. 39

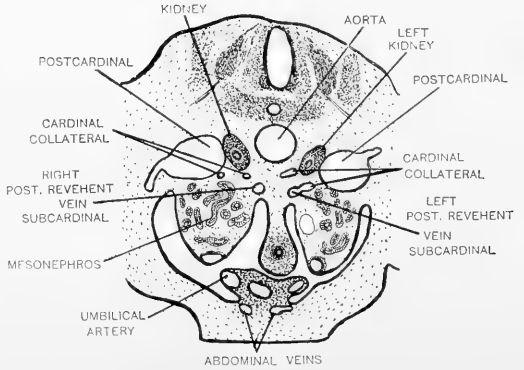


FIG. 40

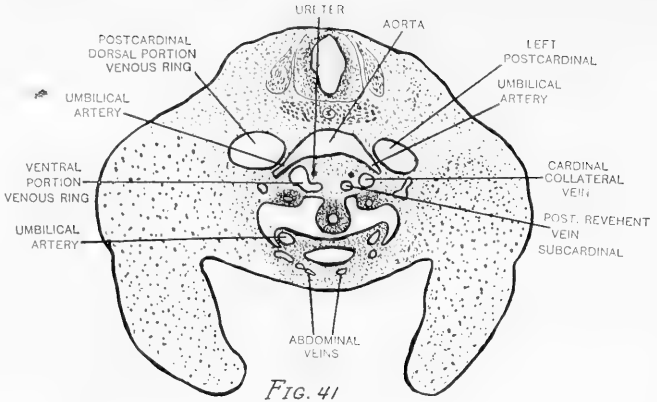


FIG. 41

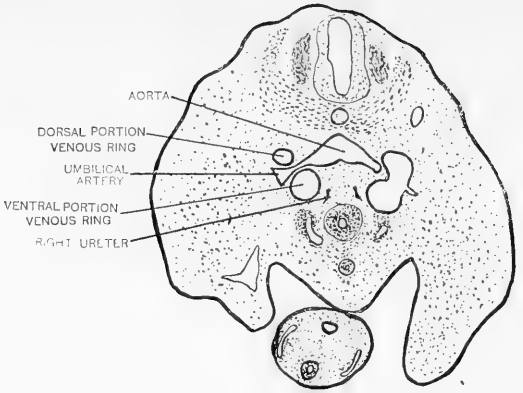


FIG. 42

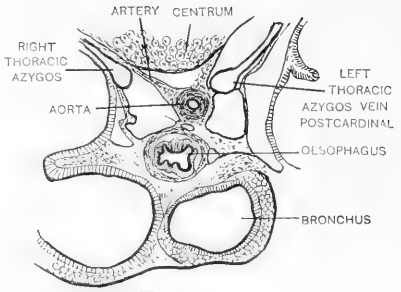


FIG. 43

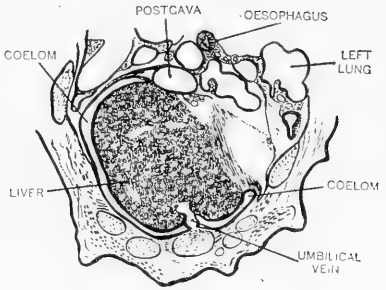


FIG. 44

PLATE III

FIG. 45

FIG. 46

FIG. 47

FIG. 48

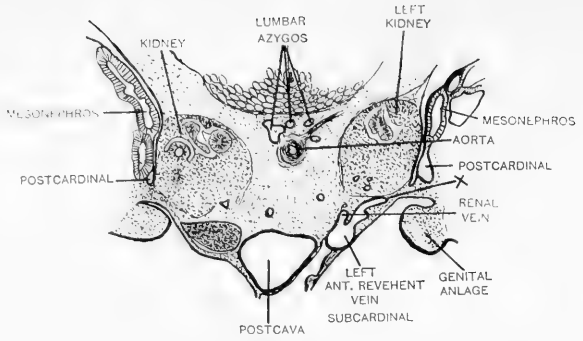


FIG. 49

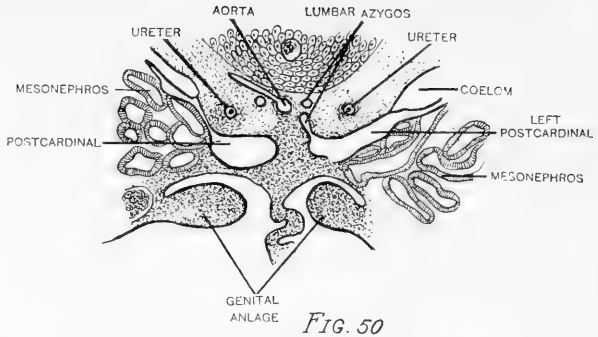


FIG. 50

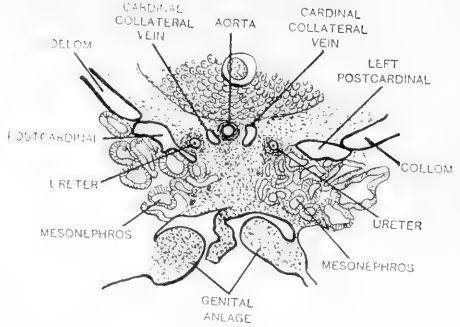


FIG. 51

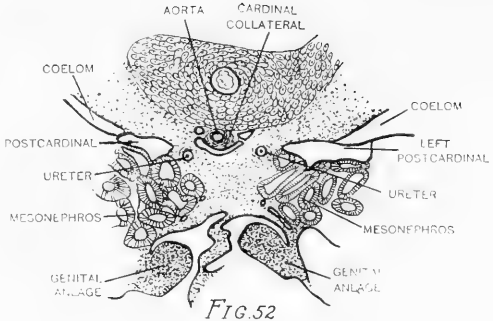


FIG. 52

PLATE IV

FIG. 53

FIG. 54

FIG. 55

FIG. 56

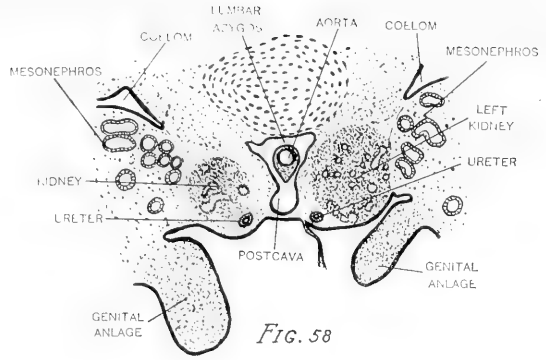


FIG. 58

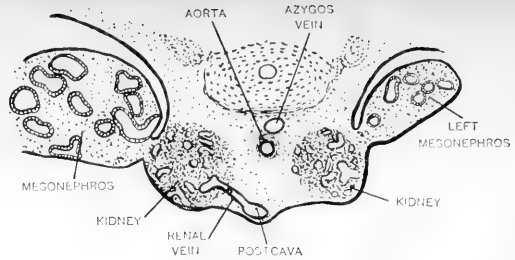


FIG. 57

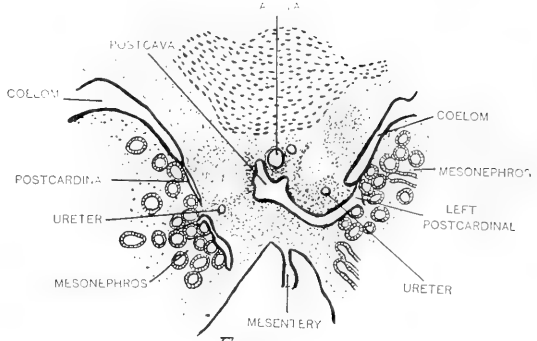


FIG. 59

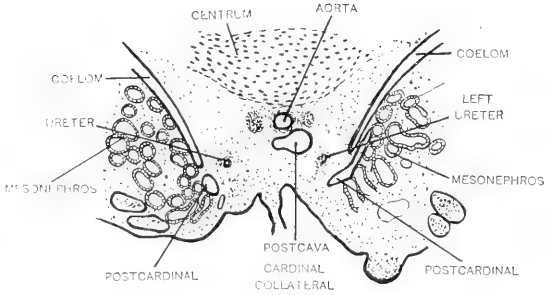


FIG. 60

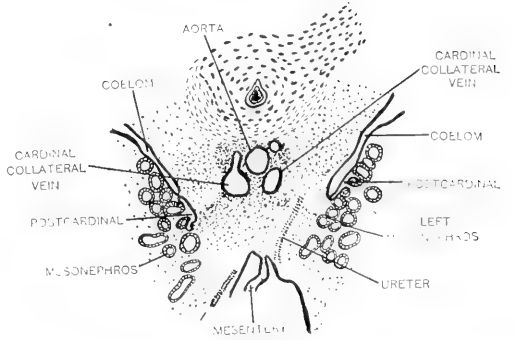


FIG. 61

PLATE V

A STUDY OF THE STRUCTURAL UNIT OF THE LIVER.

BY

FRANKLIN P. MALL.

From the Anatomical Laboratory of the Johns Hopkins University.

WITH 74 FIGURES AND 7 TABLES.

In studying the structural development of an organ it is necessary to consider the systems within it as a whole and to determine their relations to one another. Analytical methods, which must precede synthetical methods, have shown that organs are built up of like parts, or structural units, which are analagous to the leaves of a tree. However, in the growth of an organ the units are not thrown off annually, but are gradually shifted and transformed into new units. It follows that in a study of the kind proposed it is always necessary to consider the unit in relation to the organ as a whole throughout its development, and to do this we must constantly resort to reconstruction. Of course this cannot be done with much success, in the ordinary sense of the term, but for the present purpose, the tables may be considered as reconstruction. Geologists, geographers, archæologists and anatomists each have their own methods of reconstruction, and I have utilized them all, more or less, in the present study.

I undertook the study of the structural development of the liver on account of its well-known and sharply-defined lobule. It was thought at the beginning that this lobule was the simplest of all the structural units and therefore the most suitable for a study of this kind. It soon became evident that the lobule was not the structural unit, and that both lobules and units were extremely difficult to follow in their development, for they are constantly blended with adjacent lobules, or units as the case may be. Furthermore, lobules or units once formed do not remain, but sprout, fracture and rearrange themselves, thus making the various pictures obtained complex and difficult to interpret.

The work has been carried on during a number of years, after being laid aside in order to take up the same question in other organs. These secondary studies,—usually made by others connected with me,—have aided my work for the liver materially, which I now venture to present in a more or less connected form.

Historical Note.

In 1664 Wepfer described lobules in the liver of the pig, and two years later they were again described by Malpighi who gave them their name. Malpighi states that the livers of all vertebrates are conglomerate glands, being composed of lobules which in turn contain acini. For a long time after this the capital problem in the anatomy of the liver was the study of the structure of the lobules and their relation to one another.

In 1733 Ferrein described these lobules as being composed of two substances, brown and yellow (substantia fusca and substantia flava) which formed respectively its medullary and cortical portions. In general this description was accepted by anatomists, sometimes, however, with a reversal of the arrangement of the colors in the medullary and cortical portions of the lobule.[1]

In 1832 E. H. Weber showed that the two colors of the lobule are due to an unequal distribution of blood in it, and a year later Kiernan, in his classic paper, denied altogether that the lobule was composed of two substances as described by Ferrein. We owe to Kiernan our present conception of the lobules; he described their shape and relation to one another, the amount and character of tissue between them, and what is more, their relation to the vascular system; he also introduced our present nomenclature. The defining line around a lobule was broken up into spaces and fissures, spatia interlobularia when three lobules came together, and fissuræ interlobulares between each two adjoining lobules. The spaces and fissures, which were not always easy to demonstrate, were no doubt included by Ferrein in the cortical portion of the lobule. It is seen that Kiernan's interlobular spaces and fissures form a network between the lobules, and for this reason Theile calls them the substantia reticularis, and the lobule proper the substantia granosa. It was also shown that the order of the reticular and granular substances are reversed in hepatic congestion; in it the brown reticulum encircles yellow granules, pseudogranules, as Theile calls them. The yellow "pseudolobules" are tough and more consistent than the true brown lobules.

Before the time of Kiernan the usual confusion of terms naturally arose. For instance, Autenrieth accepted Ferrein's cortical and medullary portions of the lobule, only he reversed the order of their colors. Evidently he was describing "pseudolobules." Merkel, who also no doubt studied hyperæmic livers, did the same. Krause took the "happy mean" course

[1] See Kiernan, Phil. Trans., 1833; Theile, Handworterbuch der Physiologie, II, 1844; and Oppel, Lehrbuch der Vergleichenden Mikroskopichen Anatomie der Wirbelthiere, III, 1900.

and described pseudolobules, *i. e.*, the yellow interlobular connective tissue, with hepatic veins in their center. Cruveilhier made the same blunder. Numerous other terms were used in a variety of ways, as, for instance, acinus which meant anything from a cell to an entire lobule, according to different authors.

The lobule, as described by Kiernan, received its strongest support in its having on its periphery the terminal twigs of the portal vein, hepatic artery, bile duct, and an increased quantity of connective tissue, which in the pig forms a distinct capsule. Had it not been for an occasional animal with a lobule so well outlined and a great authority like J. Müller, it is probable there would still be much confusion in spite of the "happy means" and the innumerable terms. The study of the structure of the liver illustrates beautifully the value of great minds in the study of any subject. We see during a period of two centuries that the generalizations of Malpighi, Ferrein, and J. Müller are consistent and practically correct in spite of the great amount of confusion and opposition brought from many quarters. Taking all of the facts into consideration, analysis by means of injection experiments, finally gave us a structural unit of the liver which has withstood all opposition.

Lobules of the liver are certainly not well marked in most animals and it is seen by the foregoing that the lobules were as often found encircling the portal twig as around the hepatic twig. A glance at Fig. 1 will show why either arrangement is correct. With the facts before him, it is not remarkable that E. H. Weber denied the anatomical existence of the lobule, *i. e.*, a lobule that can always be seen and is always the same. However, J. Müller, with the livers of the pig and of the polar bear as examples set the question at rest for a time.

The "psuedo lobule" of Theile, with the strong connective tissue of the portal space as a center, is tougher than the true lobule which has only a delicate reticulum to hold it together. Theile has shown that it is easy to isolate the "pseudo lobule" of the dog's or the rabbit's liver, while it is impossible to isolate the true lobule. In fact, if livers of these animals are crushed and washed in a stream of water, the whole system of lobules is isolated, clustered around the branches of the portal vein, forming a specimen which may be likened to a bunch of grapes.

Sabourin has described the liver as being composed of biliary lobules with the terminal bile ducts as their centers.[2] He has accepted the pseudo

[2] Sabourin, Le Progrès Méd., VI, 1883; Recherches l'anat. normale et pathol. de la glande biliaire de l'homme, 1888; Rev. Méd., XXI.

lobule of Theile as the true unit of the liver, which in Phoca [3] is outlined by a capsule as the hepatic lobule is in the pig. The biliary or portal lobule has been used as a basis by Berdal [4] in his histology and has been

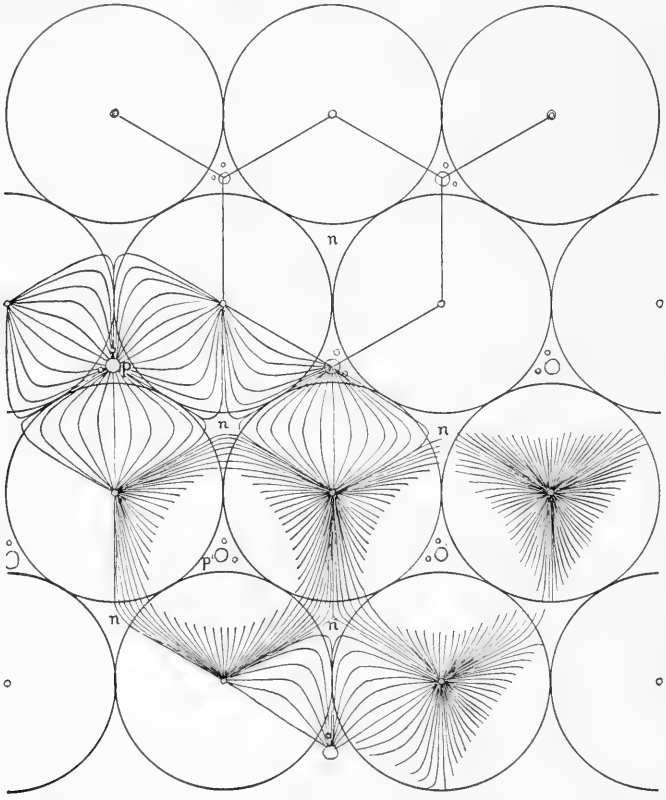


FIG. 1. Diagram of a transverse section of a group of lobules with lines indicating the course of the capillaries. The lobules as usually understood are marked with circles. *P*, a portal unit; *n*, nodal point; p^1 a portal unit outlined as in partial congestion.

advocated by myself for a long time.[5] Recently it has been discussed as the secreting lobule by J. B. MacCallum [6], and has been defended from an embryological standpoint by F. T. Lewis.[7]

[3] Brissaund et Sabourin, Compt. rend., Soc. de biol., XL, 1888.
[4] Berdal, Éléments d'histologie normale, Paris, 1894.
[5] Mall, Zeit. f. Morph. u. Anthropol., II, 1900.
[6] MacCallum, J. B., English translation of Scymonowicz's Histology.
[7] Lewis, F. T., Anat. Anz., XXV, 1904.

In all other glands we make the duct the center of the structural unit. From this center often the artery and the framework radiate. In the liver everything radiates from the so-called interlobular space,—arterial and portal blood vessels, bile duct, lymphatics, nerves and connective tissue; the liver develops from this point; physiologically everything centers there. So, viewed from any standpoint, it is the center of the structural unit.

Throughout my description I shall use the term portal structural unit, portal unit, structural unit, or unit, for the clump of tissue which surrounds each terminal branch of the portal vein. In order to avoid confusion I shall use the term lobule in its old sense,—as the hepatic lobule,—for after much discussion carried on during two centuries, it has become well established. The old idea, the idea of Wepfer, Malpighi, Ferrein, Kiernan, and J. Müller should be marked with the word lobule; the new idea, associated with the unity of structure, should be called the unit.

Vascular Proportion.

Roux has stated in one of the theses in his *Habilitationsschrift*[8] that the lobular subdivisions of the liver are due, in their arrangement and form, to the vascular system. This same idea is again brought forth several years later in the Introduction to his *Archiv*.[9] In this he says, on page 17, "Die Gliederung der gewöhnlichen, baumartig verästelten Drüsen in Läppchen erscheint durch die gestaltenden Wirkungen der Epithelien, also der specifischen Teile, bedingt und ist, so weit dies richtig ist, Selbstdifferenzierung der Drüsensubstanz. Bei der Leber dagegen, einer Netzdrüse, erscheint die normale Grösse und Gestalt der Läppchen und auch die lobuläre Gliederung selber durch die Blutgefässe bedingt, und zwar einmal durch die geeignete Kapillarlänge wie zweitens durch die Eigenschaft der letzten Verästelungen der Vena portæ, beim Wachsthum des Kapillarnetzes dichotomische Verzweigungen in letzterem auszubilden. Die acinöse Gliederung des Leberparenchyms stellt somit eine von dem Blutgefässsystem abhängige Differenzierung der Drüsensubstanz dar."

A much more extended discussion of the growth and proportion of the vascular system is given by Thoma in his numerous papers, but that

[8] Roux, Ueber die Leistungsfähigkeit der Principien der Descendenzlehre zur Erklärung der Zweckmässigkeiten des thierischen Organismus, Breslau, 1880, and Gesammelte Abhandlungen, I, 1895, p. 134.

[9] Roux, Einleitung zum Archiv für Entwickelungsmechanic der Organismen. Leipzig, 1894, p. 17.

which relates to the capillaries in particular is to be found in his brilliant study of the blood-vessels of the area vasculosa of the chick.[10] It is not possible to discuss in detail the many observations and arguments in this model research without extending this paper far beyond the space of this Journal. However, an excellent summary of Thoma's work is given in his Pathology, from which I will quote several pages from the English translation.[11] Thoma's work is summed up in three laws or histomechanical principles (page 265), as follows:

(1) "*The increase in the size of the lumen of the vessel, or what is the same thing, the increase in the surface of the vessel wall, depends upon the rate of the blood-current.* The surface of a vessel wall ceases to grow when the blood-current acquires a definite rate. The vessel increases in size when this rate is exceeded, becomes smaller when the blood-stream is slowed, and disappears when it is finally arrested.

"This law which brings the growth of the surface of the vessel wall into dependence upon the rate of the flow of the blood is, I consider, the first and most important histo-mechanical principle which determines the state of the lumen of the vessel under physiological and pathological conditions. It will be further proved, however, in many places in the general, as well as in the special parts of this book.

"A second histo-mechanical principle may be added to this, viz., *the growth in thickness of the vessel wall is dependent upon its tension. Further the tension of the wall is dependent upon the diameter of the lumen of the vessel and upon the blood-pressure.*

"The proof of this law is to be sought, in the first place, in the varying strength of the wall of the larger and smaller arteries, veins, and capillaries. In certain diseases of the vessels (arteriosclerosis, aneurism) there are apparent exceptions which will be discussed in their proper place.

"The third histo-mechanical principle has not hitherto been so completely demonstrated as the first two. It will, therefore, be put forward merely as an hypothesis, which runs as follows: *increase of blood-pressure in the capillary areas leads to new formation of capillaries.*

"The three histo-mechanical principles were, in the first place, employed to explain the developmental processes in the area vasculosa of the chick. In this flat extended area a capillary network is found at an

[10] Thoma, Untersuchungen ueber die Histogenese und Histomechanik des Gefässsystems. Stuttgart, 1893.

[11] Thoma, Text-book of General Pathology and Pathological Anatomy. Translated by Bruce. London, 1896.

early date in which no arterial and venous channels can be differentiated (Fig. 2). A few channels are, however, selected by the blood-stream in consequence of the general direction which is given to it by the position of the ends of the primitive aorta on the one side, and of the venous ostia of the heart on the other. These channels (Fig. 2, *a, b, c*) contain the more rapidly flowing streams. They, therefore, dilate and become converted into arteries and veins. (Fig. 3).

" Other channels, in which the rate of the flow of the blood has a cer-

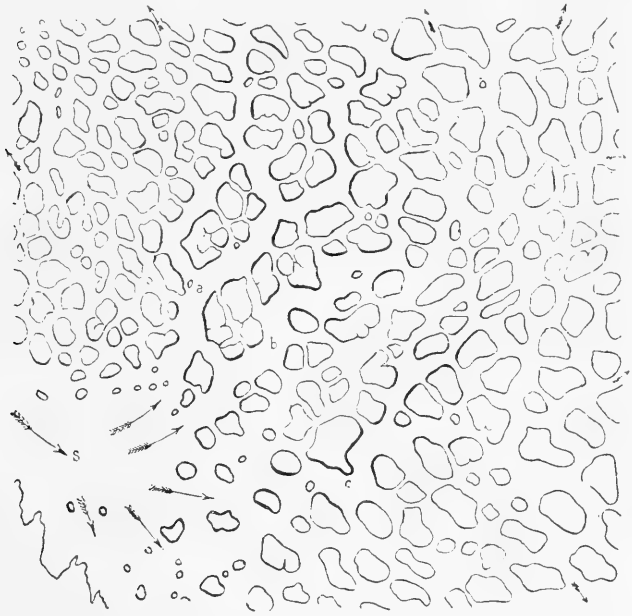


Fig. 2. Capillary channels of the area visculosa after forty-eight hours' incubation. *S*, peripheral end of the primitive aorta; *a, b, c*, selected blood-channel, × 30. After Thoma.

tain medium force, remain as capillaries, and lastly, some channels which offer great resistance to the stream, and are thus very slowly traversed, atrophy, or disappear altogether. The rapid growth of the selected channels diminishes the resistance to the blood-stream, so long as the capillary area remains unaltered. The blood-pressure in the capillary area accordingly rises and leads to new formation of capillaries. New communications are thus formed between the terminal ramifications of the arteries and veins; the capillary area is thus relieved, and its blood-pressure falls. Arteries and veins have now become wider and longer, and the capillary

area has increased in extent. A larger quantity of blood will flow into it, therefore, and this will involve a corresponding increase in the total resistance to the stream within the enlarged capillary areas. The chain of processes described may therefore be repeated until any one link in the chain becomes incapable of further increase.

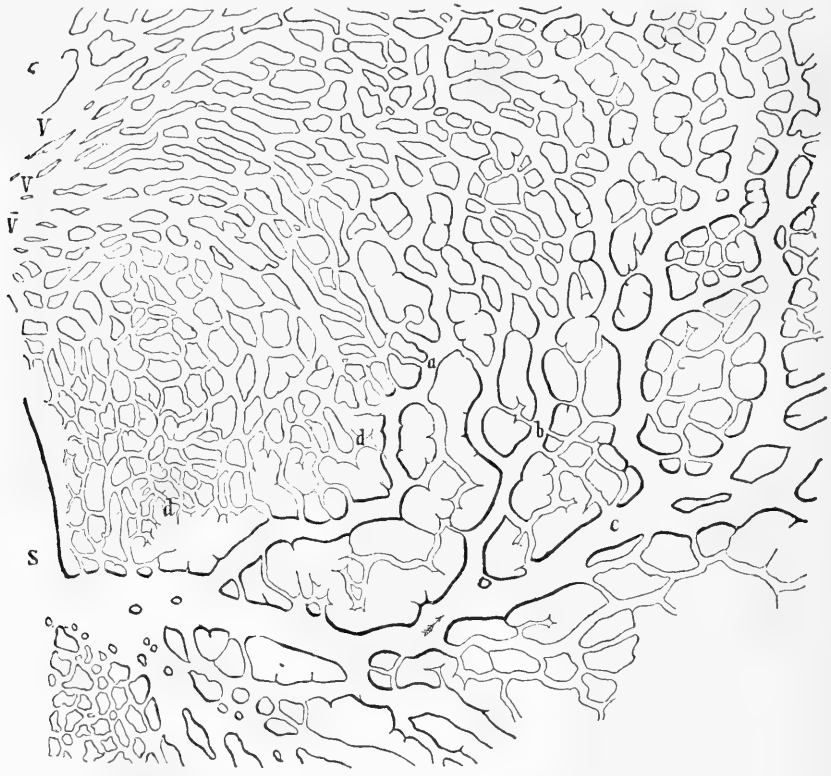


Fig. 3. Blood-vessels from the area vasculosa after fifty-seven hours' incubation. The same part as in Fig. 2. *S*, peripheral end of the primitive aorta; *a*, *b*, *c*, the selected channels of entrance to capillary network; *V*, *V*, *V*, venous exit of latter; *d*, *d*, *d*, the beginning of the second capillary network. × 25. After Thoma.

"If we consider that this chain of processes is constantly repeated within short spaces of time, and that at each time only a slight alteration of the previously existing relations is produced, we may form a fairly accurate conception of the mode of growth of the vascular system.

The details of this will not be considered here. If, however, we apply the above principles to any organ whatever which has a longer existence

than the area vasculosa, we must admit that the histo-mechanical principles justify us in assuming that, after the organ has ceased to grow, the rate and presence of the blood in all its capillaries are approximately equal.

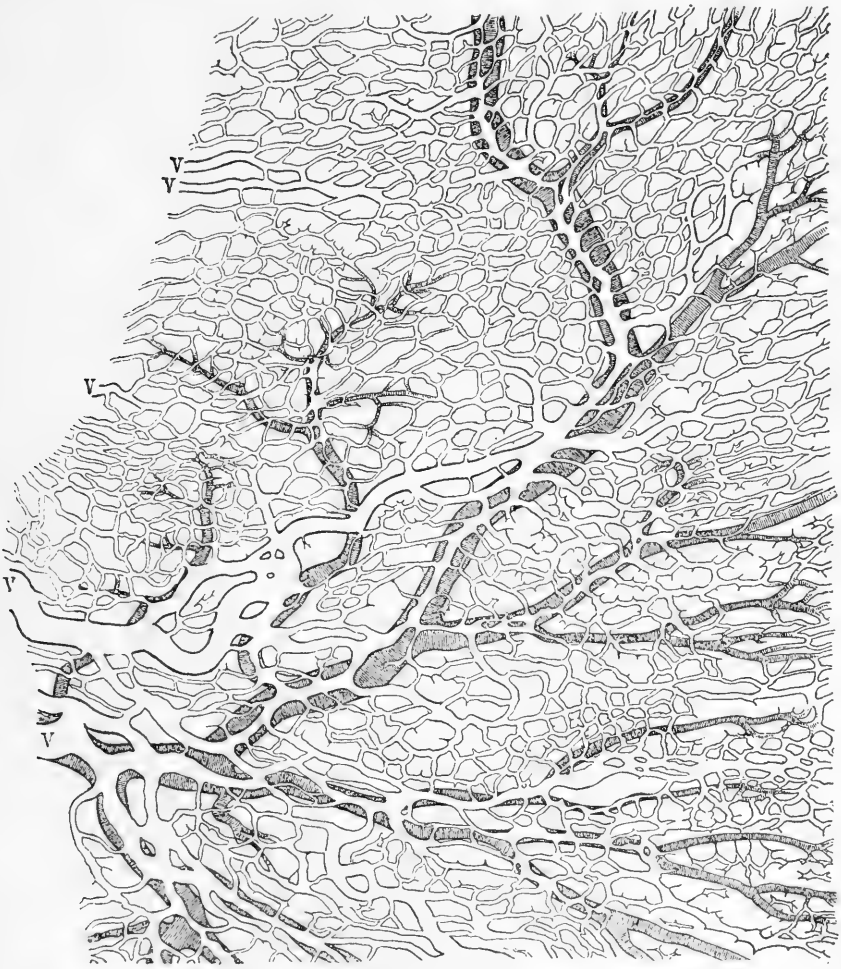


Fig. 4. Part of the area vesicular of a chick incubated seventy-four hours. The dorsal aspect is presented. *V*, *V*, veins. The arteries are dark. × 21. After Thoma.

"In this organ, according to the first histo-mechanical principle, all blood channels in which the rate of flow exceeds a certain maximum, must increase in lumen and become converted into arteries and veins. Vice

versa, all vascular channels will disappear in which the rate of the blood-stream falls below a certain maximum. If, however, the lumen of the vessel bears a fixed relation to the rate of the blood-current, the interval between the maximum and minimum cannot be great. From this it appears that, after growth is completed, the rate of flow must be fairly uniform in all the capillaries of an organ.

"The conversion of capillaries into arteries diminishes the resistance of the blood-stream, and leads to an increase of pressure in the capillaries. If, then, according to the third histo-mechanical principle, new capillaries are formed at all places in the capillary area in which the pressure of the blood exceeds a certain limit, these capillaries, again, reduce the pressure by forming new connections between the arteries and veins. The third histo-mechanical principle, therefore, implies that, during the growth of the organ, new capillaries are being formed everywhere, and that, after complete growth, the blood-pressure in all capillary areas of the same organ is fairly uniform.

"The width of the lumen of the capillary channel at the close of the period of growth must be almost the same in all areas of the same organ, since it depends on the rate of flow, and this rate is uniform in all capillaries of the same organ.

"These conclusions are in perfect harmony with the actual state of matters. It appears, however, that in the different organs there are great differences in the width of the lumen and in the number of their capillaries, in the rate of flow, and in the quantity of the blood flowing through a given area of the vessel in a given time.

"If these facts be compared with the results which were obtained above, according to which the first vascular spaces, the rudimentary capillaries, were formed by the secretory activity of the cells forming their wall, we are compelled to assume that the metabolic processes and other special characteristics of the various organs also exercise a determining influence on the peculiarities which distinguish their capillaries. It must be imagined that the individual characters of the organ, and its size in relation to other organs, decide firstly the number of capillaries in the whole organ and in a single part of the organ; further, the special relations existing between the rate of flow and the lumen of the capillary channel; and lastly, the height of the blood-pressure which will lead to the formation of new capillaries. If, for example, the growth of the capillaries is arrested in one organ at a rate of flow *a*, corresponding to a lumen *b*, in a second organ the growth of the capillary lumen might perhaps be arrested at a rate of flow *A* corresponding to a lumen *B*. Thus, in the

one organ, capillary new formation would occur when the pressure of the capillary blood exceeds the limit *c*, while, in the other organs, this limit might be higher at the blood-pressure *C*.

"The number of capillaries, their lumen, and the rate of flow of the blood-stream passing through them, determine, as has been observed, the total quantity of the blood which flows through the entire organ. We thus arrive at the remarkable result that *it is the organ iself which determines the quantity, the rate of flow, and the pressure of the blood flowing through it; and that this is effected by means of fixed relations which are expressed generally in the three histo-mechanical principles. The conditions which produce the uniformity of pressure and rate of the blood-current in all capillary areas of the same organ are included in these principles.*

"According to the generally accepted view of the problem of the circulation, which was formerly quite sufficient to serve as a basis for the account of its general disturbances, the pressure, the rate, and the amount of the blood-flow appeared to be directly dependent upon the action of the heart. According to the view given here, on the other hand, it is the metabolic processes in the organs, which determine first for the individual organs, then for the whole of the organs—that is, for the circulation as a whole—the amount of blood propelled within a given time, its pressure and its rate of flow. In this case, the working-power of the heart appears as the equivalent of the sum of the histo-mechanical demands made by the organs."

It will be seen that Thoma concludes, and I think properly, that capillaries of like component parts of an organ are of equal size and length, and that the rapidity of the circulation through them is also equal. This idea I have also tried to develop in various papers upon the structural unit of organs. It appears that each organ is broken up into units which are of equal value from anatomical and physiological standpoints. What takes place in one unit takes place in all of the rest. A good example is to be found in the intestine where the structural unit is a villus surrounded at its base with a circle of intestinal glands (crypts). In the center of the group is the main artery which passes directly to the apex of the villus and ending there divides abruptly into an umbrella of capillaries which lie at the periphery of the villus. These capillaries are about of one diameter and length, and no matter what course is taken by the blood the distance and resistance in passing from the artery to the vein is always the same.[12] Ludwig pointed out that the capillaries of an organ

[12] Mall, Abhandl. d. K. S. Gesell. d. Wiss., XIV, 1887.

were always equally favored by the circulation, and that many descriptions and illustrations of the blood-vessel, as, for example, of the villus and the glomerules, could not possibly be correct. If in reality the blood-vessels of these structures were arranged as is frequently pictured, the blood would have to take the capillaries in the course of the least resistance, while in those of the greatest resistance it would stagnate or come to a standstill. Thoma's first law explains how an equal distribution which favors no part of an organ is brought about. In development the vessels in which the blood stagnates degenerate, and in those in which the rapidity is too great the lumen is enlarged. There seems to be a tendency to maintain a "normal" flow of blood through the capillary. After capillaries are well dilated they become arteries and veins, and the thickness of their walls is now dependent upon their tension, according to Thoma's second law. These two laws are constantly at work, and regulate accurately the diameter and thickness of the walls of the arteries and veins.

Before considering Thoma's third histo-mechanical principle, it is necessary to discuss his numerous measurements as well as to give data which I have accumulated. The whole question hinges upon the cause of the new formation of capillaries for which Thoma has not found a law, but has merely put forward an hypothesis.

Thoma made many measurements of arteries and their branches and tabulated Bencke's measurements of the aorta with its branches. These measurements show that the area of all of the branches of the aorta equals about the area of the ascending aorta, being a little less before the thirtieth year of age and a little greater thereafter. Thoma gives a few measurements of small arteries in which the area of the immediate branches equals about that of the main stem. These measurements, however, are not constant in live animals, for if the observations are continued, the caliber of the branches increases out of proportion, and ultimately their area exceeds that of the main stem.[13] This change Thoma ascribes to a change in the vascular tone. In other parts of the same work,[14] as well as elsewhere, he appears to be somewhat uncertain regarding the equality of the area of a vessel and the area of all of its branches. Also in a later publication the arguments seem to accumulate against this view.[15] Thoma states, however, that the exceptional cases are found

[13] Thoma, Histogenese u. Histomechanik, 66.

[14] Thoma, Ibid., p. 86; Pathology, 275 and 276.

[15] Thoma, Ueber den Verzweigungsmodus der Arterien. Arch. f. Entwick. d. Organismen, XII, 1901.

in growing arteries, the umbilical, for instance, which is to be expected, for the peripheral bed is enlarging. After the vessels cease to grow the area of the vascular bed is about the same from the ascending aorta to the smallest arteries; the bed enlarges in the capillaries. Under such conditions (homonomous ramification) the average rate of the current is equal in all of the arteries.

Whether the ramification is homonomous or heteronomous appears to me to be of little consequence, and I have pointed out the uncertainty of Thoma's statements for my own measurement, which are quite numerous, and decidedly in favor of a heteronomous ramification. Thoma's assumption of homonomous ramification is based largely upon the measurements upon the aorta and its branches. From now on, however, the vascular bed enlarges, at first slowly, and more rapidly as the capillaries are approached. The bed has doubled itself in the arteries one millimeter in diameter and has increased about fivefold in arteries .05 mm. in diameter. A change so slight as this could barely be detected when the measurements are made in adjoining internodes. In order to obtain reliable figures the measurements must be made farther apart. For instance, it is easy to lay the intestine of the dog into a series of anatomical units to correspond with the arteries—mesenteric arches, arches to the submucosa and arteries to the villi. If the area of the superior mesenteric artery is 7 sq. mm. and that of the ends of the main branches but 12 sq. mm., it will be seen that when a trunk divides into two branches the change in area will be but slight. But when we compute the number of villi, and this is easily done, we determine at the same time the number of terminal arteries to the villi, all of which are about of the same size. At this point, as Table I shows, the artery is .0225 in diameter and the bed is nearly 60 times the area of the superior mesenteric artery. If the ramification were homonomous down to the arteries of the villi there should be but 17,000 villi, the number which can be counted upon 10 sq. cm. of mucous membrane. No matter how the following tables are compared, it is seen that there is a gradual widening of the vascular bed from the branches of the aorta to the capillaries.

TABLE I.

Giving the vascular bed of the dog's small intestine. (Mall, Abhandl. d. K. S. Ges. d. Wiss., XXIV, 1887.)

	Vessels	Number	Diameter mm.	Area of Section sq. mm.
[1]	Superior mesenteric	1	3.0	7.07
[2]	Main branches	15	1.0	11.78
	Terminal branches	45	.6	12.72
	Short intestinal arteries	1,440	.08	7.24
	Long intestinal arteries	459	.192	13.29
	Long and short intestinal arteries	....	...	20.53
	Terminal branches of short intestinal arteries	8,640	.05	16.96
	Terminal branches of long intestinal arteries	18,000	.053	39.71
[3]	Total terminal branches	26,640	....	56.67
	From the submucosa to the crypts	4,000,000	.008	201.06
	From the submucosa to the villi	328,500	.301	247.94
	Arteries of the villi	1,051,000	.0225	417.97
	Capillaries { upper one-third	31,536,000	.008	1,585.17
	Capillaries { lower one-third	15,768,000	.005	309.6
[4]	Total capillaries of crypts and villi	51,304,000	....	2,095.83
	Veins of the villi	2,102,400	.0265	1,159.57
	Veins penetrating the muscularis mucosæ	131,400	.075	580.51
	Terminal branches in the submucosa	18,000	.128	231.62
	Anastomoses in the submucosa	2,500,000	.032	2,010.62
	Terminal branches of the long and short intestinal veins	28,800	.064	92.65
	Long intestinal veins	459	.44	69.79
	Short intestinal veins	1,440	.112	14.19
	Last branches of superior mesenteric vein	45	1.5	79.52
	Branches of the mesenteric vein	15	2.4	67.56
	Mesenteric vein	1	6.0	28.27
	Muscle Coats.			
[3]	Direct muscle arteries	1,800	.03	1.27
[3]	Recurrent muscle arteries	3,600	.04	2.54
	Capillaries of the circularis	27,000,000	.003	190.85
	Capillaries of the longitudinalis	9,000,000	.003	63.62
[4]	Total capillaries	36,000,000	....	254.47
	Veins	3,600	.112	35.46

TABLE II.

Giving the vascular bed of this dog's stomach. (Mall, Johns Hopkins Hospital Reports, I, 1889.)

	Vessels	Number	Diameter mm.	Area of Section sq. mm.
	Aorta	1	6.0	28.27
	Cœliac axis	1	2.75	5.92
[1]	Gastric	1	1.7	2.27
	Splenic	1	2.5	4.91
	Hepatic	1	2.75	5.92
	To the stomach: From the gastric	1	1.7	2.27
	To the stomach: From the splenic	1	1.92	2.89
	To the stomach: From the splenic	4	.486	.74
	To the stomach: From the hepatic	1	.5	.196
[2]	Total	..	...	6.096
	From the branches of the first order to the stomach	108	.415	14.58
	Second order	740	.2	23.26
[3]	Third order	5,920	.075	26.17
	To the mucosa	76,960	.025	37.79
	Stellate arteries	615,680	.017	139.59
[4]	Capillaries	22,800,000	.006	645.24
	Subepithelial venous plexus	1,643,600	.02	493.08
	Interglandular plexus	431,649	.037	445.89
	Subglandular plexus	333,090	.049	577.25
	Branches piercing the submucosa	12,768	.0867	75.33
	Large branches in submucosa	1,480	.2	44.4
	Veins from stomach	108	.5	21.6
	Pyloric	1	2.0	3.14
	Gastro-epiploica dextra	1	2.5	4.91
	Gastro-epiploica sinistra	1	3.0	7.07
	Gastric	1	3.3	8.55
	Pancreatico-duodenal	1	5.0	19.64
	Splenic	1	6.0	28.27
	Portal	1	9.0	63.64
	In the Muscle Coats.			
	Direct muscle arteries	108	.1	.86
[3]	Recurring muscle arteries	342	.25	16.64
	Arteries of intermuscular plexus	5,016	.1	39.39
[4]	Capillaries of circularis	25,500,000	.003	180.25
[4]	Capillaries of longitudinalis	13,000,000	.003	91.89
	Direct muscle veins	108	.15	1.91
	Recurring muscle veins	342	.33	29.25

TABLE III.

Giving the vascular bed of the dog's adrenal. (Flint, Welch Festschrift, Johns Hopkins Hospital Reports, IX, 1900.)

	Vessels	Number	Diameter mm.	Area of Section sq. mm.
[1]	Arteries, first order	11-19	.17	.339
	second order	49	.15	.865
	third order	160	.13	2.123
	fourth order of cortex	532	.1	4.168
	fourth order of medulla	49	.1	.384
	Total fourth order	581 [16]	.1	4.552
	fifth order of cortex	2,320	.045	4.547
	fifth order of medulla	288	.08	1.445
[3]	Total fifth order	....	...	5.992
	sixth order of cortex	13,920	.025	6.82
	sixth order of medulla	3,168	.035	3.037
	sixth order of capsule	1,571	.018	.399
	Total sixth order	....	...	10.256
	capillaries of cortex	3,724,933	.008	186.246
	capillaries of medulla	354,742	.007	12.650
	capillaries of capsule	17,281	.007	.664
[4]	Total capillaries	....	...	199.560
	VENOUS TREE.			
	Veins, seventh order, gland	12,396	.04	15.495
	seventh order, capsule	2,156	.037	2.177
	Total seventh order	....	...	17.672
	sixth order, gland	2,590	.08	13.001
	sixth order, capsule	44	.1	.345
	Total sixth order	....	...	13,346
	fifth order, gland	390	.12	4.407
	fifth order, capsule	24	.18	.610
	Total fifth order	....	...	5.011
	fourth order, gland	57	.2	1.700
	fourth order, capsule	3	.25	.147
	Total fourth order	....	...	1.847
	third order, gland	4	.4	.502
	second order, gland	2	.6	.563
	first order, gland	2	.8	1.005
	lumbar vein	....	4.0	12.566

[16] The table is rearranged and a few corrections made according to instructions from Dr. Flint in a letter of April 14, 1905.

TABLE IV.

Giving the vascular bed of the dog's spleen.

Vessels	Number	Diameter mm.	Area of Section sq. mm.
[1] Splenic artery	1	2.5	4.91
Branches to spleen	2	1.9	5.67
[2] First order	15	.75	6.63
Second order	1,000	.1	7.85
[3] Lobular	80,000	.015	14.0
[4] Terminal	40,000,000	.008	4021.0
Pulp spaces contracted	500,000,000	.01	39,270.0
Pulp spaces dilated	500,000,000	.02	157,080.0
Interlobular veins	80,000,000	.04	100,531.0
Interlobular veins	80,000	.08	402.0
Second order	1,000	.5	196.0
First order	15	4.0	188.0
Branches from spleen	2	5.0	39.26
Splenic vein	1	6.0	28.27

TABLE V.

Giving the vascular bed of the dog's lung. (Miller, Journal of Morphology, VIII, 1893.)

Vessels	Number	Diameter mm.	Area of Section sq. mm.
[1] Pulmonary artery	1	15.5	181
Right and left branches	2	11.5	208
Lobar arteries	8	5.96	223
First order	24	3.96	293
Second order	164	2.26	656
[2] Third order	1,021	1.0	801
Lobular arteries	16,000	.3	1,120
Atrial arteries	64,000	.115	1,344
[3] Sac arteries	128,000	.165	2,688
[4] Capillaries	600,000,000	.007	23,000
Sac veins	192,000	.23	7,680
Atrial arteries	32,000	.45	6,098
Lobular veins	16,000	.4	2,000
Third order	1,021	1.22	1,194
Second order	164	2.44	765
First order	24	4.18	340
Lobar veins	8	6.12	299
Venous trunks	4	13.75	756

TABLE VI.

Giving the vascular bed of the dog's portal system.

	Vessels	Number	Diameter mm.	Area of Section sq. mm.
[1]	Portal vein	1	9.0	64.0
	Branches of the first order	6	5.0	118.0
	Branches of the second order	70	1.7	159.0
[2]	Branches of the third order	700	.8	352.0
	Branches of the fourth order	8,000	.4	1,005.0
	Branches of the fifth order	80,000	.15	1,414.0
[3]	Branches of the sixth order (interlobular)	960,000	.05	1,881.0
[4]	Capillaries	1,850,000,000	.008	92,900.0
	Sixth order of hepatic vein (central)[17]	480,000	.09	2,900.0
	Fifth order of hepatic vein	80,000	.17	1,816.0
	Fourth order of hepatic vein	8,000	.5	1,571.0
	Third order of hepatic vein	700	1.0	410.0
	First order of hepatic vein	70	2.0	220.0
	Second order of hepatic vein	7	5.0	134.0
	Hepatic vein	1	11.0	95.0
	ARTERY.			
[1]	Hepatic artery	1	2.75	5.9
[2]	Branches to the liver	2	1.18	2.2
	Branches of the first order	6	.8	3.0
	Branches of the second order	70	.3	4.9
	Branches of the third order	700	.1	5.5
[3]	Branches of the fourth order	8,000	.05	15.7
	Branches of the fifth order	80,000	.02	25.0
	Branches of the sixth order	960,000	.009	61.0
[4]	Capillaries	1,850,000,000	.008	92,900.0

[17] The area of the hepatic vein of a given order is about 50 per cent greater than that of both the hepatic artery and the portal vein.

TABLE VII.

Giving the area and ratio of enlargement of the arterial bed of six organs. The numbers in brackets refer to the corresponding numbers in the preceding tables which mark the data used in the construction of this table.

	Area of arterial bed in sq. mm.				Ratio of enlargement of arterial bed			
	[1] Main trunk.	Arteries about 1 mm. in diameter. [2]	Arteries about .05 mm. in diameter. [3]	[4] Capillaries.	From arteries 1 mm. in diameter to .05 mm.	From arteries 1 mm. in diameter to capillaries.	From arteries .05 mm. in diameter to capillaries.	From the main trunk to the capillaries.
Intestine	7.0	12	60	2,351	5	196	39	336
Stomach	4.0 [18]	6	42	917	7	153	22	229
Adrenal	.34	..	6	199	..	...	33	580
Spleen	5.0 [18]	6	14	4,021	2	670	287	804
Lung 181.0		801	2,688	23,000.	3	30	9	127
Liver	70.0 [19]	354	1,897	92,900	5	264	49	1,327

The area of the vascular trees of the six organs given in Tables I to VI is based upon careful estimations made by myself or under my direction. If the volume of the organ is carefully considered while the measurements are being taken, it is relatively easy to gain results which are very reliable. In the intestine equal sections were measured, and in the lung and the liver the lobes were measured independently of one another. Corrosion specimens were used as much as possible; the finer corrosion and thin transparent specimens were measured under the microscope. In the liver, lung and spleen the measurements were controlled by the number of lobules and their average size. In all cases for each figure given at least ten independent measurements were taken from as many different organs. The data given in Tables I, II, III and V have been published elsewhere. Those upon the spleen are in part new and in part from my article upon the spleen.[20] Those upon the liver are entirely new.

The livers of 29 dogs of medium size averaged 175 cubic centimeters. A transverse section of the lobule or a surface measurement of the lobule of a hardened liver averages .7 mm. in diameter. If a lobule is considered a cylinder 0.7 mm. high and 0.7 mm. in diameter we will find that there are about half a million of them in the liver of a medium-sized dog.

[18] Estimations from the cœliac axis.

[19] Artery and portal vein.

[20] Mall, Zeitsch. für Morphol. u. Anthropol., II, 1900.

On account of numerous calculations of the lobules, terminal portal veins and terminal hepatic veins, I have fixed upon 480,000 as the average number. The terminal lobule or structural unit, either portal or hepatic, is about one-third of a cubic millimeter in volume, and with this size as a basis I have estimated the number of capillaries in the liver. However, lobules are usually put up in clusters, as described by Kiernan, and the volume of such a cluster is about 2 cu. mm. But the clusters can easily be diminished or increased at will to two structural units or to a whole lobe.

The average diameter of the portal vein is found to be 9 mm. The branches of the first order may be considered six in number and supply respectively the six lobes of the dog's liver. They may be designated as right upper and right lower, left upper and left lower, cystic and omental branches to correspond with the terms given by Rex in his excellent paper on the morphology of the mammalian liver.[21] Each lobe usually receives two or more branches varying from 1 to 5 mm. in diameter which when bunched for each lobe will give an area represented by the single vessels from 4 to 8 mm. in diameter. So in order to round my figures without interfering with the total area of the veins of the first order I have indicated in the table that these are six in number averaging 5 mm. in diameter. This liberty is based upon measurements taken from nine corrosion specimens in celloidin and in wax, and may be controlled by the six excellent illustrations to scale given by Rex. It may be pointed out here that the left main branch ends quite abruptly in a dilatation at the point of communication with the umbilical vein; from this point veins radiate in all directions. This dilatation, the recessus umbilicalis of Rex, is even better marked in the human liver than in that of the dog (Fig. 5) where it makes its appearance during the fifth week of development (Fig. 25).

From the branches of the first order to the capillaries it is relatively easy to compute the number of vessels of a given order. To be sure a few of the branches which were bunched with those of the first order are of the second order, but they are so insignificant in number that they need not be considered. The main subdivisions of the branches of the first order may be collected and counted. One of average size may be selected and dissected out with the liver tissue it supplies. The volume of the whole liver divided by the volume of this piece will give a second estimation. The count and the estimation should not be far apart if both are made accurately. Slight variations will be neutralized in estimating the number of vessels of succeeding orders. The sixth order of

[21] Rex, Morph. Jahrbuch, XIV, 1888.

both hepatic and portal veins is to be controlled by the volume of the structural unit. In general it is found that there are two terminal portal veins for one terminal hepatic; the former are always smaller and more slender, while the latter are tortuous and end abruptly.

It is not necessary for the blood to pass through vessels of all orders in order to reach the capillaries, for often veins of a given order have

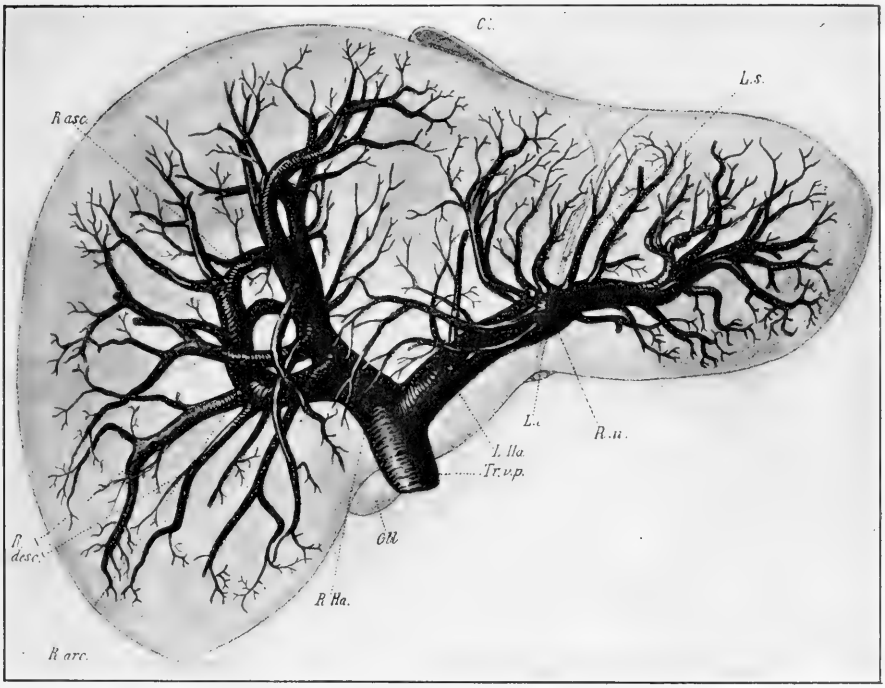


FIG. 5. Corrosion specimen of the portal tree in man. The injection was made with the liver in position. *G bl*, gall bladder; *lt*, ligamentum teres; *ls*. ligamentum suspensorium; *Ci*, vena cava inferior; *tr. v. p.* trunk of the vena portae; *r. ha*, *l. ha*, right and left main branches; *ru*, recessus umbilicalis; *r. arc*, ramus arcuatus; *r. desc*, ramus descendens; *r. asc*, ramus ascendens. After Rex.

vessels of three following orders arising side by side from the main trunk. Thus, branches of the first order have arising from them vessels of the second, third and fourth orders, and branches of the second order have arising from them veins of the third, fourth and fifth orders. The main trunk has branches as small as the third order arising from it, and these in turn have branches as low as the sixth order arising from them. Thus in special cases blood may pass directly from the main trunk into

veins of the third order and then into the terminal veins, skipping entirely the veins of the first, second, fourth and fifth orders. In general, however, most of the blood passes through veins of all orders before it reaches the capillaries. The short cut some of the blood takes is neutralized by the increased resistance due to the increased angle of the small vessel to the main trunk; very small veins always arise at right angles from large trunks, while their direction when arising from a smaller trunk is at an acute angle.

If there are 480,000 structural units in the form of small cylinders 0.7 mm. in diameter and 0.7 mm. high, it is easy to determine the number of capillaries which enter the unit in both transverse and longitudinal sections. My count in ten different injections gives 110 for the circumference of the hepatic unit and 35 for its height. These multiplied give 3850 as the number of capillaries which enter the hepatic lobule at its periphery. If all of the anastomoses within the lobule are estimated also, as they should be, this number is at least doubled. In order to err on the safe side I have taken the smaller number and multiplied it by the number of structural units, giving 1850 million as the number of capillaries which enter the periphery of the hepatic lobule of the liver.

I have arranged certain data (marked with brackets in Tables I to VI) in Table VII. It is at once noticed that the vascular bed is about five times larger for vessels 0.05 mm. in diameter than for those a millimeter in diameter. While the lumina of the vessels diminish 20 times, their number increases 2000 times. In the pulmontary artery and the portal vein, in both of which the blood-pressure is low, the vascular bed increases much more than in the arteries from the cœliac axis, down to the arteries one millimeter in diameter. In the intestine, stomach, adrenal and liver the increase in the vascular bed from vessels 0.05 mm. in diameter to the capillaries is much the same, averaging about 36 times; in the spleen it is much greater and in the lung it is much less. In the spleen with the numerous small slender vessels the resistance is enormous and in the lung it is insignificant, as is easily verified by making injections; it is very difficult to inject from an artery into the vein in the spleen, while in the lung melted wax can be injected through. In the spleen the area of the terminal arteries is 800 times that of the splenic artery, while if the pulp spaces be compared, the ratio is fully 30,000. That this number lies within the limit of probabilities is easily shown. The average volume of the spleen of the dogs used is 10 cc., which when distended increases to 80 cc.; the spleen can add to itself seven times its own volume without bursting. The area of all of the pulp spaces of

a moderately distended spleen is, as Table IV shows, 157,080 sq. mm., which multiplied by the length of a pulp space (0.02) gives 3 cc., a very insignificant portion of the entire volume of the spleen.

It is seen then that there are variations in the relation of the main vascular trunks to the capillaries in different organs, as was intimated by Thoma. This ratio in the spleen, determined by comparing arteries 0.05 mm. in diameter with capillary arterioles, is 287; in the lung it is 9; and in the liver it is at least 49. *The sectional area of the capillaries of a lobule is 49 times that of the final portal twigs which supply the lobule.*

All the lobules are equally favored, as has been frequently asserted and is easily proved by making injections with fluids of various consistency into any of the three vessels of the liver. *In each case all of the terminal vessels fill simultaneously.* The lobule most distant from the main vessel is not less favored than the lobule near to it. Thoma's laws have regulated the growth of the system of vessels and also have kept it adjusted.

According to Thoma's hypothesis, whenever the capillary pressure exceeds a certain point due to an increased exchange of substance in the growing adjacent tissue, there is a new formation of capillaries. Those portions of the area vasculosa which are favored later by the circulation seem to run ahead in their development in blastoderms from 18 to 39 hours old. It is seen that accelerated growth is accompanied with the new formation of capillaries long before there could possibly be any circulation through them.[22] A similar condition is to be seen in the human embryo. The blood vessels arise in the walls of the umbilical vesicle and grow into the embryo and form the main circle of vessels within it before the heart is fully formed. Such a condition is to be seen in the embryo described by Eternod. In exceptional pathological conditions they may develop into the villi of the chorion without the formation of a heart.

Loeb[23] has shown, by an ingenious experiment, that a very complete vascular system is developed in certain fish embryos without any circulation of blood at all. He placed the eggs of Fundulus immediately after fertilization in a solution of sea water to which 1½% of K Cl had been added, and found that they undergo a normal development without any heart beat; the chloride of potassium had paralyzed the heart. However, a complete vascular system is developed, being practically normal in arrangement within the embryo, as well as in the yolk-sac. The form

[22] Thoma, l. c., p. 28.
[23] Loeb, Pfliger's Archiv., LIV, 1893.

of the vessels was very irregular, at points forming rosettes in which narrow and dilated vessels alternated. Loeb concludes that not only did the entire vascular system develop without any circulation of blood, but also without any intravascular pressure, for had there been one, the capillaries which were developed should have been found distended. Furthermore, it is shown that the capillary buds grow independently of blood-pressure. "Die mechanischen Ursachen für das Wachsthum der Gefässwände sind deshalb nicht im Gefässlumen zu suchen, sondern in allen oder einzelen Zellen der Gefässwände und die Abgabe von Aesten ist bestimmt durch inner Ursachen in den Zellen der Gefässwände oder durch Reizursachen, die von der Umgebung ausgehend, diese Zellen treffen, ähnlich wie im Falle der Stolonenbildung von Hydroidpolypen."[24]

It is also seen from Thoma's own illustration (Fig. 3, *d*), that capillaries in which the pressure must be equal degenerate or multiply, as the case may be. In the transformation of Fig. 3 into Fig. 4, Thoma's first law on the rapidity of the circulation must have directed all of these changes. In fact, Thoma's hypothesis, on the budding of capillary cells, is not based upon intracapillary pressure alone, but also "vom Stoffwechsel der umgebenden Gewebe," which can easily be harmonized with Loeb's "Reizursachen, die von der Umgebung ausgehend, diese Zellen treffen."

In reality we can only state definitely that with the new formation of tissue new blood-vessels may grow into it, for all new tissue does not have blood vessels. Thoma's first two laws define much better and express more clearly the ideas relating to the question brought together by Roux[25] in his Inaugural Dissertation. To be sure, it is all functional adaptation, for the circulating blood arranges the irregular capillary anlage for a uniform circulation.

One would think that if the favored blood vessel dilates, a number of them would soon connect the arteries with the veins, and thus do away with the capillaries entirely. Thoma states that the reason why this does not take place is to be found in the great relative distance between the primary artery and vein, which is continued in their subsequent tree-like growth. From our present conceptions, it would appear, however, that occasionally an intervening capillary would dilate a little above the normal, and, being favored, would gradually become larger and larger. In fact, Brissaund and Sabourin have asserted that such anastomoses are quite common between the terminal portal and hepatic veins in many

[24] Loeb, l. c., p. 531.

[25] Roux, Ges. Abhandl., I, p. 19.

animals.[26] It seems to me that there must be other agencies that would prevent such a catastrophe. In fact, we have an abundance of examples of a reduction of enlarged capillaries whenever they occur. Thoma has given a satisfactory explanation of the closure of the ductus arteriosus and of the ductus venosus, but that is not quite to the point in this case. However, in the beginning of the capillary system of the liver, around the omphalo-mesenteric vein, we have an excellent case. This question will be taken up at greater length subsequently, so at present I shall be very brief. The liver grows around and into the omphalo-mesenteric vein, and while so doing we have a double circulation, a more direct one through the constricted vein and a more circuitous one through the capillaries of the liver. But, in spite of this, the vein is gradually eliminated, leaving only a capillary plexus. The aortic arches of amphibia are eliminated in a similar way. From them loops of capillaries grow into the external gills which gradually take the place of the artery. There are numerous other examples. Minot,[27] who has recognized the fundamental importance of the destruction of a main channel and its conversion into a system of capillaries, calls such vessels sinusoids, and the circulation through them a sinusoidal circulation. Not only are blood-vessels which are too large reduced, but it appears in the development of the blood-vessels in the tadpole's tail as if the new blood-vessels were always growing in the direction of the greatest resistance, for the nearest complete arch is already the shortest course from the artery to the vein; yet another and more distant one is to be formed.

The first and guiding blood-vessel is the capillary which grows in all directions, forming a plexus. Secondary changes make arteries and veins of them and their laws of growth have been discovered and clearly stated by Thoma. The normal shape of the capillary is tubular with a lumen about .008 mm. in diameter. They arrange themselves into a plexus with a tendency to come in contact with every surrounding cell. However, the tissues vary considerably in this respect, the first capillaries growing to them or past them in tufts. In general, the capillary arrangement is influenced by the tissue or organ into which it grows, but its conversion into main trunks and branches is controlled by the circu-

[26] See Oppel, Lehrbuch, III, p. 984. Brissaund and Sabourin are undoubtedly in error regarding this statement. I have made and examined hundreds of injections made with granules which would pass through small arteries but not through the capillaries, and have never seen such an anastomosis. Further, celloidin corrosions, which often include part of the capillaries of the lobule, never show such connections.

[27] Minot, Proc. Bost. Soc. Nat. Hist., XXIX, 1900.

lation. Ultimately the arrangement is such that all capillaries of an organ are equally favored by the circulation. This means that the capillaries have about the same diameter and length with about the same amount of blood passing through them during a given period of time. If too little blood passes through them, in a lobule of the liver for instance, some of the capillaries disappear; if the circulation comes to a standstill all of the capillaries are obliterated. So their very life is dependent upon a proper or normal circulation. If a capillary is too long, the resistance within it is increased, and the circulation is slowed with a subsequent reduction of length. So in order that a capillary may remain, it must have a definite lumen, a definite length and a definite amount of blood passing through it in a given time. The diameter varies from .005 to .01 mm., according to the organ in which it is located. The average is .008 mm., the diameter of the mammalian red blood corpuscles. In some of my tables the diameter is given as .003 mm., but these measurements are from hardened and mounted tissues. The length of the capillary is less than half of a millimeter, through which the blood flows in less than a second. So, morphologically, a capillary is a blood-vessel .008 mm. in diameter, .5 mm. long with a renewal of blood every half-second. If this renewal of blood is permanently diminished enough of the capillaries are obliterated to reestablish the normal circulation in those that remain. If the quantity is permanently increased, according to Thoma's first law, some of them are converted into arteries and some into veins. If the increased circulation is continued without a corresponding increase in the number of capillaries, the artery will extend into the vein just as is the case in the liver when the ductus venosus is formed.

The anlage, then, of the vascular system is the capillary; artery and vein are secondary and are differentiated out of them by the flow of blood set in motion by the beat of the heart. As the capillary bed increases the flow through the arteries increase, and the heart hypertrophies and the vascular proportion is maintained. In round numbers, in the dog, the arteries continue to grow until the rapidity of flow is 30 mm. a second in an artery .05 mm. in diameter, 150 mm. in an artery, 1 mm. and 300 mm. in the aorta. Thoma fixes the rapidity of the circulation in the aorta of man at 228 mm. a second, and over 34 mm. a second in an artery .04 mm. in diameter.

The unequal growth of different portions of an organ accounts for the unequal size of the arteries which supply them. The whole thing works from the periphery to the center. In this way a succession of organ units is formed all the way from the first divisions of the artery which supply the lobes, to its final twigs, which supply the lobules.

It is seen from what has been said above that it is undoubtedly the growth of the tissue of the organ which leads the way. Into this new-formed tissue the capillaries grow and they have an inherent power which makes them grow into an anastomosing tubular system. The density of the capillary plexus is influenced by the tissues into which they grow, but their length and arrangement is determined by the circulation through them. A vascular proportion is constantly maintained for each organ down to the minutest vascular twig. Capillaries through which the rate of circulation is below the normal shrink or disappear, and when it is above the normal they enlarge into either veins or arteries. A capillary too long will eventually cut itself off on account of the increased resistance to the circulation in its own walls. An increased flow of blood rarely causes an artery to empty directly into a vein, because the determining factor is nearly always to be found in the capillaries themselves. The growth of the capillaries causes some of them to change into arteries and veins, and the equilibrium is thus easily maintained. In rare instances, however, the amount of blood thrown into an organ may be increased greatly, as is the case when all of the blood from the umbilical vein is suddenly forced through the liver. It follows that the circulation through a chain of capillaries from the portal to the hepatic vein is much above the normal capillary circulation, and, as a result, the ductus venosus is formed.

Early Development of the Liver.

The early development of the liver has been worked out by His and others, and therefore it need not be discussed to any great extent. The liver bud, as shown in Fig. 6, is well marked in an embryo at the end of the second week (2.1 mm.). It grows rapidly and then encircles the left omphalo-mesenteric vein, in the chick and in man, and both the right and the left in the dog. At the same time that the liver tissue encircles the vein it also invades it, carrying the endothelial lining ahead of the sprouts and thus forms a series of sinuses, or the sinusoids of Minot. While this process is taking place the umbilical veins are gaining much in importance and a large share of the blood which formerly returned to the heart through the omphalo-mesenteric now returns through the umbilical veins. Figures 7 and 8, from an embryo 4.3 mm. long, and Figs. 9 and 10, from an embryo 4.5 mm. long, illustrate this point. In the embryo 4.3 mm. long large sprouts of liver tissue have invaded the common omphalo-mesenteric veins which have also extended, forming a large ring of Minot's sinusoids encircling the intestine as described by

His. At this time the umbilical veins are broken in their course, having already passed the first stage of their growth, and from now on are destined to pass through the liver rather than past it (Fig. 8). It is interesting to note that the primary sinusoidal liver—that portion which arises with the omphalo-mesenteric vein—is formed while the umbilical vein empties directly into the ductus Cuvieri. The process is at its height in an embryo of about the same age (No. 76), as shown in Figs. 9 and 10.

The growth of the liver around and through the omphalo-mesenteric veins is accompanied by the growth of capillaries from this vein into the new anlage. Hand in hand with this process the circulation through the

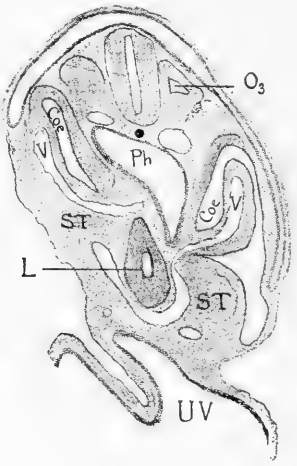


Fig. 6. Section through the third occipital myotome of a human embryo 2.1 mm. long (No. 12 of my collection). × 50. O_3 third occipital myotome; *coe*, coelom; *v*, vein; *st*, septum transversum; *l*, liver; *ph*, pharynx; *uv*, umbilical vesicle.

omphalo-mesenteric veins is further reduced by the growth and enlargement of the umbilical veins. A double force is at work: blood is diverted by the umbilical vein which is gradually assuming greater importance and by the capillaries which supply the embryonic liver. According to Thoma's first law, the diminished rapidity of the circulation is followed by a reduction of the lumen and in order to accomplish this reduction in the present case, the liver sprouts first grow into the vein instead of around it. The operation of Thoma's law in this case is so extensive that it reduces a main trunk to capillaries which forms a condition recognized by Minot as a sinusoidal circulation.

At the time the liver circulation is entirely sinusoidal, *i. e.*, about the

end of the third week, it is composed of a single lobule with the vein entering it on one side and the collecting vein leaving it on the opposite side. The turning point between the first and second stages of development is shown in Fig. 11. In the embryo represented by this figure the omphalo-mesenteric veins are broken completely into capillaries in the liver, and one umbilical vein has been transferred from the ductus Cuvieri to the lower part of the liver. The single liver lobule here is perfect; it is composed of a complete capillary network without an anas-

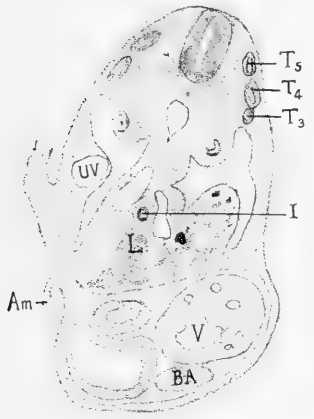


Fig. 7.

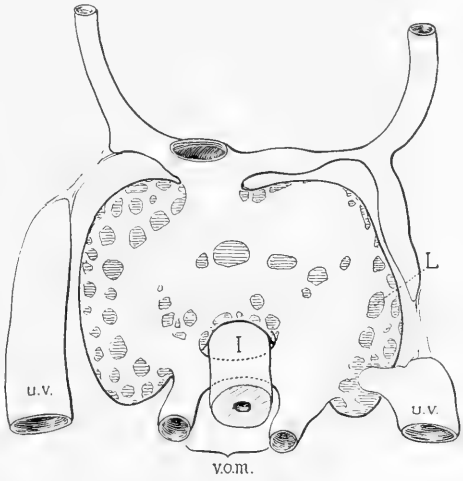


Fig. 8.

Fig. 7. Section through a human embryo 4.3 mm. long (No. 148). × 25. T_3, T_4, T_5, third, fourth and fifth thoracic myotomes; *i*, intestine; *l*, liver; *v*, ventricle; *ba*, bulb of the aorta; *am*, amnion; *uv*, umbilical vein.

Fig. 8. Semidiagrammatic reconstruction of the veins of the liver of a human embryo 4.3 mm. long (No. 148). *L*, liver; *uv*, umbilical vein; *vom*, omphalo-mesenteric vein; *i*, intestine.

tomosing vein through it. A rough estimation of the vascular proportion shows that the area of the capillaries is fully 100 times that of the entering veins. In the next two embryos, Figs. 12, 13 and 14, all of the blood from the left umbilical vein passes through the liver—the right vein having been obliterated. Within the liver it is seen that the right omphalo-mesenteric vein is open, while the main branches of the hepatic and portal veins have made their appearance. With the growth of the liver the capillary bed has increased which is naturally followed by a more rapid circulation in the distributing and collecting capillaries, and con-

sequently they are converted into veins. All of the blood from the umbilical veins now passing through the liver increases the circulation through the small liver so much that a venous channel (right omphalo-mesenteric vein) remains open, or in case it be closed it is opened up again. The two new branches within the liver care for the circulation through its left lobes, and may have been formed directly from the left omphalo-mesenteric vein. At any rate, we see in them two permanent main trunks of the liver,—the vena hepatica sinistra and the ramus angularis arising from the recessus umbilicalis.[28] In the next stage which

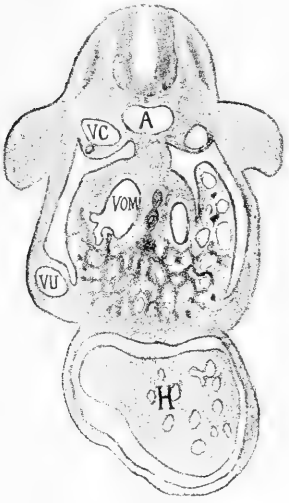


FIG. 9.

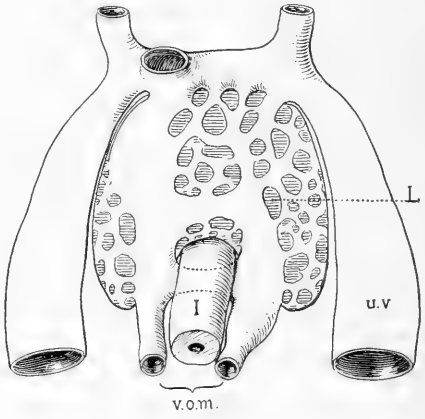


FIG. 10.

FIG. 9. Section through a human embryo 4.5 mm. long (No. 76). × 25. *VC*, cardinal vein; *a*, aorta; *vom*, omphalo-mesenteric vein; *vu*, umbilical vein; *h*, heart.

FIG. 10. Semidiagrammatic reconstruction of the veins of the liver of a human embryo 4.5 mm. long (No. 76). *L*, liver; *uv*, umbilical vein; *vom*, omphalo-mesenteric vein; *i*, intestine.

is found during the fifth week the right omphalo-mesenteric vein is obliterated and the ductus venosus is formed as a new and more direct channel. In place of the obliterated omphalo-mesenteric vein there are two new permanent veins, the ramus dextra of the hepatic vein and the ramus arcuatus et descendens of the portal system. We now have a liver of two

[28] An excellent description of the vascular system of the mammalian liver is given by Rex (Morph. Jahrb., XIV, 1888). As much as possible I have used his nomenclature.

lobules, representing the right and left lobes, with a vascular system in each identical in arrangement with that of a liver of one lobule. (Fig. 11.)

When the umbilical vein first shifts from its entrance into the ductus Cuvieri to the liver it has taken the course in its new position, of the least resistance, as a glance at Fig. 15 shows. There is a mass, if not an excess, of capillaries in the liver at this time and this vein with its loose wall makes the change suddenly as is shown in Fig. 11. This brings to the liver an excess of blood which is followed by keeping open, or opening in case it has closed, the right omphalo-mesenteric vein. The continued growth of the liver and its capillaries increases the circulation

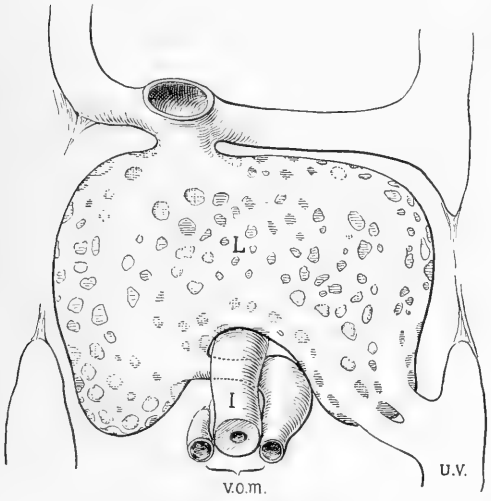


Fig. 11.

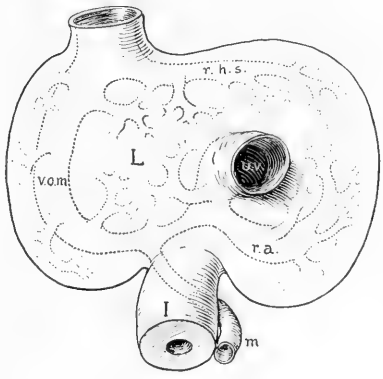


Fig. 12.

Fig. 11. Semidiagrammatic reconstruction of the veins of the liver of a human embryo 4 mm. long (No. 186). *L*, liver; *uv*, umbilical vein; *vom*, omphalo-mesenteric vein; *i*, intestine.

Fig. 12. Semidiagrammatic reconstruction of the veins of the liver of a human embryo 6.5 mm. long (No. 116). *L*, liver; *vom*, right omphalo-mesenteric vein; *uv*, umbilical vein; *m*, mesenteric vein; *rhs*, ramus hepatica sinistra; *ra*, ramus angularis; *i*, intestine.

in the distributing and collecting branches which is followed by their conversion into permanent venous trunks: first those on the left side and then those on the right side. The excess of blood is still continued and on account of the shifting of the right omphalo-mesenteric vein with the growth of the right lobe of the liver the route becomes circuitous, and a new and more direct channel, the ductus venous, is formed. This has

already taken place in the specimens shown in Figs. 17 and 20; in a later stage, Fig. 25, the omphalo-mesenteric still remains open after the ductus venous is formed.

During all this time the vascular proportion remains normal, that is, the area of the capillaries is about 50 times that of the main portal trunk. The distributing branches are on one side of the lobule, and the collecting branches on the other. With an increase of the number of lobules, however, they are no longer set parallel, but at various angles with one another. Were they continued parallel they would have to spread as a

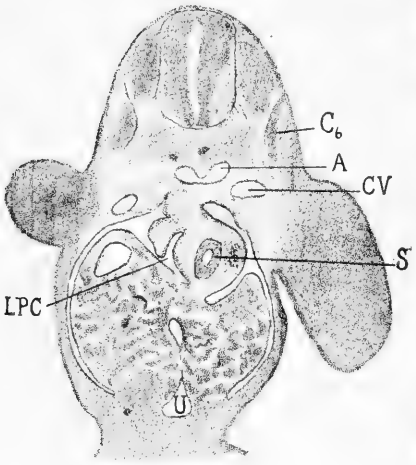


Fig. 13

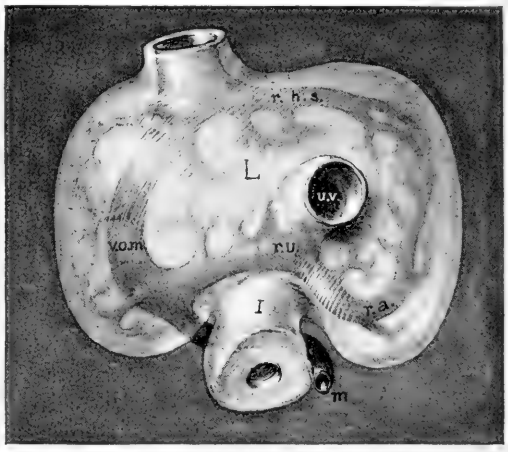


Fig. 14.

Fig. 13. Section through the liver of a human embryo 5 mm. long (No. 80). × 25. *C* sixth cervical myotome; *a*, aorta; *cv*, cardinal vein; *s*, stomach; *u*, umbilical vein; *lpc*, lesser peritoneal cavity.

Fig. 14. Semidiagrammatic reconstruction of the veins of the liver of a human embryo 5 mm. long (No. 80). *L*, liver; *vu*, umbilical vein; *r. vom*, right omphalo-mesenteric vein; *rhs*, ramus hepatic sinistra; *ru*, recessus umbilicalis; *ra*, ramus angularis; *m*, mesenteric vein; *i*, intestine.

sheet with a thickness of a millimeter, the maximum normal length of a capillary. In an embryo at the end of the fifth week, Fig. 25, two new lobules have made their appearance and the two primary lobules have begun to divide. The hepatic and portal veins are telescoping; they are beginning to dovetail with each other. The new branches of the portal have gone into the field of the hepatic and the new hepatic veins have entered into the portal field. By this process, and by this process only, can a spherical vascular organ be built up maintaining a normal vascular

proportion. All through the organ the terminal twigs of the distributing and of the collecting veins must not be over a millimeter apart, and this naturally keeps the units small and determines the ratio between the terminal twigs and the capillary bed.

In the embryo of the end of the fifth week, Fig. 25, the right and left portal twigs have begun to divide, and from the recessus umbilicalis a

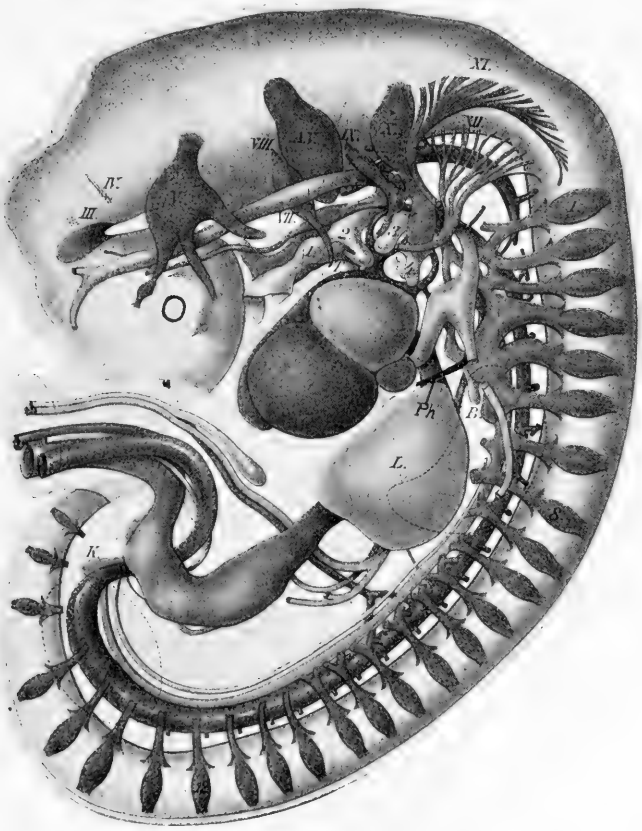


FIG. 15. Lateral reconstruction of a human embryo 7 mm. long (No. 2). *L*, liver; *ph*, phrenic vein; *1*, *2*, *3*, *4*, branchial pouches; *Roman numerals*, cranial nerves; *Arabic characters*, spinal nerves.

new group of veins have formed and radiate into the middle and left lobes of the liver. On the hepatic side the left branch has divided into two trunks and two new branches have appeared: the vena cava inferior and the vena hepatica media which has its terminal right and left branches.

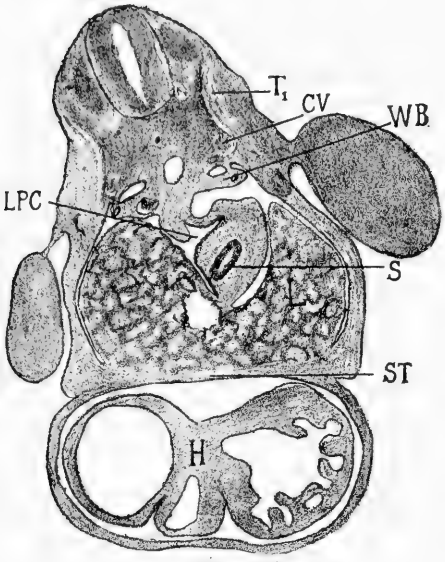


FIG. 16.

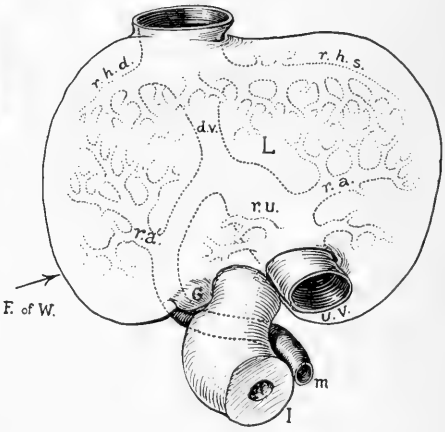


FIG. 17.

FIG. 16. Section through the embryo 7 mm. long (No. 2). × 25. T_1, first thoracic myotome; *cv*, cardinal vein; *wb*, Wolffian body; *s*, stomach; *lpc*, lesser peritoneal cavity; *l*, liver; *h*, heart; *st*, septum transversum.

FIG. 17. Semidiagrammatic reconstruction of the veins of the liver of the embryo 7 mm. long (No. 2). Viewed from in front. *L*, liver; *uv*, umbilical vein; *m*, mesenteric vein; *ru*, recessus umbilicilis; *dv*, ductuc venosus; *ra*, ramus angularis; *ra*^{1}, ramus arcuatus; *rhd*, ramus hepatic dextra; *rhs*, ramus hepatica sinistra.

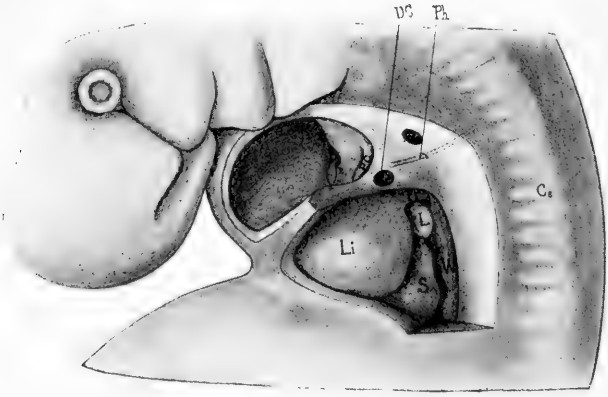


FIG. 18. Lateral view of a model of the liver in position of a human embryo 9 mm. long (No. 163). × 12½. C_8, eighth cervical myotome; *li*, liver; *l*, lung; *s*, stomach; *wf*, Wolffian body; *ph*, phrenic nerve; *pc*, pleuro-pericardial membrane; *pp*, pleuro-peritoneal membrane; *dc*, ductus Cuvieri.

The right omphalo-mesenteric vein is still present and the ductus venous is well marked. In this case the liver is formed of four main lobules, and with the subdivision of the middle and left hepatic veins into two branches each, six primary lobules are seen to correspond with the six primary lobes of the mammalian liver. In this case the vena hepatica dextra superior et inferior is represented by the open omphalo-mesenteric vein and the anlage of the vena cava inferior. In the next stage, Figs. 26 and 28, the normal arrangement of these veins is found for the vena cava inferior really belongs to the middle lobe.

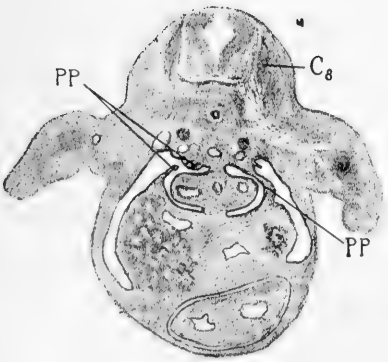


FIG. 19.

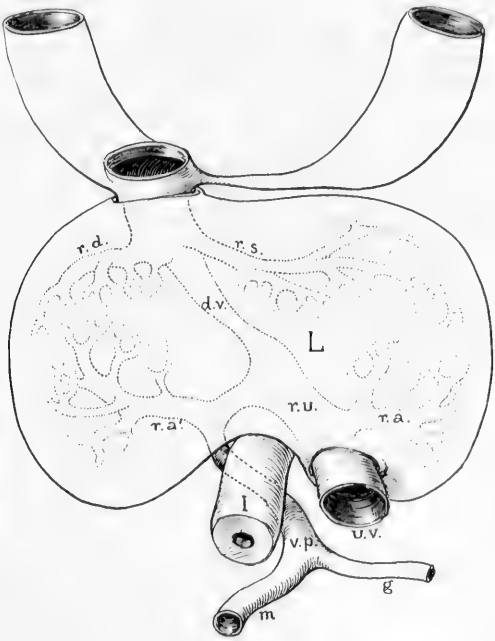


FIG. 20.

FIG. 19. Section through the embryo 9 mm. long (No. 163). × 12½. C_8, eighth cervical myotome; *pp*, pleuro-peritoneal membrane.

FIG. 20. Ventral view of the veins of the liver of the embryo 9 mm. long (No. 163). *L*, liver; *i*, intestine; *uv*, umbilical vein; *vp*, vena portae; *g*, gastric vein; *m*, mesenteric vein; *ra*, ramus angularis; ra_1, ramus arcuatus; *rs*, ramus sinistra; *rd*, ramus dextra; *dv*, ductus venosus.

With the completion of six lobules we recognize fully the adult form of the liver. Each lobule now represents one of the six lobes of the mammalian liver; each of the primary lobules is to expand into a whole lobe. The primary lobules radiate from a center and have between them the

main trunks of the portal veins; each interlobular vein at this stage is to form a main trunk in the adult. At this time we have terminal vessels to follow from stage to stage, which is impossible to do in adult specimens.

The process of sprouting and interlacing continues at a rapid pace from now on, and for the present I shall give illustrations from the livers of two embryos of the eighth week. The first (No. 22), Fig. 26, is from a wax plate reconstruction carried as far as I could conveniently, and Fig. 27 is from a photograph. The second (No. 6) Fig. 28, is from a graphic reconstruction which could be carried out pretty well, and Figs.

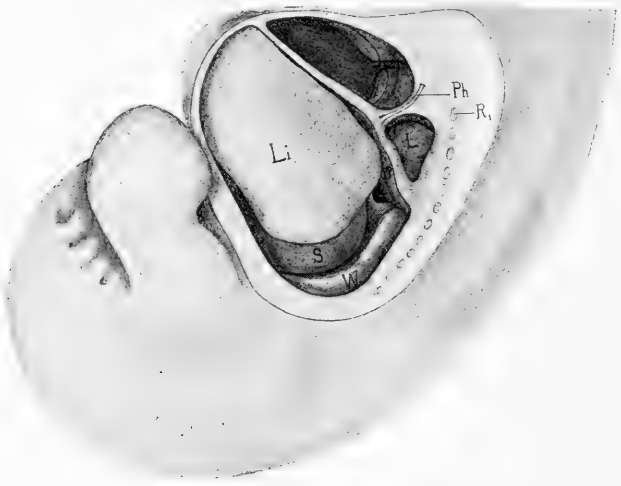


Fig. 21. Lateral view of a model of the liver in position of a human embryo 11 mm. long (No. 109). × 8½. *Li*, liver; *l*, lung; *r*, first rib; *ph*, phrenic nerve; *s*, stomach; *wf*, Wolffian body; *pp*, pleuro-peritoneal membrane.

29-31 are three views of a wax model of the exterior of the liver. These illustrations together show the form of the liver and the main vessels with their lobular branches. There are about 700 branches of the third order in the adult liver, and rough estimations made from Figs. 27, 29-31 give about this number. The lobules in these specimens are about 0.4 mm. in diameter, considerably smaller than in the adult. In general branches of the hepatic and portal veins of the same order are as far apart as possible with a tendency to run at right angles to each other. The branches of the first order or main trunks have been present from the time of the earliest differentiation of the liver, while those of the second

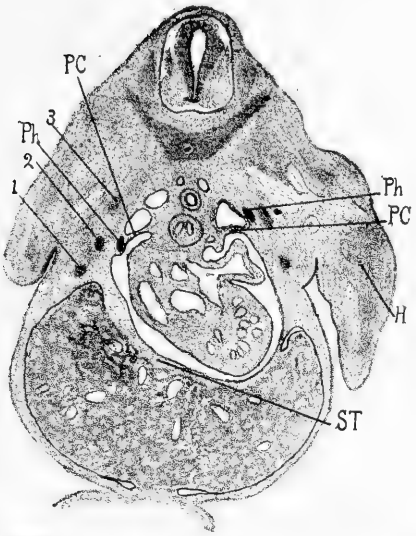


FIG. 22.

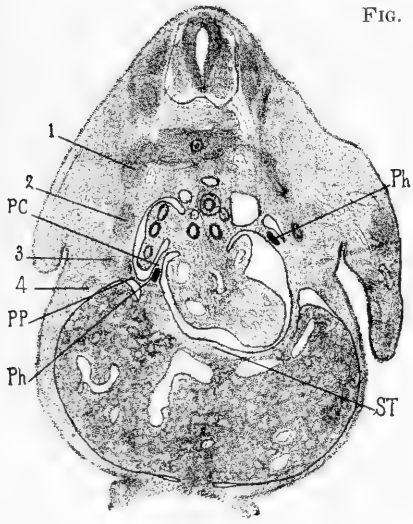


FIG. 23.

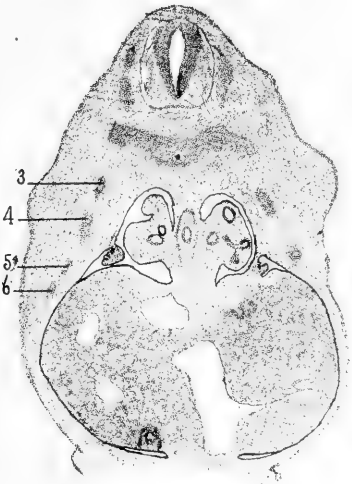


FIG. 24.

FIG. 22. Section through the body of the embryo, 11 mm. long (No. 109). × 10. The liver is attached to the septum transversum, *st; 3*, first rib; *1*, third rib.

FIG. 23. Section through the embryo, deeper than in Fig. 22. Just in front of the septum transversum in the liver is seen a section of the ramus hepatica sinistra and coming forward the ramus hepatica media.

FIG. 24. Section through the same embryo showing the umbilical vein, recessus umbilicalis and the ramus arcuatus. Behind in the liver is the vena cava, to its left the open right omphalo-mesenteric vein (see Fig. 25), in front is the gall bladder and between it and the omphalo-mesenteric vein in the ramus arcuatus.

and third order date from the beginning of the dovetailing process. A word more about the vena cava inferior. In its beginning it belongs entirely to the liver and is completely surrounded with liver tissues. In the adult liver two small branches empty into it in addition to the main branches mentioned above. The first is closely associated with the vena hepatica media accessoria. The other arises from the omental lobe and

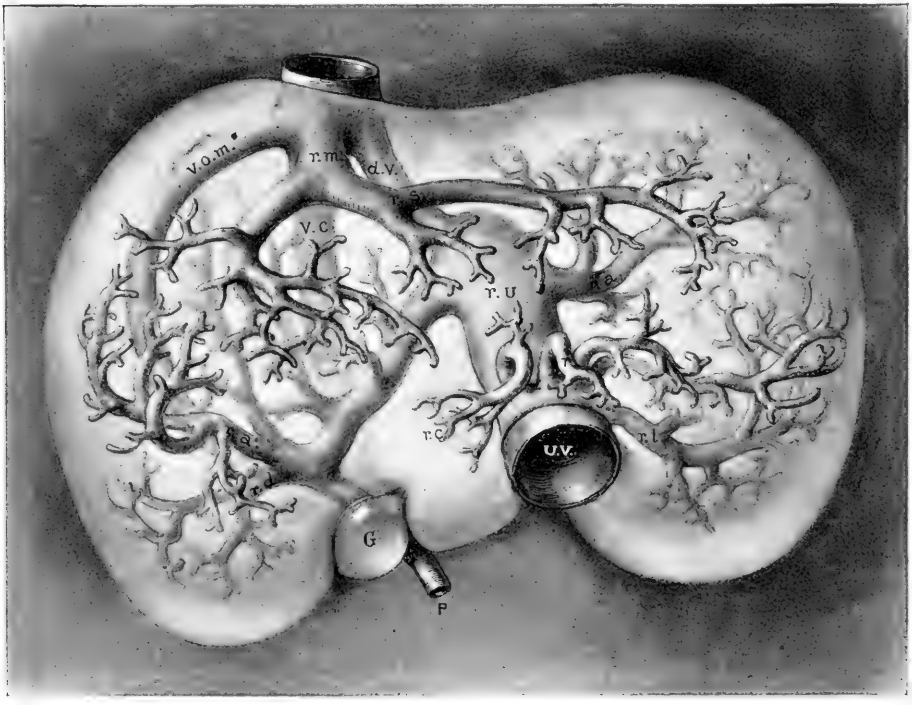


Fig. 25. Ventral view of a reconstruction of the vasicular system of the embryo 11 mm. long (No. 109). × 25. *Uv*, umbilical vein; *pv*, portal vein; *ra*, ramus angularis; *ru*, recessus umbilicalis; *rd*, ramus descendus; *ra*, ramus arcuatus (possibly ramus ascendus); *rc*, right arborization of the recessus umbilicalis; *rl*, left arborization of the recessus umbilicalis; *dv*, ductus venosus; *vc*, vena cava; *vom*, omphalo-mesenteric vein; *rm*, ramus media; *rs*, ramus sinistra.

usually goes directly to the vena cava, but occasionally communicates with the vena hepatica sinistra. It is seen that the vena cava collects blood directly from the quadrate and Spigelian lobes.

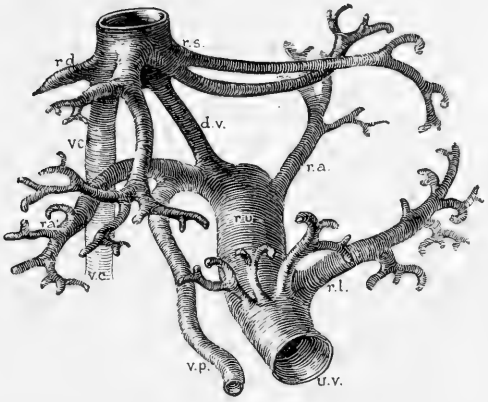


FIG. 26. Main trunk of the liver from an embryo 20 mm. long (No. 22). From a reconstruction in wax. × 12. *Uv*, umbilical vein; *vp*, portal vein; *ra*, ramus arcuatus; *ra*, ramus angularis; *ru*, recessus umbilicalis; *dv*, ductus venosus; *vs*, vena cava; *rd*, ramus dextra; *rm*, ramus media; *rs*, ramus sinistra; *rl*, left arborization of the recessus umbilicalis.

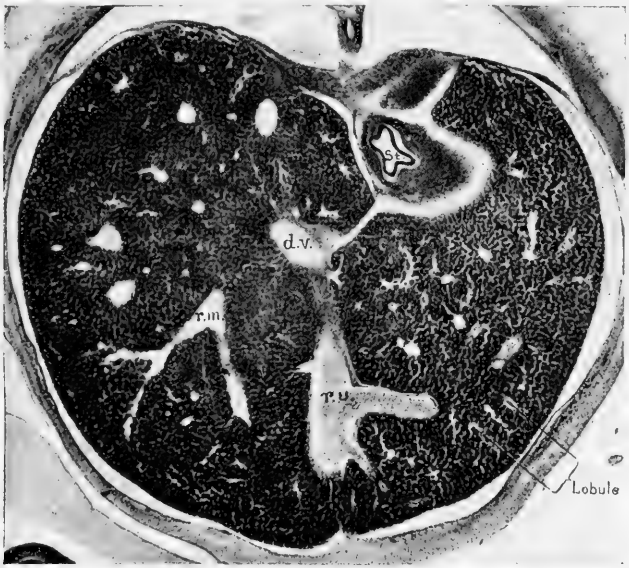


FIG. 27. Photograph of a section of the human embryo, 20 mm. long (No. 22). × 15. *Ru*, recessus umbilicalis; *dv*, ductus venosus; *rm*, ramus media. Between the two branches of the ramus media may be seen a branch of the ramus acendus cut transversely. These branches are of the second order and the terminal branches the beginning of those of the third order. In this case the lobules are .5 mm. in diameter.

The Hepatic Lobule and the Portal Unit.

It is seen from what has been said above that the final branches of the portal and hepatic veins are always as far from one another as possible throughout all stages of their development as well as in the adult liver. At all times this distance is half the diameter of a lobule and since this is in the neighborhood of one millimeter the distance is about half a mil-

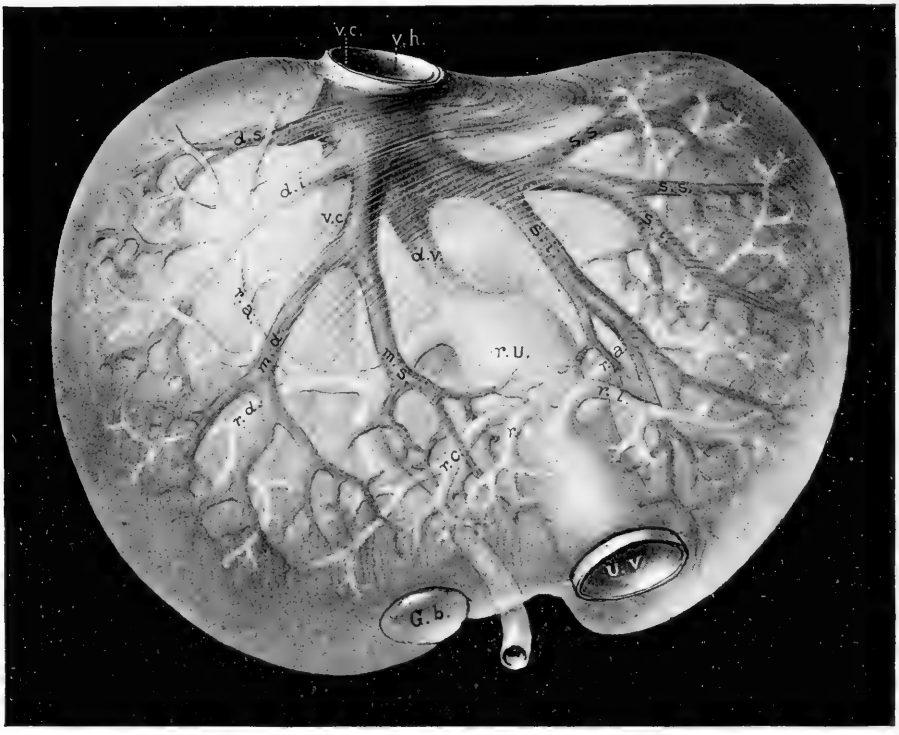


Fig. 28. Reconstruction of the vasicular system of tne liver of a human embryo 24 mm. long (No. 6). × 20. All of the important vessels are fully formed. The stage is the same as that shown in section, Fig. 27. *Uv*, umbilical vein; *vp*, vena portae; *ru*, recessus umbilicalis; *ra*, ramus arcuatus; *rd*, ramus descendens; *ra*, ramus angularis; *r*, *r*, right arborization of *ru; re*, left arborization of the recessus umbilicalis; *vh*, vena hepetica; *dv*, ductus venosus; *ds*, vena dextra superior; *di*, vena dextra inferior; *md*, vena media dextra; *ms*, vena media sinistra; *ss*, vena sinistra superior; *si*, vena sinistra inferior; *vc*, vena cava.

limeter, the normal length of a capillary blood-vessel. It is also apparent, as indicated by Fig. 1, that the liver breaks up into two sets of units arranged respectively around the terminal twigs of the two sets of veins.

That the unit arranged around the hepatic vein was finally accepted as the lobule is largely due to excessive amount of connective tissue along

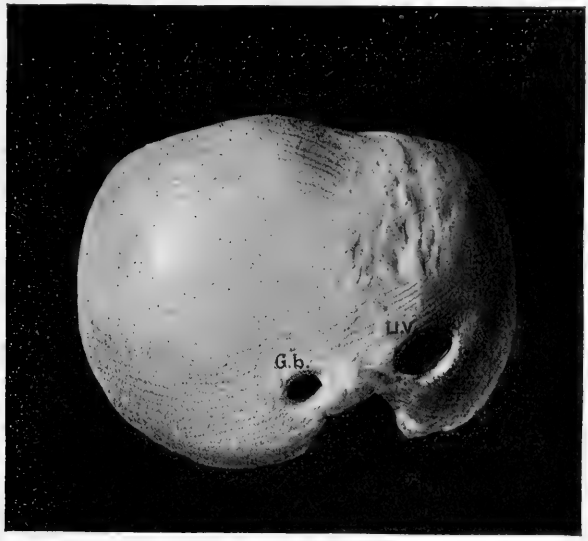


FIG. 29. Ventral view of a wax model of the embryo, 24 mm. long (No. 6) × 10. *Uv*, umbilical vein; *Gb*, gall bladder.

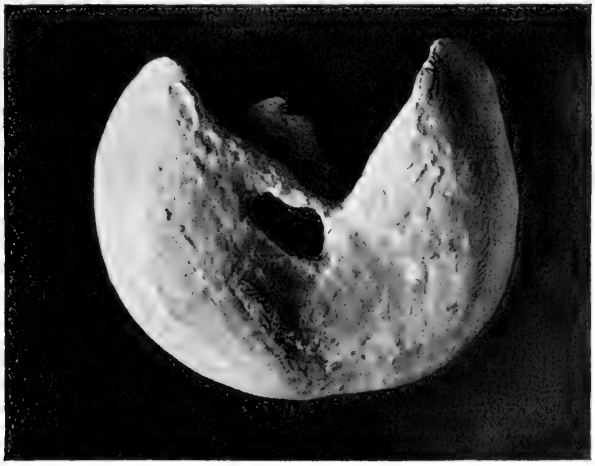


FIG. 30. Superior view of the same liver.

the portal twigs in the pig's liver, a condition almost peculiar to this animal. Had the liver of Phoca been studied in the place of that of the

pig the portal unit would have been accepted, for in general either set of lobules is only occasionally well outlined and thus marked in some mammals. In the human liver, as in the dog's, there are more terminal portal twigs than hepatic and this together with other structures which accompany the portal vein, makes it easy for practical purposes to call the hepatic unit the lobule. However, it is just as easy, if not easier, to consider the portal unit the lobule if one is so inclined.

From the standpoint of pathology, it is easy to construct a description of the liver based upon a portal lobule. Especially marked are these lobules in venous hyperæmia, in pigmentation and in cirrhosis in which

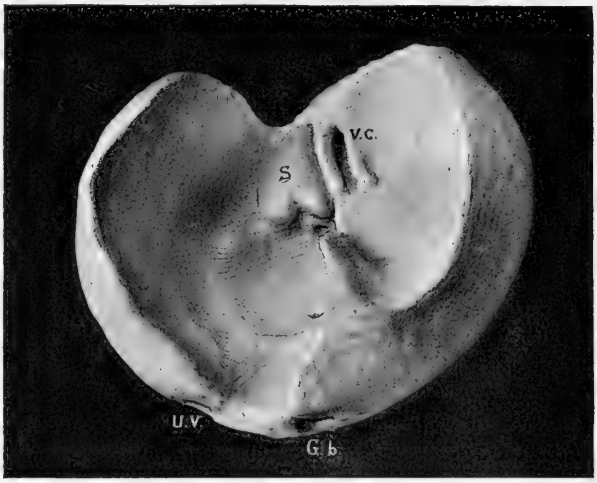


FIG. 31. Dorsal view of the same. *Gb*, gall bladder; *uv*, umbilical vein; *S*, Spigelian lobe; *vc*, vena cava inferior.

there is a marked regeneration arising from the bile ducts. Sabourin [30] has used these arguments successfully in favor of the portal lobule as the histological unit of the liver. However, his results are also not new, as may be seen by glancing over the historical note accompanying this paper. But his point is sound and shows that the liver histology may be constructed around the terminal portal veins as well, if not better, than around the terminal hepatic veins. His extensive monograph is illustrated with several hundred diagrams, many of which are fanciful, for they are defective in one respect. If a series of circles are crowded together to form hexagons and each of the angles is then used as a center

[30] Sabourin, Recherches, etc., de la Glande Biliaire. Paris, 1888.

of a series of superposed hexagons of the same size, the new series of circles will not form an equal layer, but will overlap each other. Thus, in his Fig. 227, there are six hepatic veins surrounding one portal, while in the next figure the opposite is the case. He should have had them of equal number, having an alternating space common to both systems, the nodal point, as I call it. However, the work of Sabourin is excellent and deserves much more attention than it has received outside of France.

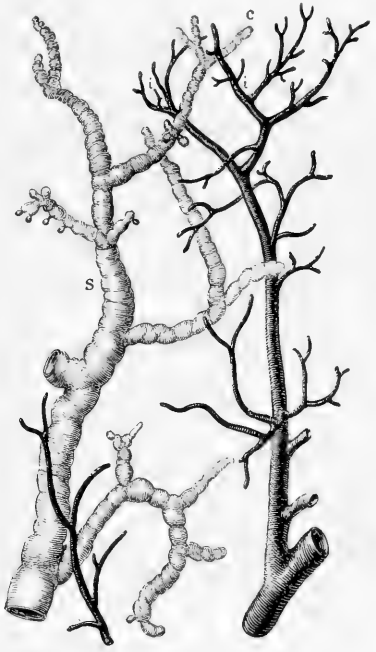


FIG. 32. From a corrosion in celloidin of the terminal branches of the portal and hepatic veins. × 20. The hepatic vein is larger and marked by many constrictions forming a "spiral valve." *I*, interlobular veins; *c*, central vein; *s*, sublobular vein.

The typical liver lobule, as described by Kiernan,[29] is based upon the study of the pig's liver, and is composed of either a single lobule, or of clusters of them. It is not clear which he considers the real unit, for there are all gradations between single lobules and compound lobules composed of at least 25 single ones. As the veins grow larger capillaries cease to arise from them, and the opened vein shows at the point of transition the bases of adjoining lobules shining through its wall; at this

[29] Kiernan, Phil. Trans., 1833.

point, according to Kiernan, the intralobular veins change into sublobular. This distinction is rather arbitrary and of little value, but has clung as a parasite to the text-books. In general hepatic veins with capillaries arising from them are called central or intralobular veins and larger veins are called sublobular; sublobular veins are hepatic veins from one to two millimeters in diameter.

FIG. 33. Photograph of a celloidin corrosion of the liver lobule and the portal unit. × 2. The dark clumps are the portal and the light anastomosing bodies are the lobules.

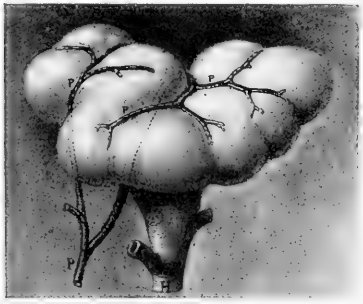


FIG. 34.

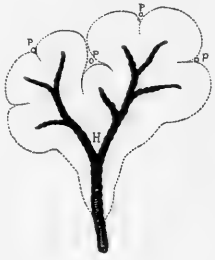


FIG. 35.

FIG. 34. Plastic diagram of a group of anastomosing lobules with the terminal branches of the portal vein, marking the centers of the portal units, added. Enlarged about 10 diameters.

FIG. 35. Diagrammatic outline of the group of lobules, shown in Fig. 34, with the hepatic vein added.

I have studied carefully the hepatic lobule in the dog's liver and found that in general I can confirm practically everything that Kiernan has said of it. Numerous injections have been made of the hepatic vein, either singly or in combination with injections of other vessels. Figure

32 is made from a celloidin corrosion specimen of both the portal and hepatic systems. The "spiral valve" of the hepatic vein, which is always present in the dog and the cat, is well shown in such specimens. From the study of granular injections of the hepatic vein with cinnabar, ultramarine blue, chrome yellow and baryta, it is found that the capillaries arise from veins which are about .17 mm. in diameter. This arbitrary point, Fig. 32, *S*, marks the beginning of the sublobular vein, although numerous capillaries still arise from it. After its walls become markedly thickened capillaries no longer arise from the sublobular vein, but in their place small veins arise which supply portions of a lobule and there-

FIG. 36. Photograph of a corrosion preparation of the portal and hepatic trees. × 2. At the × two of the terminal hepatic veins anastomose. The "special valve" may also be seen.

fore cannot be considered central veins. In my study I have called the hepatic veins in the neighborhood of .09 mm. in diameter intralobular veins, and those .17 mm. or a little larger sublobular veins. In Table VI they have been classed respectively under the sixth and fifth orders.

The form of the hepatic lobule can be well outlined by washing vessels of the liver first with saline solution, then with alcohol, after which cinnabar and lamp-black celloidin are injected respectively into the portal and hepatic veins. With considerable pressure the capillaries are injected more or less with red or with black. Figure 33 is from a specimen of this kind with red in the hepatic vein and black in the portal vein. In this specimen the lobule was but partly injected with red celloidin and in the corrosion but the center of the lobule is shown. To the extent in which

capillaries arise from the hepatic vein it is encircled by the red mass. It is seen that the celloidin entered the lobule only at the tips of the veins, that is from the intralobular veins. The sublobular veins are clear and have arising from them intralobular veins which again have clusters of lobules attached to them. The lobules are in clusters to correspond with the branching of the interlobular veins. The outline of one in perspective is given in Fig. 34, which is reduced to a diagram in Fig. 35.

While the hepatic lobules are irregular, anastomosing, and of unequal size, the portal units are regular, more spherical and of equal size. The

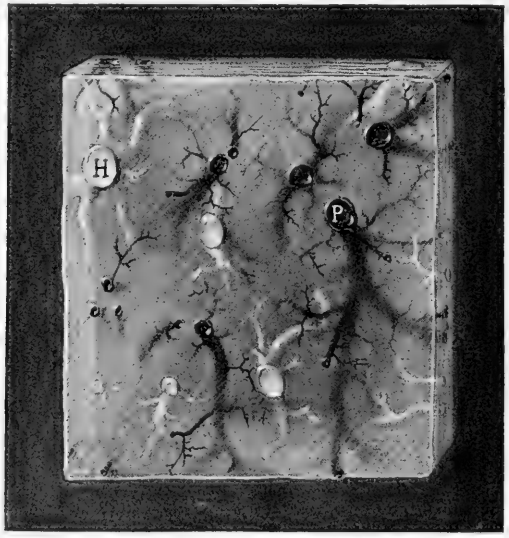


Fig. 36a. Portal and hepatic veins from a corrosion preparation of the liver of the dog. × 10. The two sets of vessels were injected with black and red celloidin mixture respectively, after which the liver was hardened in alcohol. A block of tissue was cut into very thick serial sections which were digested for a number of days in a solution of pancreatin at 37° C. After the cells were all dissolved, leaving only the connective-tisue framework and the injected celloidin the section were preserved in glycerine. The minutest twigs of celloidin are held in place by the delicate reticulum fibrils.

branches of the portal vein are much more delicate and regular than those of the hepatic. A comparison between the two is shown in Figs. 32 and 36, while the single portal branch is shown in Fig. 37. In general the portal branches are more regular than the hepatic, as far from them as possible with a tendency to run at right angles to them. The portal veins never come to the surface of the liver and never anastomose; the

terminal hepatic branches are very irregular, often come to the surface of the liver and sometimes anastomose. There is much difference of opinion regarding the statements given in the above sentence, but anyone who will take the trouble to make a few good celloidin corrosions will find them correct.

Branches of the fifth order of the portal vein are .15 mm. in diameter; those of the sixth order .05 mm. The interlobular veins are about half the diameter of the intralobular and twice as numerous. Even these

Fig. 37. Photograph of the terminal branch of the portal tree. × 2.

often branch before they give rise to capillaries. While the hepatic vein receives capillaries down to veins marked *S* in Fig. 32, the portal twigs of the same figure give off capillaries only at their extreme tips. Unless the extreme tips of the hepatic vein, Fig. 32, *C*, are taken as centers of the lobule and clusters of the tips of the portal vein, *I*, as centers of the portal units will these two correspond in number. The relative number is shown in Fig. 38, which is from a free-hand model in clay from a celloidin corrosion. The protruding points, *C*, mark the position of the tips of the central veins. The tracing, Fig. 35, shows the same.

Not only are the centers of the portal unit marked by the tips of the

portal vein, but also by the tips of the hepatic artery, as well as those of the bile duct. Furthermore, it is probable that the lymphatics arise from the same point and that the nerves and connective tissue spread from it. From time to time I have found large groups of karyokinetic figures there, which, together with the pathological evidence, make this the regenerative or growing point of the liver. The portal unit is the true structural unit of the liver.

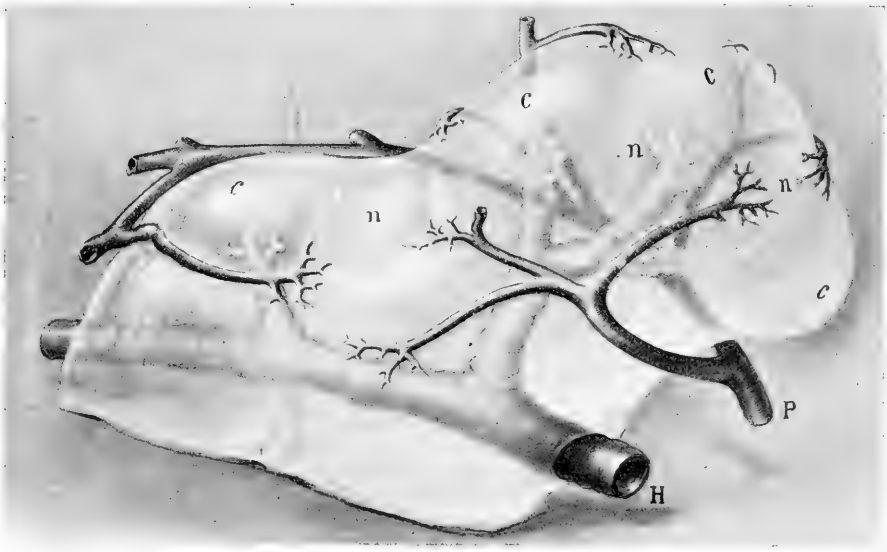


Fig. 38. Lateral view of a model of a group of lobules of the dog's liver. The outside vessels are terminal portal branches. *H*, sublobular vein; *c*, elevations in the lobule over the tip of the central veins within; *n*, nodal points; *p*, portal vein.

Growth of the Hepatic Lobule and of the Portal Unit.

When I began the study of the development of the lobule of the liver twenty years ago, I thought that I had selected the simplest and most definite lobule for investigation. It seemed then easy to follow the lobule from stage to stage and thereby gain a comprehensive picture of the histogenesis of the liver. It has turned out, however, that most of the work—the hundreds of injections, experiments and sets of serial sections—has been in vain, and but the faintest sketch remains from which to picture. The great difficulty is to recognize the same things from step to step, and this is obtained with certainty only in very young livers,

where all of the veins are of one order; the six central veins of the embryo become permanent main trunks in the adult. In development, the liver structure shifts distalwards, successively tearing off its capillary connections with the main veins, gradually rearranging the architecture of the lobules, often fracturing them and scattering them. Portions of each of the six primary lobules go to thousands of new lobules. None of the main trunks of a large liver, like that pictured in Fig. 27, give off capillaries at birth. So we must conclude that in a child the liver structure is entirely rearranged each year which calls for a destruction and regeneration of at least a billion capillaries and towards puberty ten times this number.

In the estimation of the number of blood-vessels of the liver it has been found convenient to classify the branches of the vascular tree under six orders, designating the main trunks as the first order and those directly related to the lobule as the sixth order. It is seen by studying Fig. 27, which is from an embryo of the eighth week, that but the first three orders are present with capillaries arising from all of them. By the time the liver is fully formed each of the third order, which are 700 in number, must give rise to an equal number of those of the sixth order, while the liver increases 7000 times in volume. It follows that the lobule must increase in volume as the liver grows, and, in fact, this is the case. When the lobules are well formed, as at the end of the second month, they are .5 mm. in diameter; at birth 1 mm., and in the adult 1.5 mm. Actual measurement also shows that the weight of the liver at the end of two months is .2 gms.; at birth, 75 gms. and in the adult 1500 gms.

As the lobules are shifting more distalwards, it is found that the capillaries have a greater and greater tendency to arise from a single order, so that when the liver is fully formed capillaries arise only from the sixth order of the branches of the portal vein, and from the fifth and sixth orders of the hepatic vein. It is evident then that the stretching of the liver is in all directions and that there are additions to the tip of all of the blood-vessels, both within the liver and on the periphery. Not only is the liver tissue shifted more distalward, but the vessels already laid down are stretched, and from their trunks new vessels arise to supply new lobules which have arisen from fractured portions of adjoining lobules.

Toldt and Zuckerkandl[31] have advanced ideas similar to the ones I have formulated above. They state that the younger the liver the simpler is its vascular system, and that in the youngest stage the whole liver may be

[31] Toldt and Zuckerkandl, Sitzungsber. d. K. Akad. d. Wiss., Bd. 72. Wien, 1876.

likened to a single lobule of the adult. In embryos four weeks old there is a distributing and collecting end to each of the lobules present and not until the eighth week is the adult form to be recognized in the structure of the liver. Furthermore, they state that in their growth the lobules split and give rise to numerous new lobules.

The process of expansion and rearrangement is expressed in the diagram, Figs. 39 and 40, which may be viewed to represent either a portal

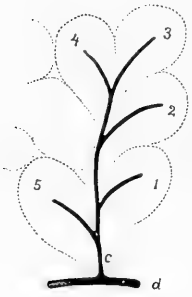


Fig. 39.

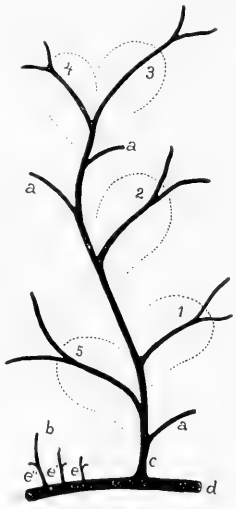


Fig. 40.

Figs. 39 and 40. Diagrammatic illustration of two stages of a growing hepatic vein. *D*, main vein; *c*, its branch; *1*, *2*, *3*, *4*, *5*, the same vessels in both diagrams; *a*, *a*, new branches from the stem c; *b*, a new branch on the main stem, *d* in which the successive stages of the central vein are marked *e*, *e'*, and *e''*.

or an hepatic twig. As the vessels represented in Fig. 39 grow, the liver tissue increases in quantity, but the liver lobules do not increase in size indefinitely, because Thoma's first law is constantly at work and will soon break up the larger lobules into a number of smaller ones. In all cases the length of the capillaries remains constant, and when they appear to be too long and too numerous it is always found that some of them have already turned into small veins and thus mark the beginning of new lobules, or of new portal units. A more advanced step is represented in Fig. 40. The lobules or units of the two stages are marked with corresponding numbers. But each of them is splitting at its end and new vessels, *a*, have also arisen from the main trunk. As there were no lobules

at the points marked *a* before, the new ones must have been differentiated from adjoining lobules. The process by which this has taken place may be expressed by the twig *b*. At a much earlier stage all this tissue was arranged in a single lobule around the vessel *d*. Then it spread over the branch *c*, and finally as the vessel *d* elongated a new lobule, *b*, arose. Its central vein was marked successively by the vessels, *e*, *e′ and e″*.

It may be considered that the Figs. 39 and 40 represent the process of growth of the liver in one dimension of space, which becomes much more complex when viewed in two dimensions, as is shown in Figs. 41, 42 and 43. The single branch, *a*, Fig. 41, becomes the main branch, *a*, Fig. 43.

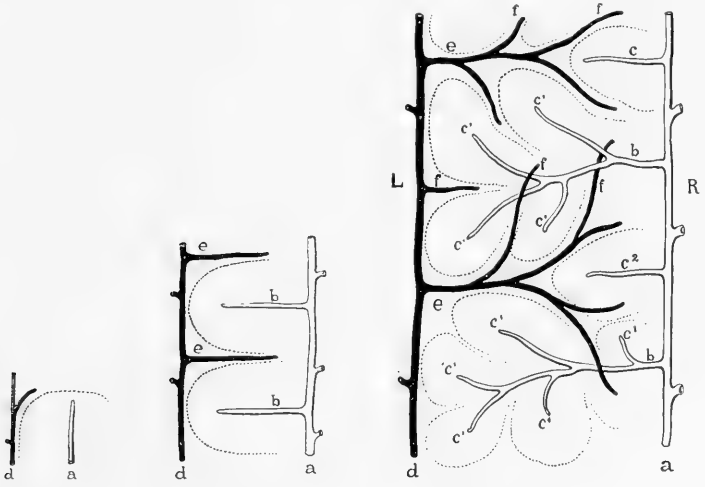


Fig. 41. Fig. 42. Fig. 43.

Figs. 41, 42 and 43. Diagrams of three successive stages of the portal and hepatic veins in a growing liver. *A*, hepatic side; *d*, portal side; *b*, and *c*, successive stage of the hepatic vein; *e* and *f*, successive stages of the portal vein.

The successive orders of new branches, *b* and *c*, explain themselves. Finally the lobule c^2 is adjoined by two different kinds of lobules, c^1 younger than itself, and *b* older. The portal vein has spread into this region, corresponding with the hepatic, and we see the branches reaching out in all directions, keeping equidistant from one another. This diagram is practically correct for most regions of the liver where there are a succession of portal and hepatic veins alternating, so that in the region, *L*, there is another vessel like *a*, and in the region *R* another like *d*. But when these vessels, *a* and *d*, are the only ones that run out to the edge of the liver, the vessel *e* must cross *a* into the field *R*, and the vessel *b* must

cross *d* into the field *L*. In fact, there is quite a regular crossing of portal and hepatic veins in many portions of the liver, and it indicates that at one time the main stems were the only vessels in that portion of the organ.

I shall not venture to describe the growth of the vascular system of the liver in three dimensions of space, but refer the reader who desires it to a good double (portal and hepatic) corrosion with the diagrams given in Figs. 39 to 43. If he takes the pains to apply them to double corrosions of the livers of very young and of old animals, I think he will find them of some aid. I wish, however, to add two points. First, if the vessel, *b*, Fig. 40, is imagined cut transversely, as represented in Fig. 1, it will be seen that the lobule represented by it must have arisen from three adjoining lobules. Secondly, if the tip of the lobules *1, 2,* etc., are considered in three dimensions of space it will be found that the growing part of the lobule is always at a certain point, which is as far as possible from both portal and hepatic tips, and is marked *n* in Figs. 1 and 38. In the diagrammatic section, Fig. 1, it is seen that there is a special arrangement of the capillaries passing towards this point, and since it is so constant, can be located in any lobule and is of such great morphological significance I shall term it the nodal point of the lobule. The nodal points are always located in Kiernan's interlobular fissures, but the fissures are not always nodal points. In Fig. 38 a line drawn through the letters *c, n, e, n, c, n, c* marks the middle of a Kiernan fissure, but only the points marked *n* are nodal points.

Meaning of the Nodal Points.

In general, it is stated that the capillaries of the lobules of the liver radiate from the central vein and in so doing branch until the periphery of the lobule is reached where they communicate with the plexus of interlobular veins, as described by Kiernan. It was shown, however, by Krukenberg and others that the terminal portal twigs do not anastomose and this in itself indicates that the usual description of the capillaries of the lobule is incorrect, for there must be some kind of collecting system for that portion of the periphery of the lobule devoid of veins. Correct illustrations of the vascular arrangement of the lobule in cross section are given by Stöhr and Bohm and von Davidoff without any description of them in the text.

A careful study of the vascular arrangement of the lobules will show that the capillaries themselves are the collecting vessels due to their own anastomosing system. A diagrammatic representation of this system in

a cross section of a group of lobules is shown in Fig. 1. It is here shown that the shortest course between the terminal portal and hepatic veins is taken by perfectly straight capillaries, and as the region away from the straight course is approached the general direction of the capillaries becomes more and more bent. It is thus seen that the deflected capillaries from several adjoining lobules come together, forming points which can easily be seen in the sections of any liver. In general the picture is sharper in the rabbit's liver than in that in any other animal I have examined, especially when it is taken immediately under and parallel with

Fig. 44.

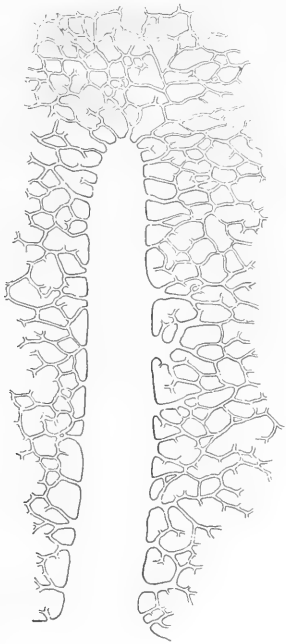


Fig. 45.

Fig. 44. Terminal distribution of a portal twig entering three portal units. × 85. The capillaries all arise from the tips of the vein.

Fig. 45. Terminal hepatic vein with the capillaries arising from it. × 85.

the surface of the liver in order to strike the lobules at right angles. Thus we have in the liver lobule two sets of capillaries, long ones and short ones, while according to Thoma's first law they should all be of the same length. This stumbling block caused me a great deal of trouble, for at first I saw no way to overcome the difficulty. An elaborate model of the vascular system of the lobule seemed to show that as much fluid,

or more, passed through the longer and less direct capillaries than through the shorter and direct ones. The capillaries passing through the nodal points, therefore, seem to be as well favored as those taking the shorter course. In the diagram it is seen that the nodal point is fed from three sources, and on account of the great number of capillaries in it the resistance to the circulation is probably diminished. It would follow that some of the main feeding capillaries should be converted into veins, and in growing livers this is the case, but every time a new vein is formed, we have a new vascular unit, or a new lobule with two new

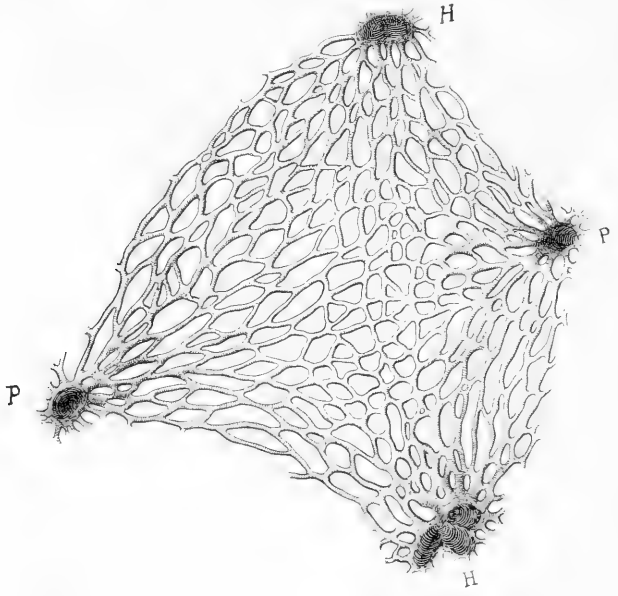


FIG. 46. Arrangement of the capillaries at the nodal point of a lobule. × 85. *P*, portal vein; *h*, hepatic vein.

but smaller nodal points to complicate the situation again. This process might be continued until the portal branches communicated with the hepatic, were there not some self-regulating force to check it. It appears that this force is due to the normal length of the capillary which is about .4 mm. in the direct course. In the indirect course, *i. e.*, through the nodal points, this distance is about .8 mm., but each nodal point is fed from three sides instead of from one, and this arrangement may account for this increase in distance without the formation of new veins.

If the hepatic and portal veins are injected with thick granular masses—cinnabar, lamp-black or ultra-marine blue suspended in gelatin—

it will be found that in successful cases these terminal veins with the capillaries arising from them are filled. Figures 44 and 45 show this distribution from the tips of the portal and hepatic veins, marking the centers of the portal unit and the hepatic lobules. Both are drawn to the same scale and show the relative arrangement of their capillaries. The portal twigs give rise to capillaries only at their ends, while in the

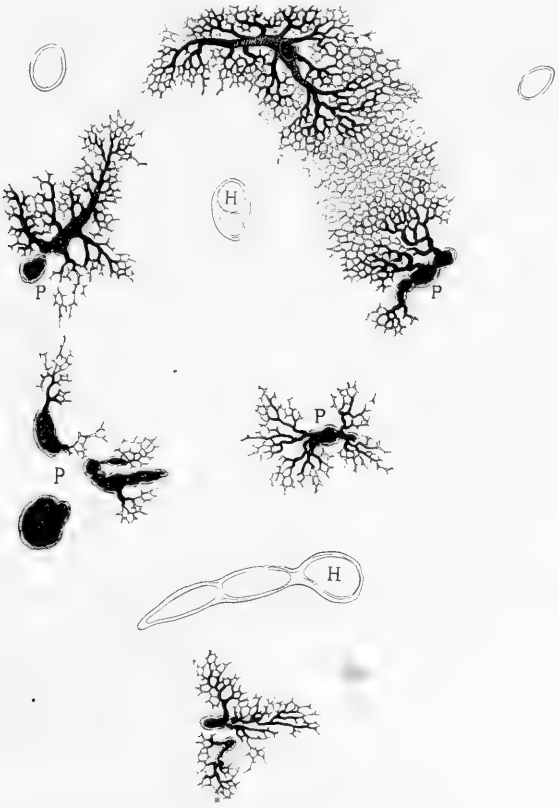


Fig. 47. Tracing of the vessels around a lobule showing the relative number of terminal and hepatic and portal branches. × 40. Portal vein slightly injected.

hepatic the origin of the capillaries extends much farther down the vein than is shown in the drawing. The portal vein lies of course in an interlobular space and its three main branches extend into the adjoining fissures. Together they mark the center of a portal unit. Figure 46 shows the arrangement of the capillaries throughout a nodal point. The

course of the capillaries is in a more direct line from the portal to the hepatic, from the portal to the portal and from the hepatic to the hepatic than in other directions.

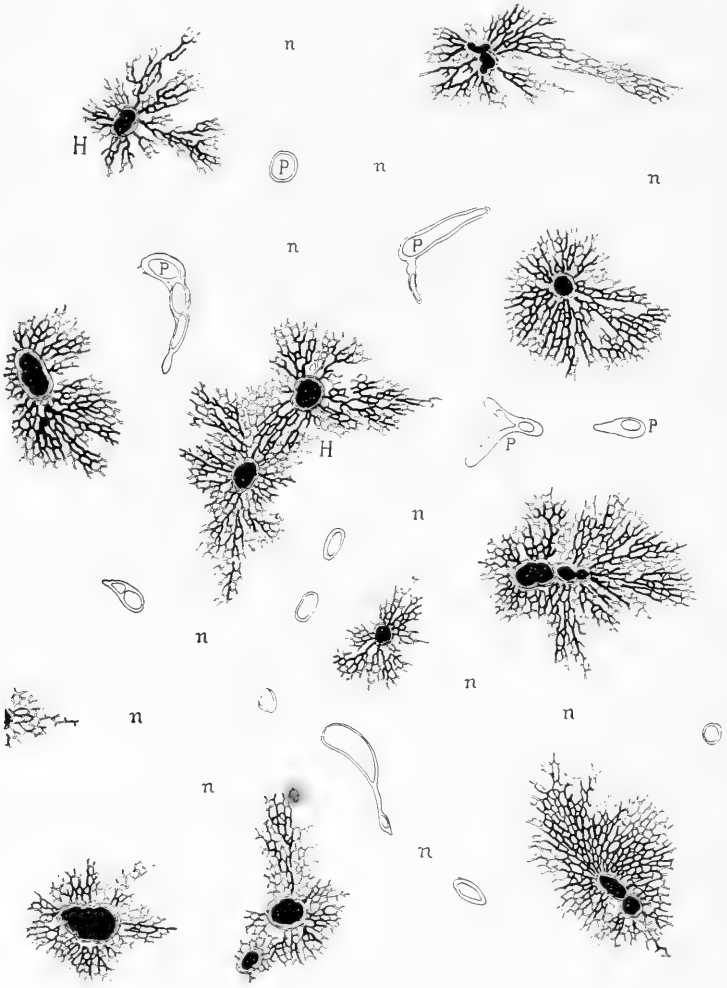


FIG. 48. Tracing of the terminal hepatic and portal veins with the nodal points marked *n*. × 40. Hepatic veins slightly injected.

Figures 47 and 48 show the first spreading of granular injections and their relation to the lobule. It is seen in both cases that the most favored vessels point toward the nodal points, *i. e.*, these capillaries are a little larger than the rest of the capillaries of the lobule; they have

grown sufficiently to favor the capillaries of the nodal point. Figure 49 is from a more extensive injection of the hepatic vein with lamp-black gelatin. It is seen that the injected area, which is sharp, is angular in shape, with projections directed towards the nodal points. We have here again the well-known picture of the beginning of passive congestion, which incidentally marks the portal units beautifully.

Enough has been said to indicate that the growth of the lobule takes place at the nodal point. That does not mean that the cells multiply at this point, but simply that the new vessels, alternately portal and hepatic

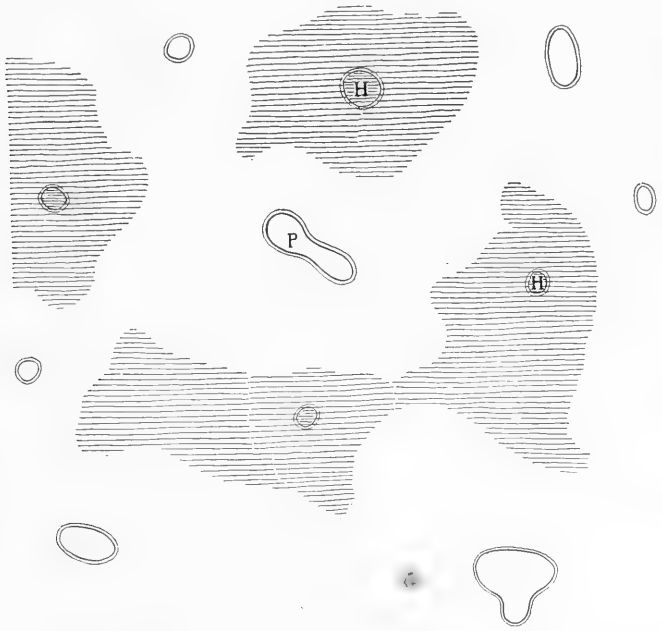


FIG. 49. Tracing to show the extent of a granular injection of the hepatic vein. × 40. The portal vein was first injected with blue gelatin and the thick granular lamp black gelatin was forced into the hepatic vein. The portal units are outlined as in chronic passive congestion.

grow toward this point and break it into fragments to form new nodal points. In order to test this question a dozen sets of sections were made of the livers of growing rabbits and fœtal pigs with more or less satisfactory results. The rabbit which is a favorable object for this kind of study has a lobule of constant size (.6 mm.) from birth, until it is fully grown. In the pig the lobule measures .8 mm. in embryos 4 cm. long until a number of months after birth; in the adult they are 1.4 mm. in diameter.

In the set of rabbits' livers, hardened in a variety of ways, it was found that whenever the distance between two adjoining portal veins is considerably greater than the average diameter of a lobule, a small portal vein grows into the nodal point which separates them. The same is true regarding the hepatic veins as shown in Fig. 50. From all appearances, the hepatic branch, *4*, is a recent one, growing into the large nodal point which had pushed apart the hepatic veins *1* and *5*. No doubt earlier in its development, the whole field of this figure formed one nodal point, and

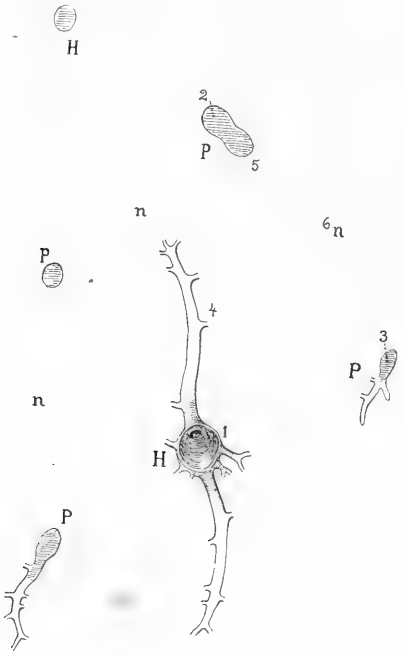


FIG. 50. Section of the liver of a rabbit one day old. × 85. Hardened in Flemming's solution. *P*, portal branch; *h*, hepatic branch; *n*, nodal point; *1, 2, 3, 4*, order of growth of the vessels; the next vessel will appear at *5*, and then it will grow towards the nodal point *6*.

then the hepatic vein, *1*, grew into it. This was followed by portal veins, *2*, then *3*, then by hepatic vein *4*. At a later stage the portal vein, *2*, will send a branch into nodal point *6*, and so on. In a measure, we can corner a bit of liver tissue at the junction of two main stems, as shown in Figs. 51 and 52. It is fair to assume that the tissue at this angle grows with the rest of the liver, for a time at least, and that the small vessel arising from the portal in Fig. 51 and that from the hepatic in Fig. 52 are new

vessels growing into the nodal point, *n*. But the growth of the liver lobules can never be proved by the study of any section or specimen, for

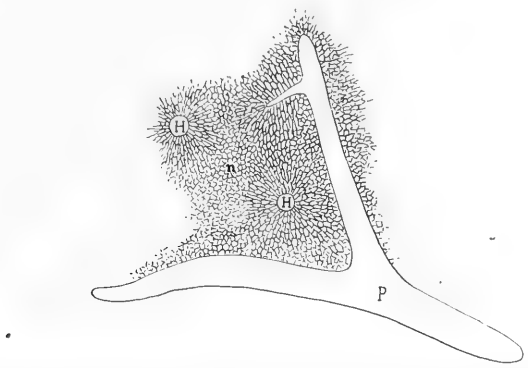


FIG. 51. Section of a rabbit's liver eleven weeks old to show the origin of a new branch from a portal trunk growing towards a nodal point. × 40.

FIG. 52. The same as Fig. 51 to show the origin of a hepatic branch. × 40.

at all times there is an equilibrium; the cells do not multiply to be followed by the growth of veins, but they go forward side by side. All grades of veins can be seen in any sections of livers, young or old, and

the imagination of the investigator can patch them together, correctly or not as the case may be. But the final evidence is obtained by studying serial sections of early livers, where the same vessel can be followed from stage to stage.

The very first vessels that grow into the nodal points in the human embryo are shown in Fig. 25 from a specimen of the end of the fifth week. The vena cava and the vena hepatic media on the hepatic side and the ramus arcuatus, the ramus descendens and the ramus angularis from the portal side are new vessels. All of the branches of the main trunks with the branches of the second order are shown in the reconstruction, Fig. 28, and in the section, Fig. 27. Here the new branches of the hepatic alternate with those of the portal, each newly-formed field, as it is formed, receiving its proper branch in the next stage. The best example is the vena hepatica media in an early stage and the rest of the main trunks and their branches as they arise in order.

There are as many nodal points as there are portal units, and more than there are lobules, unless the complex lobule is cut into blocks. The arrangement is shown in Fig. 38, which shows one lobule with six terminal portal veins encircling it. If it is considered that these veins are related to adjoining lobules, an estimation of two terminal portal veins to one hepatic, as I have it in my table, is not far from being the correct number. The reconstruction shows that terminal portal twigs run parallel, recur again, and run at right angles with the intralobular vein. If the portal units are imagined around the interlobular veins of Fig. 38, it is seen that both apex and base of the same lobule may represent the distal ends of different units. Adjacent intralobular veins and nodal points mark the outline of the portal units.

Relation of the Hepatic Artery to the Portal Units.

There always has been and still is much confusion regarding the distribution of the hepatic artery and the reason for this is very evident when it is considered that it communicates at its end directly, and possibly indirectly also, with the capillaries of the lobule. To test this question thoroughly, I have made numerous single, double and triple injections with granular and fluid substances in order to determine the courses the arterial blood may take during life.

Long ago Ferrein stated that there were two kinds of branches of the portal vein within the liver, one which conveyed blood to the lobule and

the other which collected blood from the capillaries of the hepatic artery. That the collecting veins of the gall bladder and surrounding connective tissue empty into the vena portal is easily proved by a simple injection of a granular mass into this vessel. But the conclusion that all, or even a great portion, of the blood from the hepatic artery is collected by similar, but smaller, veins which enter the branches of the vena portæ within the substance of the liver is a doubtful one, in my opinion. Theile, who made a careful study of these vessels, called them the rami vasculares (a term generally ascribed to Kölliker) and brought forth very meagre evidence that they are present in great number. In fact, his best proof is the observation occasionally of a vein which arises in the hilus of the liver and enters the quadrate lobe before it communicates with a branch of the portal vein.[32] Theile believes that the rami vasculares venosi enter very small portal branches because he was never able to see them opening into the larger branches after they had been cut open. The presence of these branches, he claims, explains why an injection into the hepatic artery enters the portal vein and why an injection into the portal vein enters the artery as well as the hepatic vein.

My own injections speak decidedly against internal roots to the portal vein, except those that arise from the gall bladder and that neighborhood, and can be recognized with the naked eye. After the arterial branches once enter the substance of the liver all of their twigs, including those of the capsule, communicate directly into the capillaries of the lobule, and from there are collected into the hepatic vein. It is usually stated that the portal branches are distributed under the capsule and collect the blood of that region, but this is incorrect. If a liver is injected with two granular masses of different colors, one into the hepatic vein and the other into the portal, it will be found that in all cases it is always the branches of the hepatic vein which come to the surface of the liver and spread out between the meshes of the arterial plexus.

From our present knowledge of the vascular system of the lobules we can easily understand how these three sets of vessels communicate freely at this point. In order to test this question in another way, I injected whipped blood into the artery of a fresh liver and found that three-fourths of it came out of the portal vein and one-fourth out of the hepatic vein. In another experiment the arterial pressure was kept constantly at 100 mm. Hg. with the same result as above. Then upright glass tubes were

[32] Theile, Handwörterbuch d. Physiologie, II, 1844, p. 342.

inserted into the cannulæ connected with the veins, with the following result:

Time	Blood rose in the tube in the Portal vein	Hepatic vein
1 minute.	5 cent.	.0 cent.
2 "	9 "	6.0 "
3 "	11 "	8.5 "
4 "	12 "	10.5 "
5 "	12.5 "	11.5 "

Further experiments show that at a given pressure it takes about as long for a liter of whipped blood to flow from the portal vein to the hepatic vein as in the opposite direction. Together these tests show that the artery communicates more freely with the portal vein than with the hepatic, apparently speaking in favor of Ferrein's venous rootlets to the portal vein. If it is considered that under normal conditions there is a high blood-pressure in the portal vein, higher than in any other vein, it is not remarkable that an injection into the artery should flow more freely from the portal vein than from the hepatic vein.

There seems to be no definite way to settle this question, except by making sections of injected specimens. If a cannula is tied into the hepatic vein and a single spurt of an aqueous solution of Prussian blue is made into it, it is found that numerous minute blue spots appear below the peritoneal surface of the liver. Sections of such specimens show that all of the fluid has entered the substance of the liver at the centers of portal units. If the injection is pushed a little farther—until the lobules are outlined—a large portion of the blue enters the terminal portal veins from the common capillary plexus of the lobule. As soon as this has taken place the fluid backs into the larger veins, forms secondary injections of other units and the picture becomes confused. In order to obviate this, I injected the artery with Prussian blue gelatin in which were suspended a large number of granules of cinnabar. The blue again flowed over into the portal and the hepatic veins, but the granules all lodged in the capillaries in the periphery of the lobule. Very few granules were found in any of the terminal portal twigs. This experiment shows at least that the bulk of the granules reach the capillaries of the lobule without passing through the portal vein. But an insignificant number of red granules are found lodged in the capillaries within the capsule of Glisson, and these encircle the bile ducts.

The first experiment, with but a spurt of Prussian blue into the artery, shows that the long delicate arteries give rise to capillaries which form a plexus around the bile ducts and then enter the center of the portal

units together with a terminal portal twig. Now these arterial capillaries communicate with the capillaries of the lobule, as do all of those that encircle the bile ducts. At no point does any of the injected mass enter the portal branches within the liver unless it is as a backward injection, in a direction the blood does not circulate normally. To test this question thoroughly the whole portal tree was first plugged with a thick granular injection mass made by mixing lamp-black or baryta with gelatin. The venous tree thus plugged did not cut off the capillaries, but prevented a second injection into the artery from spreading in the vein. In

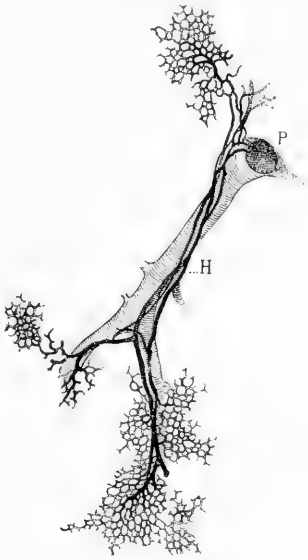


FIG. 53. Picture to show the termination of the hepatic artery. × 40. *P*, portal vein; *h*, hepatic artery. The portal vein was first plugged with a granular mass and then an aqueous solution of Prussian blue was injected into the artery. The extent of the capillary injection with the blue is shown.

general it was found that baryta gelatin for the portal vein and aqueous Prussian blue for the artery gave the most satisfactory result. All the vessels and capillaries injected with either fluid are always sharply defined. In such an experiment it is possible to force the blue fluid through all of the capillaries arising from the artery and in case they collect within the capsule of Glisson which empty into the portal vein, their beginnings should be marked. But at no place were such veins found; the blue had only percolated through the white mass in the interlobular veins. The capillary plexus around the bile ducts communicated directly with the

capillary plexus of the lobule. This condition seemed to be true down to the largest bile duct. At no time did I find a collecting vein within the substance of the liver, and I must, therefore, declare the rami vasculares venosi as mythical.

It is evident by studying good injections of the artery after the portal vein has been plugged that the artery communicates with the lobule throughout the extent of the vessels of the sixth order and possibly in part with those of the fifth order (Fig. 53). All the capillaries in the capsule of Glisson in this region communicate only with those of the lobule and they do not communicate at all with the portal vein. In those regions of the liver immediately around portal veins of the fourth order it is always found that in their immediate neighborhood there are both arteries and veins of the sixth order which ramify again, as stated above. So down to and including vessels of the fourth order all of the capillaries of the artery communicate directly with the capillaries of the lobule. By consulting the table giving the number of vessels of the various orders it is seen that this observation excludes the possibility of any veins of Ferrein arising from over a million terminal arteries and leaves them to arise from the walls of the main trunks of the first three orders; probably there are no more veins of Ferrein that empty into the vena portæ than can be seen with the naked eye.

The hepatic artery then supplies the gall bladder and the hilum of the liver, and the veins from this region communicate with the portal vein. The branches of the artery then enter the lobes of the liver with the branches of the portal vein and the bile ducts. Here the artery gives off a few branches to the bile ducts which form a capillary plexus around them, after which it communicates with the capillary plexus of the lobule. By far the greater number of arteries enter the centers of the portal units and communicate at once with the capillaries; they supply the periphery of the lobules. There are about a million of these very small terminal arteries, one for each portal unit which then spread out toward the nodal points and the hepatic veins. The branches that spread over the capsule of the liver supply the subcapsular portal units and then communicate with the hepatic veins. Some of the hepatic veins perforate the subperitoneal lobules as described by Kiernan; none of the portal twigs reach the surface. The great bulk of the arterial blood is equally distributed from the centers of the portal units and is fully mixed with portal blood before it reaches the nodal points or the hepatic veins.

Relation of the Connective Tissue to the Structural Unit.

From the very earliest appearance of the liver, from the time the sprouts of epithelial cells invade the omphalo-mesenteric vein and break it into sinusoids, it is extremely difficult to demonstrate connective tissue cells within the liver substance. In all cases the endothelial cells lining the capillaries of the lobule come in apposition with the liver cells, and there are no nuclei between them. The cells which have been described as connective tissue cells have been abundantly proved to be von Kupffer's stellate cells, and these are, according to von Kupffer's last paper, the endothelial cells of the capillaries.[33]

It is impossible to demonstrate sharp outlines to the lining cells of the capillaries of the liver lobule with nitrate of silver. Successful injections show the markings in the portal vein until it reaches the lobule and there they stop. From now on the endothelial cells form an extensive syncytium with large openings through which the blood plasma comes in direct contact with the liver cells. However, there is a framework of fibers which has been described from time to time during the last fifty years as delicate, naked fibers which encircle the capillaries as they pass through the lobule.[34] This adventitia capillaris of His can be demonstrated by brushing fresh sections, but clear pictures were not obtained until special methods were invented for this purpose by Oppel[35] and by myself.[36] Oppel isolated the net-work by his special precipitation method which showed the thickness of the fibrils and their relation to the surrounding tissue. By my method all the cells were destroyed by digesting fresh sections in pancreatin, leaving only the fibrils which for special reasons were classed with the reticulum fibrils of the lymph node as well as those of other organs.[37] It is now generally admitted that the *Gitterfasern* and my reticulum are identical and that they form the framework of the lobule.[38] That they are the same is shown by digesting a section upon the glass slide by the method of Spalteholz when pictures of reticulum identical in form and arrangement with the *Gitterfasern* are obtained. Upon the network of fibrils of reticulum which encircle the

[33] The extensive literature upon this subject may be found collected with a critical discussion in Oppel's Lehrbuch, III, 1900.

[34] Kupffer, Arch. f. Mik. Anat., LIV, 1899.

[35] Oppel, Anat. Anz., V, 1890; VI, 1891.

[36] Mall, Abhandl. d. K. S. Gescell. d. Wiss., XVII, 1891; and Johns Hopkins Hospital Reports, I.

[37] See also Mall, On the development of the connective tissues from the connective-tissue syncytium. Amer. Jour. Anat., I, 1902.

[38] Hoehl, Arch. f. Anat., 1897; and Oppel, Lehrbuch, III, p. 1009, 1900.

capillaries the syncytium of endothelium lies. We have, therefore, but three elements within the liver lobule, liver cells, a syncytium of endothelial cells, and a network of reticulum between them.

I have been unable to find any of the fibrils of the reticulum of the liver in embryo pigs less than 2 cm. long. If frozen sections are made of fresh livers at this stage it will be found that they are very delicate and can be crushed under the coverglass very easily. When such preparations are stained by allowing a solution of magenta to run under the coverglass, it is seen that a network of stained fibrils lies between clumps of liver cells. In any such sections it is easy to determine that all of the fibrils of the young reticulum surround the capillaries and are in intimate connection with Kupffer's endothelial cells. The fibrils, or rather the syncytium is delicate, and can be broken easily by slight pressure upon the coverglass. Frozen sections are easily broken into granules by shaking them slightly in water, showing that the reticulum is not strong. When digested for a short time, at room temperature the liver cells disintegrate, leaving only the delicate syncytium to which many small granules adhere. Pressure upon the cover glass shows that the reticulum is very elastic. Acetic acid does not cause it to swell and become transparent.

It is not difficult to obtain fresh specimens with all of the capillaries surrounded with this delicate reticulum with the endothelial nuclei imbedded in it. The continuity of the endothelial cells with the embryonic reticulum is complete, and thus forces us to the conclusion that the fibrils are developed from the endothelial cells in the same manner as I have shown that they are developed from the connective-tissue syncytium elsewhere.

This observation, however, is entirely out of harmony with the development of connective tissues in general, for they arise from mesenchyme, while the reticulum of the liver arises from the angioblast. However, the reticulum fibrils are in no way connected with the liver cells and there is no third group of cells in this neighborhood. It is possible that these fibrils reach into the lobule from distant interlobular spaces where connective tissue cells may be found.

If the liver of a dog is carefully crushed with the fingers in a stream of water all of the lobules are gradually destroyed and the portal and hepatic trees may be separated. At the end of the tips of the portal tree there are small enlargements which correspond with the portal units; no corresponding lobules are found to adhere to the tips of the hepatic veins. The stronger tissue—the capsule of Glisson—is thus isolated, while the

framework of the lobule is destroyed. Sections of the tips of the two systems of vessels show that more connective tissue extends to the periphery of the lobule along the portal vein than along the hepatic vein. However, in nearly all animals it is not the entire lobule which is surrounded by an increased amount of connective tissue, but only that which marks the center of the portal unit.

From the study of the connective tissue of the liver by various methods, it is found that it is impossible to draw a line of separation between the interlobular and the intralobular tissues. One appears to be continued into the other. However, there are stronger bundles which take a direct course toward the hepatic vein, not always following the capillaries. These are the so-called radial fibers. More delicate fibrils communicate with them and form a dense network around the capillaries. In general the fibrils radiate from the centers of the portal unit towards the nodal points as well as towards the terminal hepatic veins. They are of course arranged as are the capillary blood-vessels, which were discussed above.

While the liver is growing it is evident that with the destruction and transformation of the lobules and portal units the reticulum must be constantly tearing and shifting. This is possible with such a delicate embryonic tissue, and it may be that the connective tissue around the portal vein in a given stage lay within a lobule in an earlier stage. Veins of the third order are terminal in embryos about 2 cm. long, *i. e.*, they are interlobular and intralobular, while later on these same veins become main trunks. In the pig's liver there is no indication of any connective tissue capsule of the lobule before birth, and it probably does not appear until the liver is fully formed. Sections of the liver of pigs two months old show the lobule outlined by marked bands of cell radiating from the centers of the portal units to the centers of the nodal points. Possibly at this time the reticulum between the lobules is a little denser than that within the lobule. However this may be, the capsule of Glisson is but slightly marked, having within it but few nuclei of connective tissue cells. It is evident that the connective tissue of the liver must be studied anew from the standpoint of an ever-changing lobule during development.

The Lymphatic Ducts.

Hand in hand with the development of the capsule of Glisson, the lymphatic ducts grow into the liver. According to Professor Sabin, they arise from the receptaculum in embryo pigs and grow along the trunks of the artery and portal vein. How rapidly they grow and function has

not yet been determined, but it is certain that in the cat and the dog they do not extend beyond the center of the portal unit. I have been able to trace them in numerous specimens beyond portal vessels .06 mm. in diameter, and this naturally puts them into the center of the unit. One great obstacle in the way of studying the finer lymphatics is that the amount of connective tissue and the size of the portal vein seem to vary in inverse ratio, and when the veins of the sixth order are distended to their maximum the surrounding connective tissue is compressed, and consequently the lymph ducts are completely obliterated. It is not easy, therefore, to obtain clear pictures of the lymphatics from their very beginning down to the main trunks.

The origin of the lymphatics of the liver was first definitely determined by MacGillavry,[39] who studied this subject under the direction of Ludwig. Long before the work of MacGillavry it had been observed that ligature of the bile duct was followed by passage of bile over into the lymphatics, and the artificial filling of the lymphatics naturally followed, by injecting a colored fluid into the bile duct. Sections of liver, in which the lymphatics had been filled with Prussian blue, or with asphalt, showed that the fluid injected into the bile ducts leaves them at the periphery of the lobule to enter spaces surrounding the blood capillaries, the so-called perivascular lymph spaces. These spaces communicate at the periphery of the lobule, that is, in the center of the portal unit, directly with the interlobular lymph channels. Frequently there is an extravasation of the injection mass into the blood capillaries of the lobule.

These observations were subsequently confirmed by numerous competent investigators, using the method employed by MacGillavry as well as that of direct injection of Prussian blue into the walls of the portal and hepatic veins. In successful injections made in this way it is found that the Prussian blue injected enters the center of the portal unit and from there radiates and encircles its blood capillaries.[40] Such injections, however, are always accompanied with numerous extravasations of the injected material into the surrounding tissues, and often there is a secondary injection into the blood capillaries. This fact has raised an objection to the direct injection of the lymphatics from the bile capillaries. It appears more probable, the opponents say, that the extravasation of bile, or the injected material into the center of the portal unit enters the lymphatic radicals of the capsule of Glisson, and from them the larger lymph channels and the perivascular spaces of the capillaries are filled. Fur-

[39] MacGillavry, Wiener Sitzungsber., 1864.

[40] Budge, Ludwig's Arbeiten, 1875.

thermore the injected mass may pass from the pericapillary spaces directly into the capillaries, thus accounting for their frequent injection.

According to Fleischl,[41] all the bile is taken up by the lymphatics after ligature of the bile duct, and in case the thoracic duct is also ligated no bile or only a trace of bile ever reaches the blood. The observation of Fleischl has been confirmed by Kunkel,[42] Kufferath [43] and Harley.[44] It is extremely difficult to understand why the bile does not enter the blood capillaries in case it passes from the bile capillaries over into the perivascular spaces before it reaches the interlobular spaces after ligature of the bile duct. A further objection to the idea that the perivascular spaces first take up the bile, after ligature of the duct, is the fact that fluids injected into the bile duct pass with ease over into the lymphatics but only with difficulty into the bile capillaries. In all cases it appears as if the main origin of the lymphatics is at the center of the portal unit and that the radicals communicate freely with the perivascular lymph spaces. Furthermore, it appears that the course the bile takes after ligature of the bile duct, or of a fluid injected into the bile duct in passing to the lymphatics, is well within the center of the portal unit and not within the lobule. This idea is greatly strengthened since we know that the walls of the capillaries of the lobule are extremely porous, being composed of a dense basketlike layer of reticulum fibrils [45] upon which lie the endothelial or Kupffer's syncytial cells. This layer of reticulum fibrils encircling each capillary has been isolated by Oppel [46] and by myself [47] and is sufficiently described above. The capillary walls then are very pervious, blood plasma passing easily from them out into the perivascular spaces to bathe the liver cells.

It is well known that a large quantity of lymph is constantly passing from the liver, much more than from any other organ. That this lymph comes directly from the blood is indicated by its high per cent of proteid matter, nearly equal to that of the blood, and from two to three times that of the lymph from other parts of the body.

The course the lymph takes from the blood capillaries to the lymph radicals, *i. e.*, its natural course, can easily be marked by injecting colored

[41] Fleischl, Ludwig's Arbeiten, 1874.
[42] Kunkel, Ludwig's Arbeiten, 1875.
[43] Kufferath, Arch. für Physiol., 1880.
[44] Harley, Archiv für Physiol., 1893.
[45] Kupffer, Arch. f. Mik. Anat., 54.
[46] Oppel, Arch. Anz., 1890.
[47] Mall, Abhandl. d. K. S. Ges. d. Wiss., XVII, 1891. See also Johns Hopkins Hospital Bulletin, XII, 1901.

gelatin into any of the blood-vessels of the liver. I have usually found it most convenient to inject the gelatin into the portal vein, but it is just as easy to fill the lymphatics by injecting either the hepatic artery or hepatic vein. In all cases the colored fluid reaches the main lymph channels in the same way. The colored gelatin flows with great ease from the capillaries at the center of the portal units as well as from those around the smaller hepatic veins into the lymphatics. After the lymphatics have all been filled it is well to inject a small quantity of fluid of different color into the blood-vessels. A much better method of making double injections is to mix red granules with a glue gelatin or blue granules with

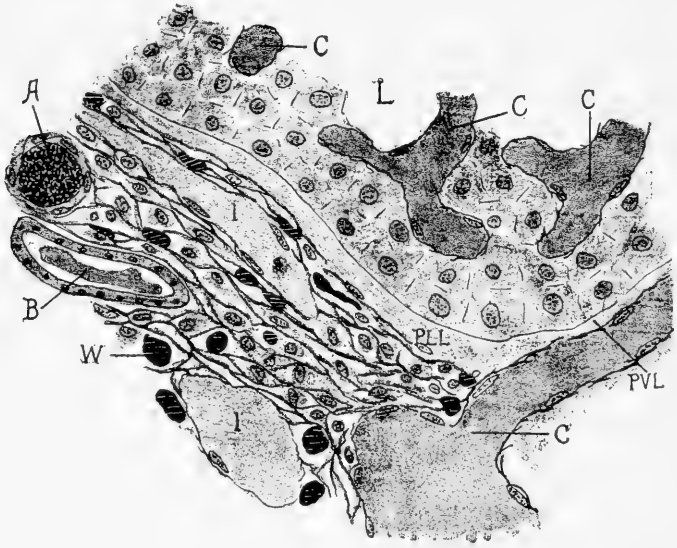


FIG. 54. Section through the center of a portal unit of a cat. × 500. Stained by Van Gieson's method. The hepatic artery was injected with cinnabar gelatin, and the portal vein with Prussian-blue gelatin. *L*, lobule of liver; *c*, capillaries; *a*, artery; *l*, lymph vessel; *plv*, persivacular lymph space; *pll*, perilobular lymph space; *w*, bundles of white fibrous tissue between which are loose connective tissue fibrils and cells.

a red gelatin, the fenestrated lining membrane of the capillary acting as a sieve which allows the fluid to pass but holds back the granules, as is the case with the blood corpuscles and plasma in life.

When the portal vein is injected with Prussian-blue gelatin at a low pressure, it is found that in a few minutes the lymphatics are all filled with the blue mass. Livers injected in this way are best hardened in formalin and then cut by the freezing method, for alcohol causes the

gelatin to shrink. Such sections show that the blue fluid has entered the lymphatics at the center of the portal unit. The specimens are more instructive when the injection is stopped just as the first lymphatics are filling with the colored gelatin. By following the larger portal veins and lymphatics back into the liver substance it is found that the interlobular connective tissue is entirely filled with blue where the lymphatics are injected, but only partly colored blue when they are not. In other words, the blue extravasates from the capillaries at the center of the portal unit and invades the connective tissue to reach the beginning of the lymphatics, when of course it is carried rapidly from the liver. The nearest course from the capillaries to the lymphatics is at the center of the portal unit where the amount of connective tissue is small, for as colored fluid begins to enter lymph channels only the tips of the capsule of Glisson are entirely tinged, while the larger portal spaces are encircled by a zone of color. Furthermore, it is found that in certain instances, where the injection was too brief, that the blue did not enter the lymphatics at all. In such specimens all of the interlobular spaces are surrounded by a zone of colored gelatin which does not enter the main lymph channels.

A successful injection of the lymphatics is illustrated in Fig. 54. The granular blue enters the capillaries of the lobule, *c,* with ease, and from them the liquid blue is filtered through the capillary walls to enter the perivascular lymph space, *pvl.* This space communicates at the center of the portal unit directly with a large lymph space between the liver cells and the capsule of Glisson, which may be called the perilobular lymph space. These spaces, *pll,* in turn communicate with the lymph radicals.

It is further shown by injecting the liver with aqueous Prussian blue that there are no capillaries between the periphery of the lobule and the interlobular connective tissue. The liver cells come in contact with the capsule of Glisson. An injection of brief duration with blue gelatin soon fills the perilobular lymph spaces, so that it appears as if all groups of liver cells at the periphery of the lobule were separated from the interlobular connective tissue with capillaries. In case cinnabar granules are mixed with the blue a few of these granules are found in the perivascular and perilobular lymph spaces, the openings in the walls of the capillaries being large enough to allow a few of the smaller granules to escape. As the injection is extended the blue invades the connective tissue spaces from the lymphatic radicals more and more until a lymph channel is reached, when of course it rapidly fills all of the larger ducts. Were there a direct channel from the perilobular lymph spaces the blue

would flow through it at once without further filtration through the interlobular connective tissue spaces. The course the cinnabar granules take also speaks against a direct channel between the perilobular lymph spaces and the interlobular lymph channels. A few of the granules enter the perilobular lymph spaces, but none of them reach the main lymph channels. All of my specimens without exception force me to the conclusion that there are no direct channels connecting the perivascular and perilobular lymph spaces with the lymphatics proper other than the ordinary spaces between the connective-tissue fibrils of the capsule of Glisson. These spaces, however, are relatively large, permitting of a rapid transfusion through them.

Injections with a hypodermic syringe into the walls of the smaller portal veins naturally fill the surrounding lymphatic vessels, and when no valves are in the way the injected fluid passes to the origin of the vessels, or lacunæ, which are located in the center of the portal units. From here the fluid passes through the main connective-tissue spaces into the perilobular and perivascular lymph spaces, and frequently from them into the blood capillaries. When the injection of the lymphatics is made through the bile ducts I have always found that there is an extravasation at the center of the portal unit, although the bile capillaries are often injected to the nodal points. The extravasation does not take place from the bile capillaries, but only from the duct as it communicates with the capillaries as well as from the larger bile ducts. Such extravasations naturally are then taken up by the lymphatics and carried from the liver. If after ligature of the bile duct the bile enters the perivascular lymph space within the lobule it may still be carried to the lymphatics, as the direction of the current of lymph is constantly from the blood capillaries to the lymphatics.

That the blood capillaries of the liver communicate more freely with the lymphatics than do the bile ducts is proved by injecting the bile duct and the portal vein with fluids of different color under the same pressure at the same time. In all the experiments I made the fluid injected into the vein appeared in the lymphatics first. In many instances beautiful injections of the lymphatics were obtained from the vein while the fluid injected into the bile duct did not extravasate at all, showing at least that the veins communicate with the lymphatics much more freely than do the bile ducts.

It is seen from the above description that the lymphatics of the liver do not drain all portions of the liver lobule, but only those portions that are formed by the centers of the portal units. There are no lymphatics at

the center of the lobules nor at the nodal points. At the center of the portal units there is a very free communication between the blood capillaries and the lymph radicals in the tips of the capsule of Glisson. So the lymph circulation is marked at this point, *i. e.*, the center of the portal structural unit, while in the rest of the unit it is insignificant or wanting altogether.

Relation of the Bile Ducts to the Structural Units.

All of our knowledge of the growth of the liver lobule indicates that the multiplication of the cells is in those portions of its periphery which mark the centers of the portal units. However, this statement is extremely difficult to prove. It is probable that the bile ducts communicate with the capillaries of the lobule throughout the whole length of the ducts of the sixth order, much as is the relation of the hepatic artery with the blood capillaries of the lobule, and unlike that of the portal vein, which gives rise to capillaries only at its tip.

Much has been done to gain a clear understanding of the development, growth and regeneration of the liver cells, but the results are very meager, for only in rare instances are karyokinetic figures found in them. Frequently, however, cells with two nuclei are found and these appear to be scattered quite evenly throughout the lobule. I have studied many sets of serial sections of livers in all stages of development and have nearly always failed to find karyokinetic figures. In the few specimens in which cell divisions were present they were in groups of several hundred around the terminal bile ducts. MacCallum [48] has also found a specimen in which there were numerous karyokinetic figures at the periphery of the lobule together with indications that the cells are being destroyed around the central vein. Ponfick [49] has shown that such figures are very numerous in the early stages of regeneration after removal of a large portion of the liver. In his specimens the dividing cells were found distributed evenly throughout the lobule which, on account of its growth, has become much enlarged with a disarrangement of the radiating strands of cells. Recently Schaper [50] has discussed the question from a broad scientific standpoint and concludes that when the regeneration of the liver tissue forms typical lobules, the growth has taken place entirely within the minuter bile ducts. This conclusion is admitted by MacCallum [51] for only those cases in which

[48] MacCallum, W. G., Jour. Amer. Med. Assoc., 1904.
[49] Ponfick, Vir. Arch., CXXVIII, Supplement, Heft., 1895.
[50] Schaper, Arch. f. Entwicklungsmechanik, XIX, 1905.
[51] MacCallum, Johns Hopkins Hospital Reports, X.

the liver cells have all been destroyed; then the epithelial cells of the gall ducts take upon themselves the more complicated process of regeneration of liver tissue. At any rate, it is now well known that liver cells often contain more than one nucleus and that in a variety of pathological disturbances as well as in normal development the bile ducts have a tremendous power of growth. The aberrant bile ducts have been known since the time of Ferrein, and probably represent liver tissue which was present and active at some earlier stage of development.[52]

It has been proved quite conclusively by Toldt and Zuckerkandl in their excellent study on the growth of the liver that degeneration takes place in one part of the organ while it is growing large in another portion. The vasa aberrantia mark those portions which have degenerated as along the left lateral ligament and the region of the vena cava and the gall bladder. For example, the gall bladder in its growth encroaches upon the substance of the liver and causes its atrophy. The liver lobule in degenerating is often reduced to small islands which have the portal vein on one side of them and the hepatic on the other, returning to its early embryonic state. It is probable that a similar but diffuse degeneration is taking place in many portions of the growing liver, for vessels which are of equal size in a given step are often found very unequal subsequently. It is also known that pressure by foreign bodies, by exostoses and by the ribs in excessive lacing may produce atrophy of the liver which is always marked by aberrant bile ducts, with hypertrophy elsewhere in the organ.

The striking experiments of Ponfick first showed us to what extent the liver may regenerate. His valuable communication also illustrates most beautifully that liver lobules do not hypertrophy, but sprout and give rise to new lobules, a conclusion which he thinks he disproves. Ponfick finds that after a portion of the liver has been removed the lobules in the remaining portion coalesce, and are not sharply defined as they should be in their hypertrophy (p. 86). He repeatedly states that it is difficult to find enlarged lobules, but in their stead he finds heart-shaped or clover-leaf-shaped lobules (pp. 104, 107), exactly what is to be expected in a growing liver. However, he does state that when the liver hypertrophies evenly in all directions the circumferences of the lobules are increased, two, three, or even four, times. This statement he illustrates with a figure of a lobule (Fig. 2) which is compound and on account of the large veins in it must be from its base, a condition which may be found in any liver which is not growing. His figure 7 which is

[52] Toldt and Zuckerkandl, Sitzungsber. d. Wiener Akademie, LXXII, 1876.

enlarged to the same scale as Figs. 1 and 2, and Fig. 3, which is on a larger scale, are not given to show that the lobules hypertrophy when the liver regenerates and are therefore found to be about of normal size. It is proved by Ponfick's experiments, it seems to me, that in regeneration of

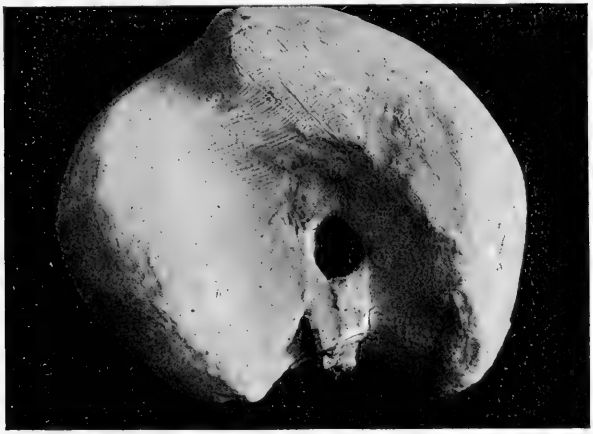


FIG. 55.

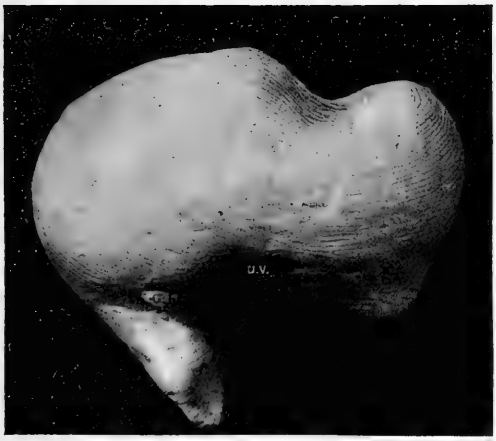


FIG. 56.

FIG. 57.

FIGS. 55, 56 and 57. Three views of a model of the liver of a human embryo, 17½ mm. long (No. 9). × 16. *Gb*, gall bladder; *uv*. umbilical veins.

the liver, the lobules do not enlarge, but sprout and give rise to new lobules, as is the case in the growing liver.

By comparing the livers of three embryos (Figs. 29-31 and 55-60) it is seen that only their upper surfaces are regular in form from stage to

stage; the processes extending into the abdominal cavity are irregular, to fit into the spaces that there are for them to grow into. Thus, in its

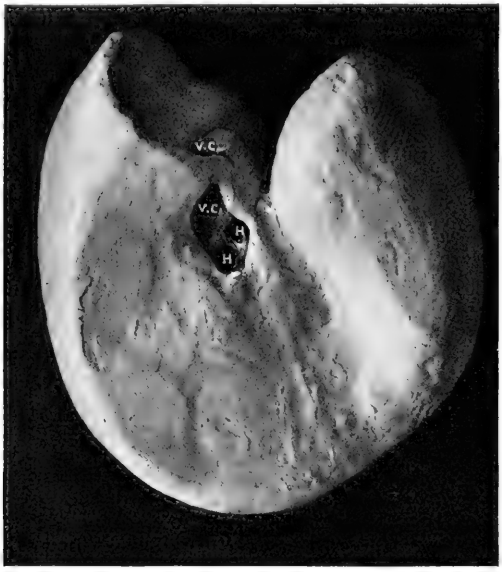


FIG. 58.

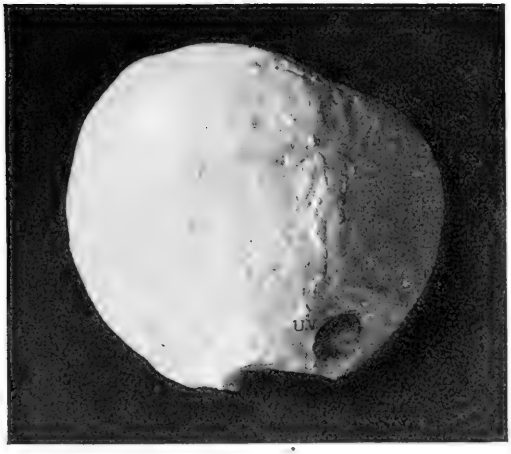


FIG. 59.

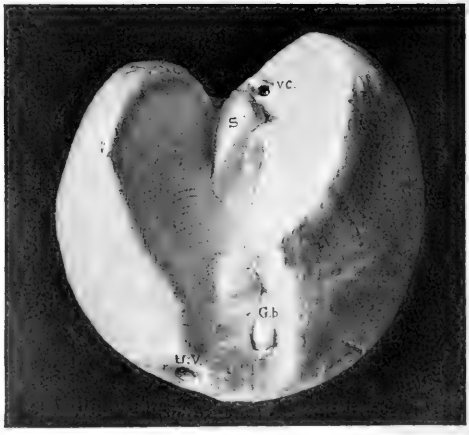


FIG. 60.

FIGS. 58, 59 and 60. Three views of a model of the liver of a human embryo, 24 mm. long (No. 10). × 8.

growth the liver may atrophy at one portion and expand in another, the aberrant bile ducts marking those portions of the liver which have been

shifted; they are present in those portions of the liver which had to make way for encroaching organs. Not only must large masses of the liver disappear entirely, but also smaller areas throughout the liver, especially along the trunks of the main vessels, as the liver is growing from its center towards its periphery. Hand in hand with this change, the plexus of bile ducts which surrounds the main trunks of the portal vein shifts towards the periphery, leaving only a single vessel in its place, which, of necessity, becomes very variable, as has been pointed out by Rex.

In a liver which has been well formed, as in the rabbit's liver shortly after birth, the cells radiate from the terminal bile ducts towards the nodal points and the central veins, as indicated by the lines in Fig. 1. The point of juncture between the bile ducts and liver cells is not sharp and the younger the liver the more difficult it is to determine it. In fact, in young embryos it is extremely difficult to follow the bile ducts into the structural units, *i. e.,* to the lobules. Injections show that the younger the specimen the more extensive is the plexus of bile ducts around the terminal veins, which indicates that an intermediate tissue, neither true lobule tissue nor true bile ducts encircle the terminal portal veins in growing livers, as is shown beautifully in a pig two months after birth. When the liver is finished, this tissue is reduced to a minimum. When the liver begins to regenerate, it becomes conspicuous again as "newly-formed bile ducts." With a plexus of bile ducts encircling a portal vein of the sixth order throughout its whole extent we have the most intimate connection between the bile ducts and the center of the portal unit, from which additions can be added to the unit. As the cells are added they seem to pile up in the nodal points, for the distance between the terminal portal and hepatic veins does not increase but remains constant. Hand in hand with the growth of the nodal points the capillaries follow, and on account of their increased number the resistance to the circulation through them is diminished, and, according to Thoma's first law, veins from both sides are extended into them; these alternate, as the observations above described have demonstrated.

To obtain an additional key by which we may unravel the growth and architecture of the liver units numerous tests have been made in the Anatomical Laboratory of the Johns Hopkins University, by Hendrickson, Sudler, Johnson, Sabin, Hill, and myself, with more or less satisfactory results. At present we are able to follow in a connected way the formation of the bile ducts and capillaries in embryos from 5 cm. long upward. Possibly at some later date they may be followed back to their earliest appearance. Furthermore, it is probable that some simple methods will soon be found, by which the history of the blood-vessels can be

determined much better than it is given now, and since it appears that the development of the artery and bile ducts are parallel the study of the one will help to clear up the other.

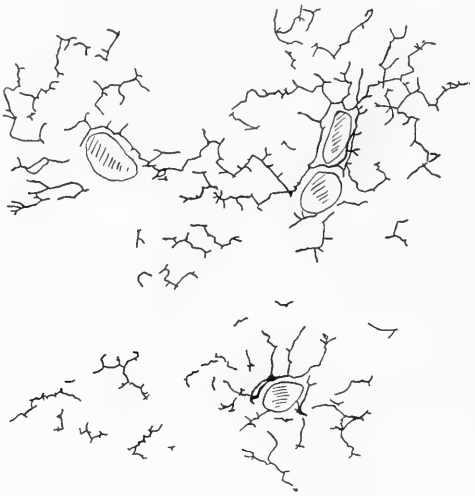


Fig. 61.

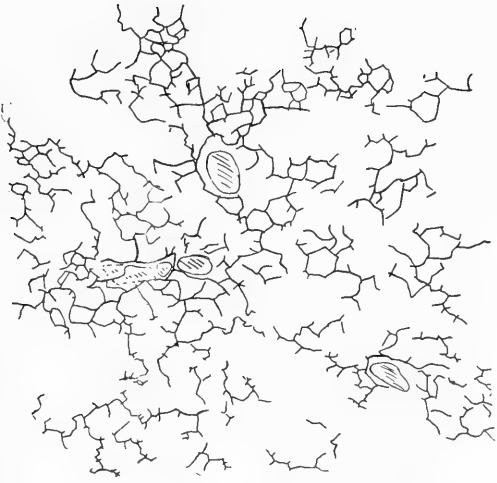


Fig. 62.

Fig. 63.

Figs. 61, 62 and 63. Golgi specimens of the livers of human fœtuses, 5 cm. and 10 cm. long, and at term. × 53. After Hendrickson.

Bile capillaries and ducts can be outlined beautifully by Golgi's method in human embryos 5 cm. long, or longer, as Figs. 61 to 63 show.

But it is difficult to interpret these specimens, for it is not easy to determine which vessel is a portal vein, unless reconstructions are made, which is often out of the question. In the earliest stage, Fig. 61, the capillaries encircle both hepatic and portal veins, the vessel to the left being an hepatic vein. The same is probably true in an older embryo, Fig. 62, while in a fœtus at birth the bile duct pictured lies at the junction of two portal veins. When the terminal ducts are arranged in order, as shown in Figs. 64-67, it is seen that the first bile ducts are formed around the portal veins from bile capillaries. Longitudinal sections, Figs. 68-72, indicate the same. This interpretation of the specimens, which was

Fig. 64.

Fig. 65.

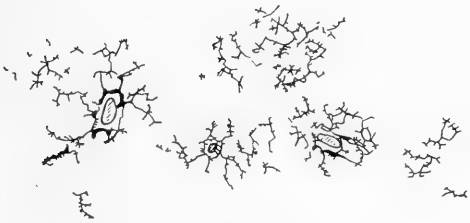


Fig. 66.

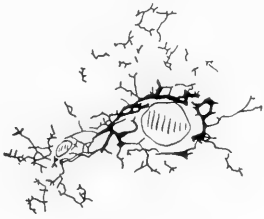


Fig. 67.

Figs. 64, 65, 66 and 67. Golgi specimens of the livers of fœtal pigs, 5, 6, 7 and 8 cm. long. × 53. The portal twigs are shown in transverse section. After Hendrickson.

first given by Hendrickson[53] is rational, and subsequent observations, which I have been able to make from some of Mr. Eben Hill's skillful injections of the bile ducts in the embryo, corroborate Hendrickson's view. The untimely death of Dr. Hendrickson made his preliminary report his final publication upon this subject, and it is now a pleasure to me to carry out in part one of his desires. The obstacle at the time of his publication was a lack of knowledge of the vascular tree and complete pictures of the bile ducts, especially in young embryos. Mr. Hill has supplied the latter by filling the bile ducts of a pig's embryo 10 cm. long. In the early stages diluted Higgin's India ink was injected di-

[53] Hendrickson, Johns Hopkins Hospital Bulletin, 1898.

rectly into the stomach from which it flowed over into the intestine and backed up into the bile duct. Within the liver it filled the main trunks which correspond with those of the portal vein and then filled a capillary network around portal branches of the first order. An illustration of this specimen is given in Fig. 73. This figure shows that the vessels

FIG. 68.

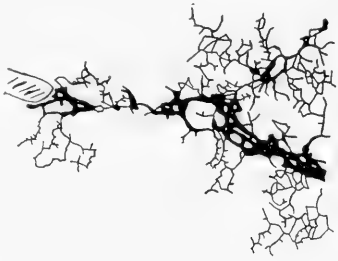


FIG. 69.

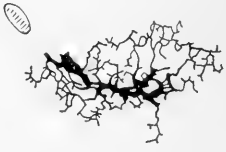


FIG. 70.

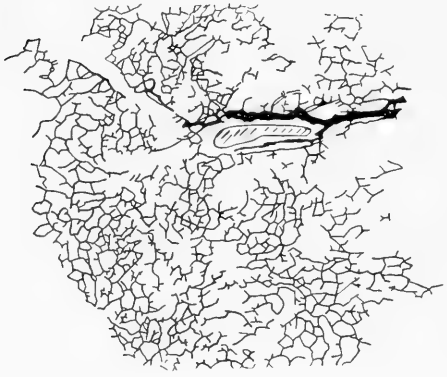


FIG. 71.

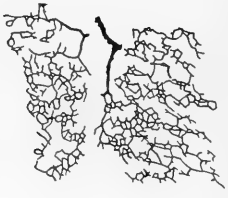


FIG. 72.

FIGS. 68, 69, 70, 71 and 72. Golgi specimens showing the terminal portal twigs with their surrounding bile ducts and capillaries in longitudinal section in fœtal pigs 8, 16, 19, 21 cm. long, and in the adult pig. × 53. After Hendrickson.

pictured in Figs. 65-67 are probably of the second order, while that in Fig. 64 is of the first order and, therefore, represents a main trunk.

The bile duct-system can be injected with greater ease in older embryos, for in embryos over 15 cm. long the injection may be made directly through the gall bladder. It is unnecessary to give all intermediate stages, for Fig. 74, which is from an embryo 20 cm. long, helps to tell the whole story. With it may be compared Figs. 68-71, for one gives the main trunks and the terminal plexus and the other gives the terminal plexus and the bile capillaries.

In the adult liver the artery communicates with the lobule throughout the extent of the vessels of the sixth order; the connection between bile ducts and bile capillaries is probably even more extensive. No bile capillaries arise from bile ducts of the fourth order, the liver tissues in their immediate neighborhood being drained by the plexus of the fifth and

Fig. 73. Bile ducts of a pig, 10 cm. long, injected with India ink. × 60; II, portal branches of the second order; III, portal branches of the third order. From a specimen made by Mr. Eben Hill.

sixth orders. So in the center of the portal unit the branch of the portal vein ramifies, while along a portion of its axis the artery and duct spread out. The unit is bounded on its periphery by a number of intralobular veins and nodal points, as shown in Fig. 1.

In the development of the liver the shifting of the peripheral plexus

of bile ducts is of great importance and helps to clinch much that has been said above about the development of the liver. In an embryo 10 cm. long this plexus encircles portal branches of the second order (Fig. 73). In an embryo twice as long the plexus has passed the veins of the third order and now encircle completely those of the fourth or fifth orders. So as the liver tissue is shifting towards the periphery

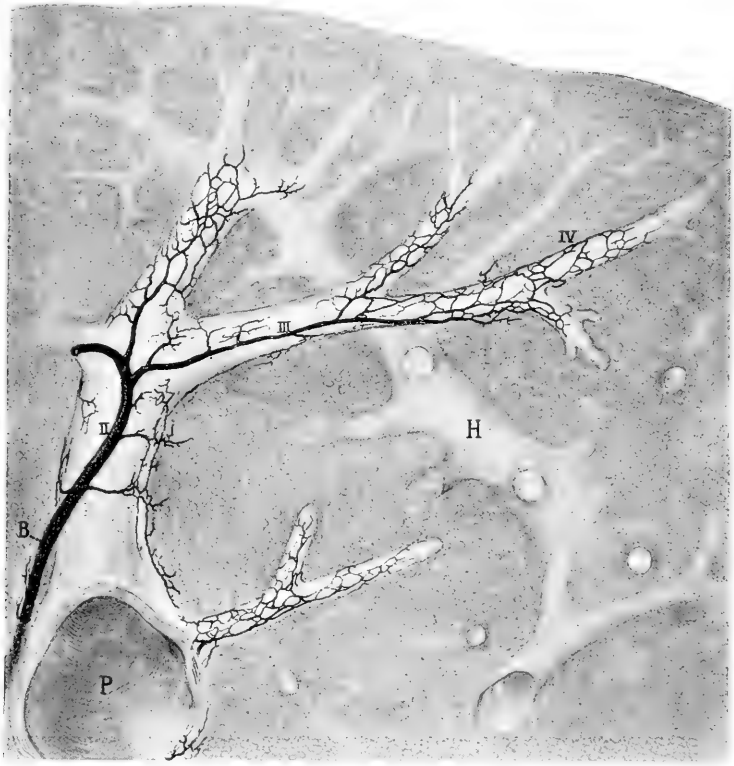


FIG. 74. Injected bile ducts of the liver of a pig, 20 cm. long. × 50. *B*, bile duct; *p*, portal vein; *h*, hepatic vein; II, III, IV, respective order of the branches. From one of Mr. Hill's specimens.

branches which were once central in every respect, are reduced entirely to main trunks, and throughout this process of growth the structural units remain practically of one size. Thus from one vein encircled by one structural unit a million are formed in the dog. Throughout this growth the vascular proportion is constant. Within the center of the unit the duct expands into a plexus from which regeneration takes place. The periphery of the unit is marked by nodal points which in one sense are embryonic units.

THE DEVELOPMENT OF CHROMATOPHORES IN NECTURUS.

BY

ALBERT C. EYCLESHYMER.

From the Anatomical Laboratory of St. Louis University.

WITH 7 FIGURES.

The questions regarding the origin of pigment in the epidermis of vertebrates is one of deep interest not only to the histologist, but also to the pathologist.

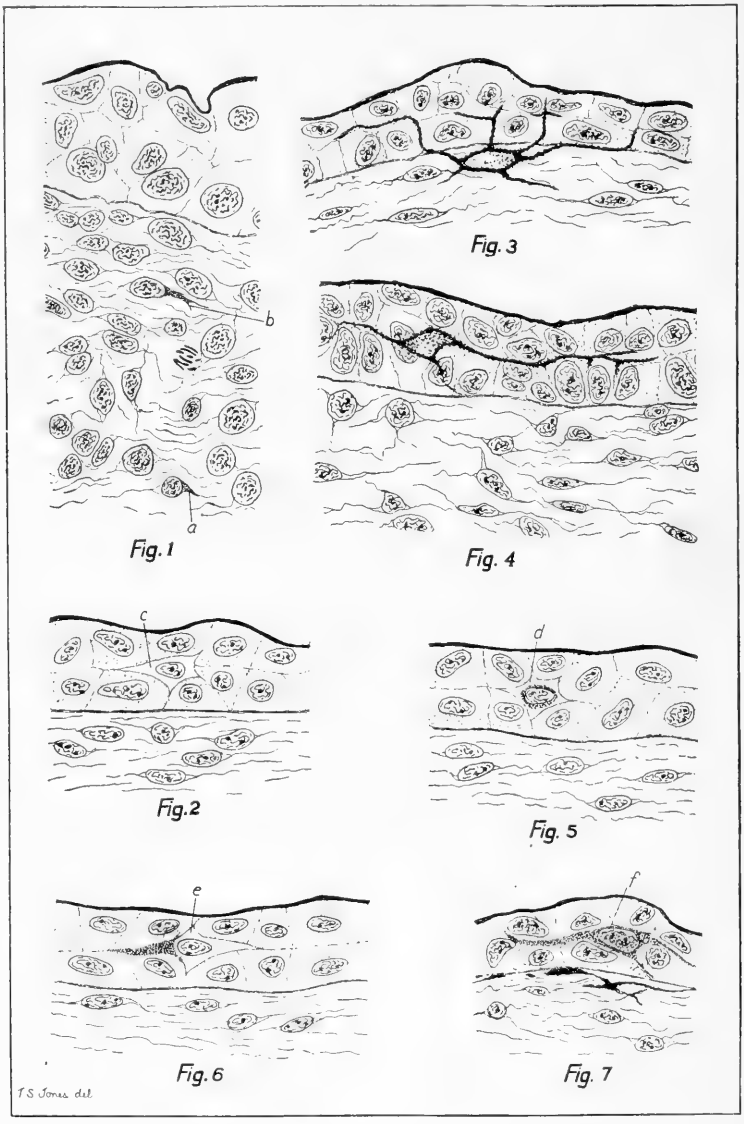
The literature shows that two different conclusions have been reached. One group of workers (Aeby, Kœlliker, Ehrmann, Ribbert and others) have regarded the epidermal chromatophores as modified mesenchymal cells, which have wandered into the epidermis. Another group (Kodis, Jarish, Kromayer, L. Loeb, Strong and others) have considered the epidermal chromatophores as modified epithelial cells which have differentiated *in situ*.

In the course of my studies of the larvæ of Necturus, I have been repeatedly attracted not only by the peculiar movements of the chromatophores in the normal embryo, but also by the striking changes in their character brought about through decapitation.

These casual observations led to a new method of studying the origin and movements of chromatophores. The method was briefly the following: The larvæ were placed in a bed of cotton in the fibers of which they soon became entangled, and were thus held in a given position for an indefinite period. The water in the receptacle containing the cotton was of course frequently changed. The larvæ were then observed under the higher magnification of the binocular dissecting microscope, and the movements of the living chromatophores followed. This study was then supplemented by a study of serial sections of larvæ of corresponding stages.

Larvæ 11-12 mm.—The first appearance of pigment is found in the larvæ of this length. When observed under the binocular microscope, this pigment appears as minute black dots lying deep down in the transparent connective tissues. If these minute structures be persistently watched for a few hours, or even examined at short intervals, it is easily seen that they gradually increase in size and slowly approach the surface. When first observed, the pigment seems to be confined to the body

of the cell, but it soon extends to the protoplasmic processes. It is then possible to follow the constantly changing positions of the processes.



This amœboid movement becomes more clearly defined as the chromatophores approach the surface.

Serial sections of larvæ of this stage show that the chromatophores are

not restricted to any particular locality. In the vicinity of the myotomes they are found for the most part in the intermuscular mesenchyme. Anterior to the myotomes they are irregularly scattered and although a few are found in the outer portion of the dermis, they are for the most part located in the deeper mesenchyme.

A study of the formation of pigment in the mesenchymal cells shows that it first appears in the immediate vicinity of the nucleus, and from this locality extends into the cytoplasmic processes. The pigment granules are at first separate and distinct, but as the cells become more deeply pigmented, the granules become less distinct. It is thus possible in a general way to differentiate between the younger and the older chromatophores. Fig. 1 is taken from a section through the head of a larvæ of the above length, and shows two stages in the growth of the chromatophore. The deeper cell (*a*) represents one of the first stages in the formation of the pigment. The pigment is here largely confined to the region of the nucleus, having extended but slightly into protoplasmic processes. The cell nearer the surface (*b*) represents a later stage in which the pigment has extended farther into the protoplasmic processes. Other sections show various stages in the formation of pigment from its first appearance in the region of the nucleus to its extension into all the protoplasmic processes of the cell.

The epidermis at this time is made up of different kinds of cells. The first and most numerous are the ordinary polyhedral cells, which contain fine yolk granules. The second and less numerous are the large oval or spherical cells which contain very large yolk granules, and probably form the unicellular glands. The third and least numerous are certain cells which possess more or less extended cytoplasmic processes and which, from their granular contents, staining capacity, and general form, closely resemble mesenchymal cells. Such a cell is shown in Fig. 2, *c*. lying among the epidermal cells. Whether these cells are modified epithelial cells or are mesenchymal cells, which have wandered into the epidermis at some earlier stage, cannot be definitely determined. As will be seen later cells of this type give rise to one group of epidermal chromatophores.

Larvæ 15-16 mm.—In the preceding stage but few of the chromatophores were at the surface of the dermis, but in the present stage large numbers of them have reached its outermost surface and through their widely branching processes form an open meshwork. These superficial chromatophores are most numerous over the dorso-lateral surfaces of the head, but they are also scattered along the body, being confined for the most part to an irregular dorso-lateral band which extends from the region of the gills to the posterior limb buds.

In sections of this stage one frequently finds conditions such as that represented in Fig. 3, in which the protoplasmic processes of the dermal chromatophores extend among the cells of the epidermis. Other sections show widely branched chromatophores lying wholly within the epidermis, as shown in Fig. 4. From the study of sections alone, one would readily infer that these epidermal chromatophores are simply the dermal chromatophores which have wandered into the epidermis. I have examined many sections with the hope of finding a chromatophore in which the cell body lay partly in the dermis and partly in the epidermis, but such a cell has not been found. All doubt, however, is dispelled by using the binocular microscope under which one can readily see the dermal chromatophores pass outward into the epidermis.

In the epidermis one frequently observes the peculiar type of cells described under the preceding stage. These cells may be as yet unpigmented or they may show varying degrees of pigmentation, often the pigment is confined to the region of the nucleus as shown in Fig. 5, *d*, again the pigment has extended to one or more of the cytoplasmic processes, as represented in Fig. 6, *e*.

Larvæ 17-18 mm.—The chromatophores show a marked increase in number over the preceding stage. In a number of larvæ, they have extended well down over the upper surface of the yolk. In the head region there is a median dorsal line which is almost free from chromatophores. On either side of this line chromatophores have extended downward to the upper margin of the nose and eye. The upper margin of the retina is now deeply pigmented, and not infrequently numbers of chromatophores are observed directly over the lens. They have extended to the base of the gills, although but few are seen in the gill bars. A few are present in the dorsal surfaces of both the anterior and posterior limbs. The dorso-lateral veins are present along the dorso-lateral surface of the yolk. Even at this early stage, the chromatophores are becoming more densely aggregated along the lines of these veins. Now as in the preceding stages, one can see in the region sparsely pigmented a continual migration of the dermal chromatophores from a deeper to a more superficial position. In those regions which are densely pigmented, one can readily see an increasing number of the dermal chromatophores passing into the epidermis.

Serial sections of this stage show a continued formation of the dermal chromatophores in the deep mesenchyme, and especially in the intermuscular spaces. As they pass toward the surface the pigment extends into the cytoplasmic processes and they become more and more branched. The greater number of these chromatophores pass to the outer layer of

the dermis and there remain, but a considerable number can be followed directly, as they pass into the epidermis.

The epidermis in addition to the increased number of chromatophores of this type also shows a considerable increase in the chromatophores of the second type which are as yet found in all stages of formation from the earliest condition to the condition shown in Fig. 7, which represents the complete formed chromatophore.

Conclusion.

The chromatophores found in the epidermis are of two kinds. One is but slightly branched, taking on in general a pyramidal form. The other is highly branched, taking on a mossy appearance. The former becomes pigmented *in situ* within the epidermis. They may be mesenchymal cells which have wandered into the epidermis before becoming pigmented, or they may be modified epithelial cells. The second type is derived from the mesenchymal cells which wander into the epidermis after becoming pigmented.

ON THE NATURE OF THE GRANULE CELLS OF PANETH IN THE INTESTINAL GLANDS OF MAMMALS.

BY

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WITH 5 FIGURES.

The recent activity in the investigation of the chemical and physiological properties of the succus entericus, and the discoveries of new enzymes which are produced by the intestinal mucous membrane, create a renewed interest in the structure and relationship of the elements composing the intestinal epithelium and glands, which are the sources of this secretion. It becomes a fundamental problem of intestinal histology to determine as far as possible the cytological and microchemical characters of these elements and to compare them in these respects with similar elements of known function from other sources. Of special interest in this connection are the peculiar, coarsely granular cells which occupy the deeper ends of the glands of Lieberkühn, and which were first observed in 1872 by Schwalbe, **72**, in fresh material from the intestine of the rat.

For some reason Schwalbe's description attracted little attention, and it was not until 1888 when Paneth, **88**, rediscovered them and described at some length their microscopic and chemical characters, that these cells became generally recognized as constant constituents of the intestinal glands of certain mammals. For this reason they are generally known as the granule cells of Paneth.

Paneth regarded these granular cells as a specific kind of gland cell wholly different from the globlet cell. Concerning their origin he was somewhat in doubt, although he favored the view that they were derived from indifferent mitotic cells farther up the gland, because of the fact that the granules became fewer towards the middle of the gland where the mitoses occurred.

Paneth described the granules, as observed in fresh preparations of the mucous membrane, as moderately refractive structures, although not so refractive as fat. Distilled water and solutions of caustic potash had no effect on them, although they shrank somewhat in the latter and became

more refractive. Ether and alcohol on the other hand dissolved the granules slowly and in diluted acids they disappeared instantly. By means of osmic acid and of picric acid he succeeded in fixing the granules and the cells which contained them. Flemming's fluid gave unsatisfactory results. He regarded the cells in question as a special kind of glandular cell different from the goblet cell. Concerning the fate of these cells Paneth did not express himself definitely although he inclined to the view that they are completely used up in secretion and are replaced by mitotic cells farther up the gland. His attempts to show that this was the case by observing the effect of physiological stimulation, however, did not lead to the desired result, inasmuch as the cells were as numerous in the animals which had been fed as in those which were examined in a state of hunger.

Nicolas, 91, also studied the granule cells and described at considerable length the different varieties of cells to be found in the bottom of the gland of Lieberkühn. Some of these he regarded as secretory phases in the history of the cell of Paneth. Of these he recognized several, of which the following may be mentioned: (a) indifferent cells with clear protoplasm; (b) fine granulations appear in the protoplasm-primary granules; (c) the granules contain a safraninophilous body in the form of a crescent or semicircle, the rest of the granule staining in Flemming's fluid; (d) the secretory activity has attained its maximum and the cell is completely filled with granules containing a safraninophilous body; (e) the cells expel the granules; (f) the cell contracting after expelling its contents assumes the aspect of the small narrow cell with deeply stained protoplasm; (g) the cell recovers itself and assumes the appearance of stage *a*. Nicolas also observed that in the later stages of secretion the nucleus became smaller, often irregular in shape, and stained diffusely, whence he concluded that the nucleus participated in the secretory activity of the cell.

Bizzozero, 93, did not acept the conclusion of Paneth and Nicolas that the granule cells were specific glandular elements, but attempted rather to bring the facts with regard to them into accord with his theory that the glands of Lieberkühn were not in reality true glands, but merely foci for the regeneration of the surface epithelium, and to convert the apparently adverse fact of the occurrence of peculiarly organized elements in the bottom of the glands into an additional proof of the validity of his theory. He claimed to have found, in material from the intestine of the mouse stained in safranin and hæmatoxylin after fixation in Hermanns' fluid, what he considered to be transitional forms between Paneth cells and goblet cells. In these preparations the mucin in the goblet cells

was stained a violet color while the Paneth granules became red. The cells which were regarded by Bizzozero as transitional cells contained both small red granules and large blue mucin granules. As these cells were observed in an intermediate position in the gland between the Paneth cells below and the goblet cells above it seemed probable to Bizzozero that the mucin in them had been produced by the transformation of Paneth granules. He assumed, therefore, that the Paneth cells were young goblet cells.

Subsequent investigators, however, among whom may be mentioned Möller, 99, Zimmermann, 98, Zipkin, 04, and Schmidt, 05, have failed to find the transitional elements described by Bizzozero, and have accepted the conclusion of Paneth and Nicolas that the granule-cells are specific elements engaged in a special kind of secretion.

Schaffer, 91, described and figured these structures in the glands of Lieberkühn of the duodenum and jejunum of man although he did not succeed in staining the granules. Zimmerman found that the granules stained strongly in iron hæmatoxylin in sections of human small intestine fixed in sublimate. He regarded the cells of Paneth as serous cells.

Möller, 99, studied the structure of the glands of Lieberkühn of a large number of mammals, chiefly in material fixed in a formaldehyde bichromate mixture, and stained in the Ehrlich-Biondi mixture, although he also used other fixing fluids for purposes of control, and applied the iron-hæmatoxylin method with good results. Möller found that the cells of Paneth occurred in the intestinal glands of the mouse, guinea-pig, rabbit, ox, sheep, and horse. His results were negative as regards the cells of Paneth in the pig, cat, and dog, although he regarded the failure to find them in the first-named animal as due to a failure to fix the granules. Möller also found that the granules in different cells often exhibited different affinities for the stains employed, so that, for example, in sections stained in the Biondi-Ehrlich mixture some granules stained red, others yellow, greenish-yellow, or dark olive green. This difference he thought to be due to different functional conditions of the cells, the changes which the granule underwent from the time of its first formation in the cell to the time when it reached its mature form being indicated by its staining properties. In some cells he found indications of the fusion of the separate granules to a common mass which in part occupied the meshes of the cell framework, in part the wide lumen of the gland. These facts he regarded as undoubted indications of a real secretory activity on the part of these cells. He found no transitions between Paneth cells and goblet cells.

Zipkin, 04, describes the Paneth cells of Inuus rhesus as present without exception in the bottom of every crypt, often lying beside one another in considerable numbers. The protoplasm of these cells always stain more deeply than that of the surrounding cells.

Oppel, 97, described, in the glands of Lieberkühn of Echidna, cells, at the bottom of the gland, the inner segment of which was finely granular. The granules diminished in number as the mouth of the gland was approached and in the upper portion of it were wholly lacking.

Schmidt, 05, studied the distribution of the cells of Paneth in the human intestine and confirmed the observations of Bloch, 03, who found them in practically every gland of the ileum and jejunum, as well as of the duodenum. In addition, Schmidt found Paneth cells frequently present in the glands of the vermiform appendix, although he was not able to find them in other portions of the large intestine except in three cases of pathological conditions. Concerning the occurrence of Paneth cells in the large intestine of the infant where Bloch claims to have observed them, Schmidt records a negative result in five newborn children.

For the differentiation of goblet cells from Paneth cells Schmidt used mucicarmine by means of which he obtained a sharp distinction even in the foetal intestine. As far as the function of the Paneth cells is concerned he regards the fact of their absence from the intestines of even young carnivora as opposed to the conclusion which might be drawn from Bloch's observation of their occurrence in large numbers in the large intestine of suckling infants, that they have something to do with the secretion of a substance which is active in the digestion of milk. He is rather inclined to the view that inasmuch as they are constantly present in the glands of herbivorous animals they affect some constituent of the vegetable food.

As far as the occurrence of Paneth granule cells in lower classes of Vertebrata is concerned comparatively few references can be found in the literature. Nicolas, 91, in the article already referred to mentions their occurrence in the lizard without stating the species examined, and E. Bizzozero, 04, has described, in the depressions between the folds of the intestine in Teleostomes, cells which contain numerous granules stainable in haematoxylin but differing in their characters from the young goblet cells which occur in the same location.

The last decade has been particularly fruitful in researches dealing with the morphology and microchemistry of glandular cells. As a result of these, new methods have been devised and new criteria established for distinguishing between zymogenic cells and mucous cells. In particular

may be mentioned in this connection the basal filaments of Solger, 94, which have been shown by the researches of Bensley, 96, Garnier, 00, Cade, 00, Zimmermann, 98, and others to be a structure common to many sero-zymogenic cells. Bensley, 96, 98, has also shown that the basal filaments correspond to the chromatin of the nucleus in their staining reactions and like the latter contain iron in the form of an organic compound and thus represent morphologically the substance presumably of nuclear origin which Macallum, 95, long ago discovered in gland cells of various sorts by means of staining reactions, and subsequently confirmed by means of the microchemical reaction for iron. As far as mucin is concerned no microchemical reaction has been, as yet, discovered, which is effective in recognizing this substance in isolated cells in sections. The work of P. Mayer, 97, has, however, provided us with a number of new staining solutions which, while they do not permit us to say whether a given cell does or does not secrete mucin, yet furnishes evidence which may be of much value when taken in connection with that from other sources.

Up to the present no special attention has been directed to the question of the presence of Macallum's prozymogen in the cells of Paneth, either in the form of basal filaments or as a diffused compound in the base of the cell, although several observers, notably Zipkin, 04, and Nicolas, have called attention to the deeper staining of the protoplasm of these cells as compared with neighboring cells. The results, moreover, of attempts to discover experimentally, differences in the aspect of these cells corresponding with phases of physiological activity have not been decisive. Accordingly, at the suggestion of Professor Bensley I undertook the reinvestigation of these structures in the hope that the application of new staining and microchemical methods would reveal new facts which would be of assistance in forming an opinion as to their nature and their relationship to other intestinal epithelial elements.

At a very early stage in the investigation the discovery was made that in the opossum, Didelphys virginiana, the cells of Paneth occurred not only in the glands of Lieberkühn but also mingled with other epithelial elements on the sides of the intestinal villi even at their very tips. This remarkable fact, which possesses no parallel in any other mammal, so far as is known, possesses so much significance in the interpretation of the Paneth cells that a somewhat extended description is called for.

The small intestine of the opossum is characterized by extremely long villi and a very thin tunica mucosa. Corresponding to the latter the glands of Lieberkühn are very short and contain a scarcely recognizable

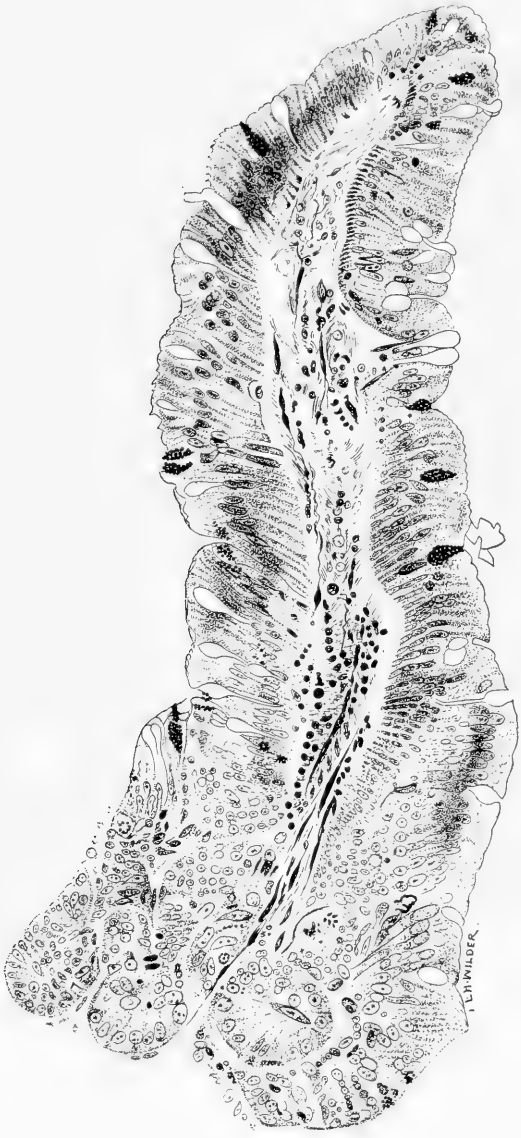

Fig. 1.

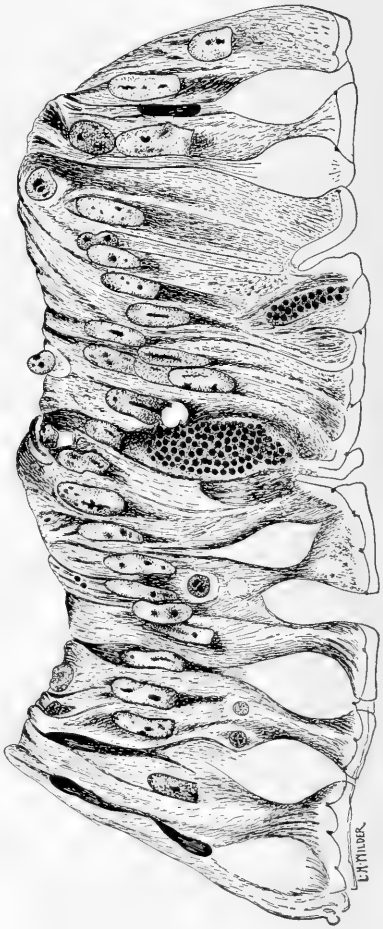

Fig. 2.

Fig. 1. Villus and subjacent glands of Lieberkühn of the opossum. From a preparation stained in iron-alum hematoxylin and mucicarmine.

Fig. 2. A portion of the epithelium of the villus shown in Fig. 1 as seen under Leitz Homog. Imm. 1/12, Oc. 4.

lumen. Fig. 1 illustrates fairly well the nature and distribution of the three kinds of epithelial cells in the glands and in the villi. The preparation is from material fixed in Bensley's bichromate-sublimate-alcohol fluid and stained with iron haematoxylin followed by mucicarmine. By this means the granules are stained blue-black in the cells of Paneth, red in the goblet cells. Thus a sharp differentiation is obtained between these cells even in the glands of Lieberkühn where the young cells contain comparatively little of the secretion-antecedent. The Paneth cells on the side of the villus are large and resemble very closely typical goblet cells (fig. 2). The theca is filled with large discrete granules which do not react with mucicarmine but on the contrary stain intensely in iron haematoxylin. These granules also stain strongly in the neutral gentian mixture recommended by Bensley, **02,** for staining zymogen granules, the mucous goblet cells remaining colorless. The granules in the Paneth cells occupy the meshes of a network which is formed by the cytoplasm separating the granules. At the proximal end these cells are narrower and contain a nucleus which is somewhat elongated in the direction of the long axis of the cell and often slightly cupped on the side next the theca. The basal protoplasm is small in amount and uniformly more deeply stained than that of neighboring cells, with the exception of the narrow cells which are obviously undergoing degeneration. Tested with Macallum's reagents for the detection of organic iron a positive result is obtained but not enough to be convincing evidence of the presence of prozymogen in the cell. The search for basal filaments also proved without result although the positive outcome of these observations in the guinea-pig, to be described presently, gave ground for the belief that had a more abundant material been available, and had it been possible to examine it in different physiological states, a positive result might have been obtained.

The glands of Lieberkühn in the opossum are remarkable for their low grade of development, and, although the three main types of cells are present, the amount of secretion which the Paneth cells and goblet cells contain indicates that they are to be regarded rather as young elements than as cells already functioning as secreting organs. Indeed, in some respects, these glands present but little advance over the epithelial buds to be found in the intestinal epithelium of Batrachia, and to this extent realize Bizzozero's idea of a gland of Lieberkühn which serves merely as a place for the production of new cells which ultimately migrate to the free surface and there reach their full functional development. Many mitoses are always present in the glands, some in mucin-

holding cells, others in the cylindrical cells. I have not been able to assure myself definitely of the occurrence of mitoses in the cells of Paneth. The small size of these cells and their more irregular shape and arrangement make the exact determination of the nature of the mitotic cells somewhat difficult.

The distribution of the cells of Paneth in the intestinal epithelium of the opossum, the occurrence of the fully loaded cells in the surface epithelium and of immature cells in the gland cannot be reconciled in any way with Bizzozero's view that they are young cells which only achieve their full development as mucus-secreting goblet cells, nor, indeed, with any view except that they are specific elements engaged in the production of a special secretion.

The material from the guinea pig proved the most fruitful in results as regards the cytological characters of the cells of Paneth and in this animal results were obtained which bring the cells of Paneth into line with other sero-zymogenic cells such as the cells of the parotid gland, the chief cells of the fundus glands and the pancreatic cell.

At first, considerable difficulty was experienced in obtaining accurate fixation of the granules. Aqueous sublimate, Bensley's alcohol-bichromate-bichloride mixture, and Kopsch's formaline bichromate mixture, were tried with only partial success. A few of the cells at the very edge of the section, in these imperfect fixations, would be found to have retained the granules while from the majority of the cells they had either been removed entirely or only retained in an imperfect and distorted form. Very frequent in these cases were the crescent-shaped granules described by Nicolas in the Paneth cells as one of the stages in the secretory history of the cells. A great deal of the work was done on material fixed in 10 per cent formaldehyde which penetrated somewhat better than the other fluids mentioned above, although in this fixing fluid the crescentic-shaped granule was common. When the work was nearly completed we succeeded in obtaining complete fixation of the granules by means of a combination of equal parts of alcoholic sublimate and Kopsch's fluid. In preparations fixed in this mixture the granules retained their round form and were perfectly fixed in all the cells of the material.

For staining, Bensley's neutral gentian was employed with good success to differentiate Paneth cells, the granules of which stained intensely violet, from goblet cells which remain colorless or faintly violet. Another method which has rendered great service is staining in iron haematoxylin followed by mucicarmine. By this method the granules of the Paneth cells are stained deep blue-black, those of the goblet cells carmine red.

To demonstrate prozymogen as basal filaments or as a diffuse chromophile substance in the base of the cell toluidene blue was employed in saturated aqueous solution. Better results, however, were obtained by a method devised by Bensley of staining in toluidene blue, orange G, and acid rubin. This method is as follows: the sections cut as thin as possible in paraffin and fastened to the slide by the water method are passed through benzole, absolute alcohol, and graded alcohols, to water. They are then stained for a period of one minute with a mixture containing equal parts of the saturated aqueous solutions of Orange G, and acid rubin. Then wash in water and stain for one minute in saturated aqueous solution of toluidene blue. Wash in water; transfer to absolute alcohol; clear in benzole, and mount in balsam. The result as far as the distribution of the toluidene blue is concerned is much the same as that obtained by staining with this dye alone. The intensity of the blue stain, however, is much increased, and in addition the method offers the advantage of the contrast stain produced by the rubin and orange. By this method chromatin and prozymogen (or basal filaments) are stained intensely blue, protoplasm faint bluish, zymogen granules red, and the contents of goblet cells remain unstained.

Confirmatory evidence of the presence of prozymogen in the Paneth cells was sought by means of the microchemical reaction for organic iron introduced by Macallum.

In the guinea pig Paneth cells are very abundant in the glands of Lieberkühn of the small intestine. They occupy chiefly the deep ends of the gland where they often form a continuous layer which is interrupted by comparatively few goblet cells. A few also occur on the sides of the gland but the upper ends of the glands are wholly free from them.

The structure of these cells depends on the stage of physological activity. In the animals which are kept constantly supplied with food of which they are allowed to partake at will, the cells are cylindrical in shape, the outer end being somewhat broader than that which is directed towards the lumen. In each cell two zones of about equal width are easily recognized. The distal zone directed towards the lumen of the gland is occupied by fine granules which are so closely crowded that it is often difficult to recognize the thin laminae of cell-protoplasm which separate them from one another. In material fixed in aqueous sublimate, however, many cells may be found from which the granules have been removed and here we find the distal zone occupied by a fine meshwork which corresponds in the size of its spaces to that of the granules, indicating that each granule occupies a small space in the protoplasm, a thin

lamina of which separates it more or less completely from its neighbors. The granules stain intensely in iron hematoxylin and in neutral gentian but remain quite unaffected by muchaematein or mucicarmine. The proximal or basal zone of the cell contains an oval nucleus which is surrounded by a small quantity of protoplasm which takes a slightly deeper stain than that of neighboring cylindrical cells. Some of the cells contain a larger quantity of this basal protoplasm and in a few of the cells this exhibits a distinct radial striation, in which case the deeper stain is largely confined to the striae. The presence of these basal stria-

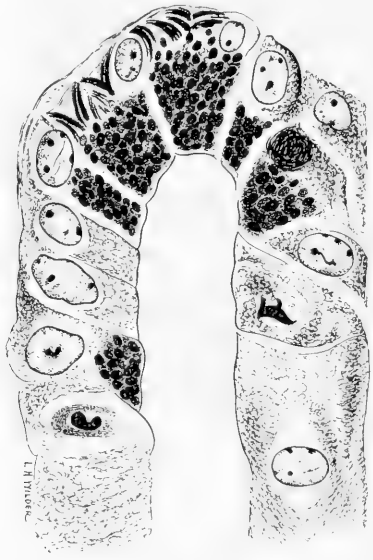

Fig. 3. Bottom of gland of Lieberkühn of the guinea-pig after twenty-four-hour fast. From a preparation stained by orange-rubin-toluidene blue method. Leitz 1/12, Oc. 4.

tions was, however, more easily demonstrated in those animals which were protected from an excess of physiological stimulation by controlling the amount of food taken and supplying it at regular intervals. Fig. 3 represents the lower end of the gland from the small intestine of a guinea pig which had fasted for twenty-four hours after receiving a mixed meal of carrots and oats. The aspect of the cells in this case is very different from that seen in the animal feeding irregularly. In the first place the granules are more than twice as large, and there appears to be a larger number, although, for obvious reasons, it is difficult to be sure of this. In sections of the intestine stained with toluidene blue alone, or with

toluidene blue and orange-rubin, the basal cytoplasm of practically every cell exhibits a radial striation which is exactly similar to that described in various sero-zymogenic gland cells by Bensley, Solger, Garnier and others. This character is well illustrated in fig. 3 which is from a specimen stained in the toluidene blue-orange-rubin method. The basal filaments stain intensely in toluidene blue, less intensely in iron haematoxylin, but may be observed without difficulty in sections stained with alum-haematein. The most effective method of demonstrating the basal

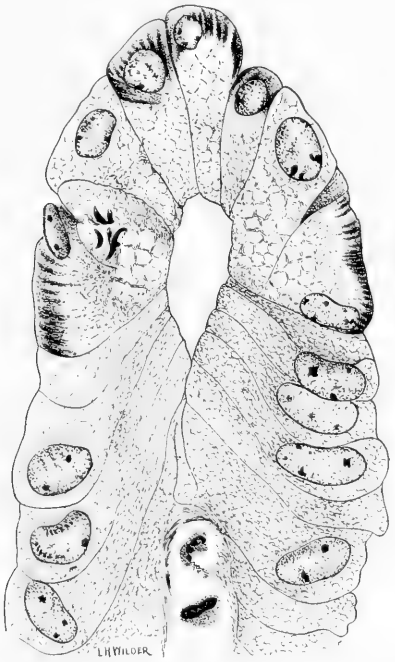

Fig. 4. Gland of Lieberkühn of guinea-pig. From preparation treated by Macallum's method for the detection of masked iron; Leitz 1/12, Oc. 4.

filaments is by means of the microchemical reaction for organic iron of Macallum, because the result is not confused by the faint protoplasmic stain which is obtained generally in staining with toluidene blue. This method consists in liberating the iron from its organic combinations by treatment of sections from material hardened in alcohol with a solution of sulphuric acid in alcohol for several hours at 37.5 C. and then demonstrating the iron at the point of its liberation by means of haematoxylin (see Macallum, 95). The result, as far as the Paneth cells are concerned, is a strong reaction in the substance of the basal filaments and in the nuclear chromatin (fig. 4). In some of the Paneth cells a more

diffuse reaction is obtained indicating that the specific substance on which the staining reaction of the basal filaments depends is present although not definitely organized in the form of filaments.

The absence of the basal filaments from the majority of the cells of the animal which has been kept constantly supplied with food is doubtless due to the fact that in this case the cells are subjected to a physiological stimulus which is practically continuous, which results in a constant drain on the reserve substances of the secretion, both the granular zymogen and its antecedent prozymogen.

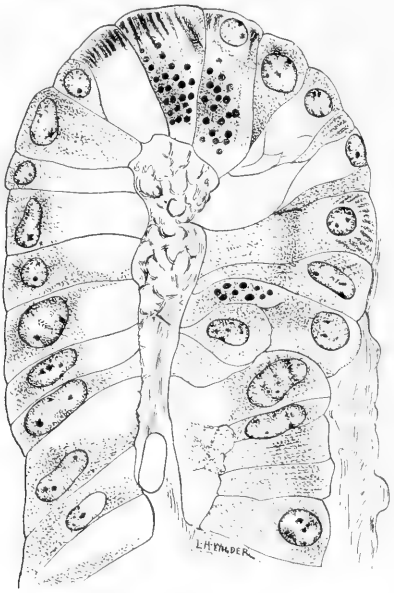

FIG. 5. **End of gland of Lieberkühn of guinea-pig six hours after food. Preparation stained in iron-alum hematoxylin and mucicarmine; Leitz 1/12, Oc. 4.**

In order to determine the effect of physiological stimulation on the Paneth cells I have examined them in sections taken at gradually increasing intervals after feeding, from animals which have previously fasted for twenty-four hours in order to bring the cells to the condition of maximum loading. The results of these experiments show beyond a a doubt that the cells of Paneth respond to the stimulus of food, although considerable differences were found in the rate of disappearance of the granules in different sets of experiments. The condition of the cell in animals which have fasted for twenty-four hours has been already de-

scribed and figured (Fig. 3). As early as four hours after feeding, a change in the number and size of the granules may be noticed. They are obviously reduced in number and are somewhat smaller. At six hours the condition represented in Fig. 5 is presented. The cells are longer and narrower and the granules are small, few in number, and occupy the distal segment of the cells only. Indeed, at this stage the cells have assumed very much the same appearance as they present in the animal which is feeding irregularly, although the amount of prozymogen is greater in the six hour stage. At no stage of secretion have I found the fusion of granules into large masses described by Nicolas, if a fixative was employed which was effective in fixing the granules in all parts of the tissue. With some of the fixatives mentioned at the beginning of this paper great differences were found in granules in different cells both in staining power and in form. Very common in these imperfect fixations were the granules composed of a faintly stained central mass with a deeply staining crescent at one border corresponding to the safraninophilous body of Nicolas. I have no doubt that these appearances are entirely due to imperfect fixation, and that the crescent-shaped granules have no existence in the living cell. On the other hand, the different behavior of granules towards the same fixative even in the same cell indicates clearly that some of the granules differ from others either chemically or physically, and it is probable that this difference is due, as Nicolas supposed, to a change in the granule preparatory to its solution and extrusion from the cell as a part of the secretion.

As regards the prozymogen the experiments did not result in a great change in its amount. It is probable that an equilibrium is established between the rate of production and use of this substance which results in the amount in the cell being kept fairly constant.

Summary of Results.

The results recorded show clearly that the cells of Paneth correspond in their structure and microchemical reactions to the enzyme-producing cells of other granular organs, such, for example, as the cells of the parotid gland, the chief of the fundus glands of the stomach, and the cells of the pancreas.

In common with these cells the cells of Paneth contain granules which do not stain in the specific stains for mucin, such as mucicarmine and muchamatein, and which react like zymogen granules to such stains as iron hematoxylin, neutral gentian and acid rubin. In addition they contain in their basal segment a substance which is distinguished by its

affinity for basic dyes, such as toluidene blue, sometimes as a diffused substance in the base of the cell, generally in the form of basal filaments. The failure of previous observers to find this substance is probably to be explained by the fact that the study of these cells has generally been undertaken in herbivorous animals which like the guinea pig show the effects of an almost continuous secretory activity. Furthermore, this basal substance, whether diffused in the basal cytoplasm or in the form of the so-called basal filaments, gives when treated by Macallum's method a decided reaction for iron. For these reasons it seems certain that we have to do here with a substance exactly comparable to the prozymogen of other zymogenic cells.

In the opossum the cells of Paneth are found not only in the glands of Lieberkühn, but also on the surface of the mucous membrane. Indeed, when the small size of the cells in the glands, their large size on the villi, and the generally rudimentary character of the glands in this animal are taken into consideration, it seems probable that the cells are formed in the glands, but only reach physiological maturity after migrating to the surface in the way described by Bizzozero. This is of course only true, as far as we know at present, of the opossum, although it is possible that the examination of other polyprotodont marsupials might reveal similar conditions in them. In placentals the cells of Paneth appear to be confined to the bottoms of the glands of Lieberkühn, which thus function as true glands as maintained by Oppel.

Whether the condition found in the opossum is the primitive condition for mammals or not it is impossible to say, although this view presents many interesting possibilities. Nicolas' observation that they occur as a part of the general intestinal epithelium in a lizard points in this direction. The observations bearing on the occurrence of Paneth cells in lower vertebrates are, however, as yet, too few to enable any opinion to be offered as to their phylogenetic source.

The distribution of the cells of Paneth in the opossum absolutely excludes the possibility of the cells of Paneth being young mucous cells, and equally opposed to this view are the facts brought out by Nicolas, Möller, and the writer, as to the indications of active secretion in the structure of the cells and as to their response to physiological stimulation.

The cells of Paneth of the guinea pig respond to physiological stimulus of food by secretion as indicated by changes in the form of the cell and reduction in the number and size of the granules.

The crescent-shaped granules of Nicolas and others are due to imperfect fixation and have no previous existence in the cell.

It seems clear from these facts that the cells of Paneth are specific elements engaged in the secretion of a special substance, probably an enzyme which is of use in digestion. It is not, however, possible to connect the Paneth cells with the formation of the new substances, secretin, erepsin, and enterokinase, because these substances are also found in the small intestine of carnivora in which Paneth cells do not occur. Nor is it possible to connect them definitely with any particular class of food, as Möller suggests, because they have been found not only in herbivorous and granivorous animals, but also in insectivora.

In conclusion, I wish to express my indebtedness to Professor Bensley, at whose suggestion and under whose direction the work was undertaken, for friendly guidance and advice during the progress of the investigation.

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SOME PHASES OF THE GASTRULATION OF THE HORNED TOAD, PHRYNOSOMA CORNUTUM HARLAN.

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WITH 15 TEXT FIGURES.

The following account of the gastrulation of this very interesting iguanid is the outcome of investigations carried on at the University of Cincinnati in 1899 and 1900 and at Trinity College in 1900 and 1901. The embryos were collected at Austin, Texas, in 1892-1894. Except for the cleavage and the beginning of gastrulation the collection embraces the complete embryology of *Phrynosoma conutum,* but in this paper we present only the general phenomena of the gastrulation as interpreted from the stages in our possession. The breeding and nest building habits of the horned toad have been described by Edwards, 96. Breeding takes place in the months of June, July and August. Contrary to all previous accounts in which species of the genus *Phrynosoma* are given as viviparous[1] it was observed that at least in the case of *P. cornutum* at Austin, Texas, the eggs, numbering in some instances as many as twenty-five, are deposited in nests formed in a chamber at the end of a tunnel in the ground which the female burrows, and then, after laying the eggs, carefully refills with the loose pellets of earth. At the time the eggs are laid the embryo has attained the stage in ontogeny equivalent to that represented by Peter, 04, Taf. II, Fig. 17, as **N. T. Nr. 68** for *Lacerta agilis.* The *Phrynosoma* embryo when it leaves the oviduct is 2 mm. in its greatest length. The head bend forms a wide acute angle at the projecting mid-brain, while the neck bend forms an obtuse angle. The body is slightly curved, the caudal end being twisted around to the right. In the open space of half the length of the body between the tip of the fore-brain and the tail lie the well marked, distended allantois and the strongly curved, prominent heart.

In order to obtain the earlier stages, it is necessary to take the eggs

[1] This error, repeated by Gadow, 01, p. 533, was corrected by Edwards, 03, p. 826.

from the oviduct of a gravid female immediately after she has been chloroformed. The oblong eggs are cream-colored and when laid the soft, moist, semi-transparent membraneous shell allows the embryonic area to show through, forming a dark pinkish oval area on the upper side of the egg. The egg shell becomes tough on drying, but not brittle or stiff. To fix the embryos free from yolk and separated from the egg shell, a disk somewhat larger than the embryonic area was cut out. In removing this a considerable portion of the yolk immediately beneath was carried with it to sustain the embryo until it could be supported on all sides by physiological salt solution. By the careful use of a current from a pipette, the yolk was removed and then the shell membrane and the vitelline membrane. Sometimes, in very early stages, it was found desirable to allow the shell membrane to remain on the blastoderm for its support. Usually a drawing was made of the unstained embryo with the aid of about sixty diameters magnification, in order to facilitate the interpretation of sections In general, Fleming's mixture of chromic-aceto-osmic acid was employed for fixing followed by successive alcohols. A modification of Mayer's hæmalum was found superior to hæmatoxylin and other hæmatin stains, both for sections and specimens *in toto*. For the latter Mayer's hæmalum diluted with twenty parts of ammonia alum was employed. The specimen was decolorized in 1-10 of 1% hydrochloric acid made up in 70% alcohol. Alcoholic cochineal also gave good results *in toto*. Benzopurpurin was advantageous in older stages. Orange G was used as a plasma stain in sections. Clearing was accomplished by the use of anilin, or clove oil, removed subsequently by xylol

Owing to the radially symmetrical appearance of the embryonic area in the earliest stages, they were extremely difficult to orient. They were embedded in celloidin and then the celloidin was pared down until the embryos could be observed under the low power of a microscope when triangular blocks were cut with definite relation to the anterior and posterior end of the blastoderm. These were reëmbedded when sectioned. The celloidin also protected the delicate embryos which had become brittle after several years in alcohol.

The youngest embryo in this collection has passed through cleavage and the first phase of gastrulation being in the second phase of gastrulation as first worked out for the lizard by Wenckebach, **91**, p. 75.

This stage is represented in surface view in Fig 1. Superficially it resembles a like stage of *Lacerta viridis*, according to the drawings of Will, **95**, *b*, Fig. 6, Pl. 1; also of the turtle embryo as represented by Mitsukuri, **94**, Fig. 1, Pl. 6, and of Tuatara (*Hatteria*) as figured by Dendy, **99**, Fig. 1. The embryonic area is pushed up above the surrounding

blastoderm in the form of a cap with vertical margins leveled toward the center of the crown and having a deep recess in the posterior edge where the blastopore is located. The length of the area is about 4 mm. The width is slightly less. At the anterior margin the cap tapers to a round point which indicates more rapid growth in this plane. The recess at the posterior edge indicates either an ingrowth of the upper layer cells at this point or a backward movement of parts on either side of it. Sections prove that the former is true.

This stage is represented as seen in longitudinal section in Figs. 2 and 3. These sections are slightly diagonal. Fig. 2 passes through the blasto-

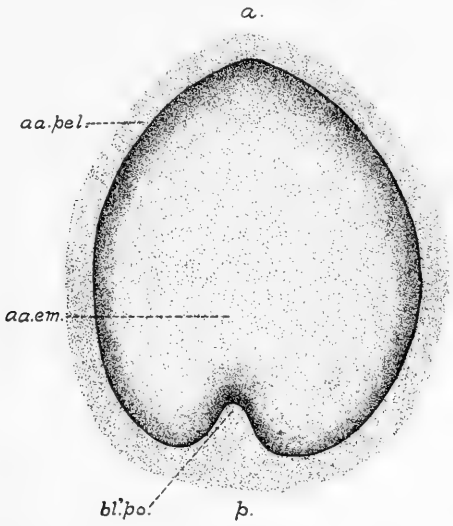

FIG. 1. Dorsal view of a very early stage in which probably the mesoblast sac has not broken through into the subgerminal space. *a.*, anterior; *aa. em.*, area embryonic; *aa. pel.*, area pellucida; *bl. po.*, blastopore; *p.*, posterior; × 74 diameters.

pore, but runs to the left of the median plane in the anterior region of the embryonic area. The extent of this rotation may be judged from the fact that Figs. 2 and 3 are the 2d and 3d sections which pass through the embryonic area, there being 9 sections included in this area. All the structures of interest are to be found in these two sections.

The elevation of the cap above the surrounding blastoderm is apparently due to the rapid increase in thickness and extent of the epiblast over this area. The accumulation of the mesoblast may take part in this elevation, but the absence of mesoblast in the greater part of the anterior

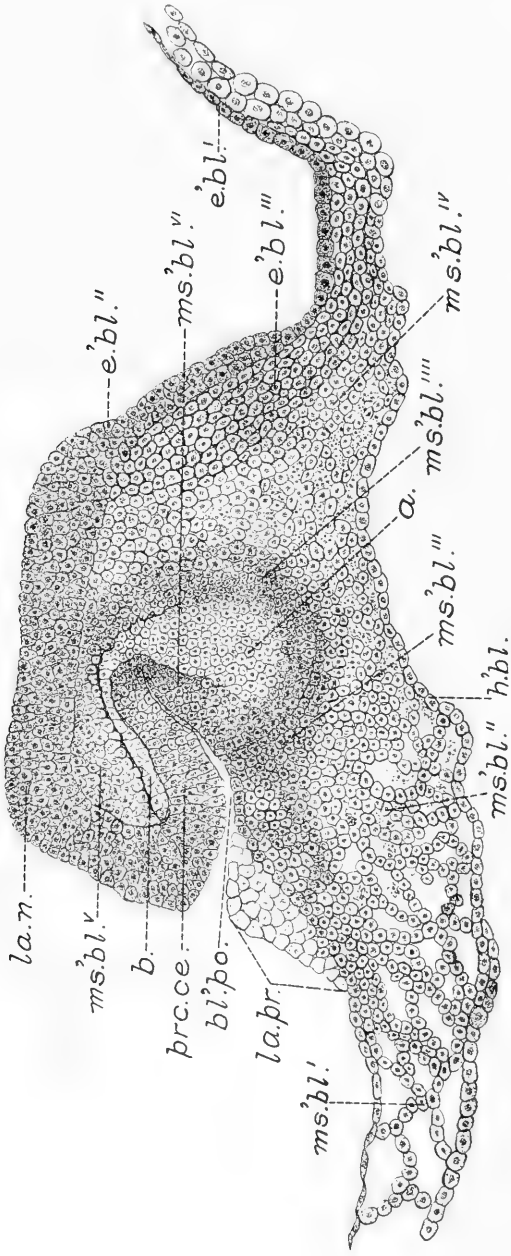
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Fig. 2.

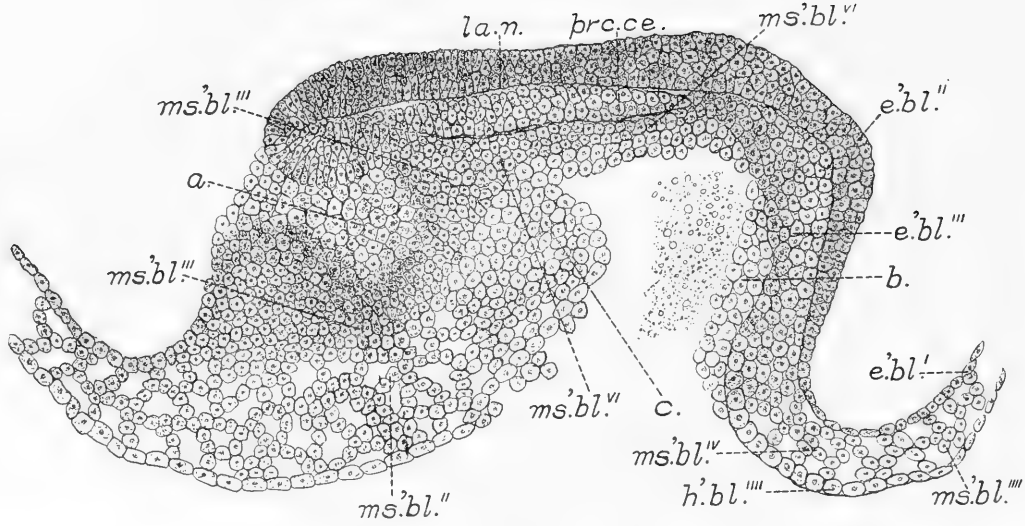

FIG. 3.

FIGS. 2 AND 3. Longitudinal sections of the blastoderm seen in surface view in Fig. 1. Fig. 2 is median through the blastopore but runs to the left of the middle line in front. Fig. 3 is the next section to the right of Fig. 2. In Fig. 2, *a*, *b*, and *la. pr.*, and in Fig. 3, *a*, *b*, *c*, are represented tissues which appear only at deep focus. *bl. po.*, blastopore; *e. bl.*, epiblast; *h. bl.*, hypoblast; *la. n.*, neural plate; *la. pr.*, primitive plate; *ms. bl.*, mesoblast; *prc. ce.*, head process; × 200 diameters.

embryonic area proves that some other agent maintains the elevated position of the thickened epiblast.

As in other reptiles the upper and lower layer are connected by a mass of mesoblast at the posterior edge of the embryonic area. Here by the invagination of the primitive plate of Will is formed the mesoblast sac (Mesodermsäckchen of O. Hertwig, 03, p. 828). That all of the mesoblast in Figs. 2 and 3 was derived from the proliferation and invagination of the region of the primitive plate and blastopore, can not be positively affirmed, yet it seems highly probable. The mesoblast at the anterior margin in Fig. 3 is in direct connection with the mesoblast under the primitive plate.

The mesoblast cell masses are continuous with the epiblast of the posterior and lateral lips of the blastopore which leaves little doubt as to their origin. From these two sources there is a lateral and a forward extension of the mesoblast cell masses. From the floor of the mesoblast sac one mass extends forward in the middle region (Fig. 2, ms'bl.^{vi}) It is continuous laterally with the lateral extensions, the floor of the mesoblast sac contributing to the lower portion of these also. The inturned epiblast, or lateral roof of the mesoblast sac is continued laterally and forward by a mass of mesoblast (Fig. 3, ms'bl.^{iii}) having a high position. Laterally this mesoblast bends down and back (Fig. 2, ms'bl.^{iiii}) to unite with the mesoblast from the floor of the mesoblast sac (Fig. 2, ms'bl.^{iii}). It thus forms a pouch on either side which is continuous posteriorly with the mesoblast sac in the median line. The floor of the mesoblast sac is continued anteriorly for a short distance under the chorda anlage into a sheet of mesoblast. In a middle position it spreads laterally over the lateral pouch above mentioned, while farther back, as the floor of the mesoblast sac, it is continuous with the ventral wall of the lateral pouches.

Under the region designated primitive plate (Fig. 2, la. pr.) the mesoblast is in the form of a network, and, while it seems to be spreading from these centers of origin, it is not being encroached upon by new additions, or proliferation, *inter se*. Mesoblast of this nature can be traced from the hollow pouches extending laterally on either side of the primitive streak, to the anterior margin of the embryonic area where it fills in the space between the epiblast and hypoblast (Fig. 3 ms'bl.^{iiii}). According to Will a similar forward growth of mesoblast on each side of the chorda takes place in *Lacerta agilis, L. muralis* and a like condition exists in the turtle (Mitsukuri, 94), snake (Will, 98) and in *Sphenodon* (Dendy, 99).

The mesoblast sac becomes a canal reaching far into the mass of meso-

blast, almost to the middle of the embryonic area. At the mouth it is funnel-shaped, being wider perhaps laterally than vertically. Its dorsal wall is the thick infolded epiblast destined to become the chorda. The epiblast of the depressed ventral wall of the blastopore becomes mesoblast deeper in the canal, for this mesoblast is produced by the forward growth of epiblast cells. Since the dorsal and ventral walls of the mesoblast canal contribute to the lateral mesoblast by infolding and lateral growth, the mesoblast sac may be regarded as having no lateral limitations, but extending between the above named mesoblast anlagen. However, the sections of this stage only indicate this for a short distance in front of the blastopore. The head process is four or five cells thick where it is continuous with the epiblast at the blastopore. It is continuous laterally at this point with the ectodermic folds that give rise on either side to the dorsal limbs of the mesoblastic pouches. Anteriorly it becomes a wide belt of cells, three or four deep, and thin at the edges (Fig. 3, prc. ce.) extending quite to the anterior limit of the neural plate. As in Fig. 3, anterior sections do not have mesoderm adjacent to the chorda laterally. A part of the tissue under the chorda in Fig. 3 is mesoblast (Fig. 3, ms'bl.^{vi}) of primary origin, but under the greater part of it is mesoblast (Fig. 3, ms'bl.^{ii}) which is derived from the primary mesoblast. In the adjacent sections the hypoblast is easily traceable to the limits of the embryonic area and there is no mesoblast lateral to the chorda in the forward end.

In Figs. 2 and 3 the epiblast represented is not all in the same plane (e'bl.^{ii} and e'bl.^{iii}); e'bl.^{iii} is cut in a diagonal plane and e'bl.^{ii} is cut vertically. In Fig. 2 (*a, b* and la. pr.) and Fig. 3 (*a, b, c*) are represented tissues which appear only at deep focus. The epiblast over the whole top of the embryonic cap is four or five cells thick, but it thins out rapidly toward the base of the uprising wall, becoming a thin one-celled layer in the extra-embryonic area (Figs. 2 and 3, e'bl.^{i}). The hypoblast is uniformly one cell thick over the whole embryonic area, except under the chorda where it appears to be two or more cells thick, but this condition is of limited extent. Extra-embryonic mesoblast separates these two germ layers but a short distance from the embryonic area (Fig. 2 ms'bl.^{i} Fig. 3, ms'bl.^{iiii}). From these observations we may conclude that at a time when the epiblast is in the form of a much thickened cap the mesoblast sac forms a canal stretching forward into the mass of tissues that are then accumulating at the posterior part of the embryonic cap and perhaps opening into the sub-germinal cavity in front of this mass of tissues. The mesoblast which is being produced around the blastopore, stretches antero-laterally from it in the forms of two hollow sacs of

closely packed cells, one on each side, which act as feeders to surrounding parts, there being an extension of it along each lateral margin to a position well in front of the embryonic cap. The cavities of these sacs are in communication with the blastopore. The epiblast turns in at the blastopore and gives rise to the chorda and mesoblast. The latter also receives accessions below from the primitive plate region, in fact from epiblast cells of the floor of the mesoblast sac as we shall endeavor to prove later.

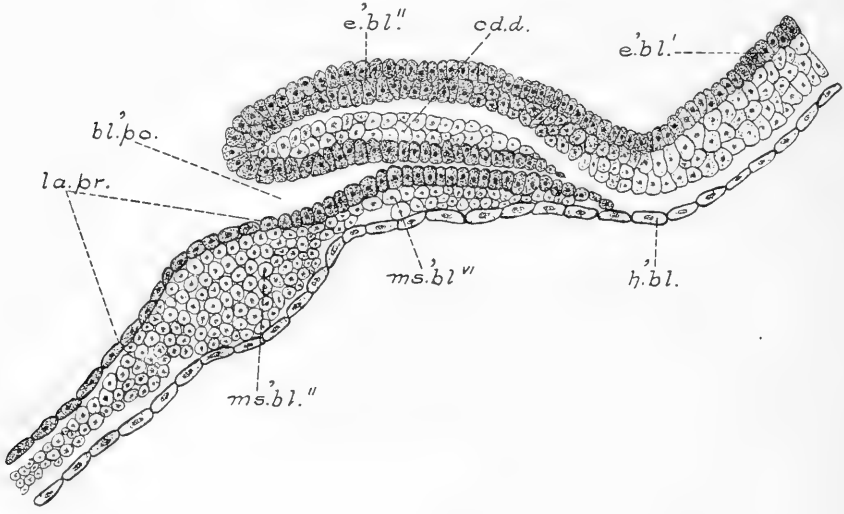

Fig. 4. Longitudinal section of an embryo in which the cap-like condition is less marked but in which the mesoblast sac has not yet fused with, or broken through, the hypoblast. *bl.'po.*, blastopore; *cd. d.*, chorda dorsalis; *e.'bl.*, epiblast; *h.'bl.*, hypoblast; *la pr.*, primitive plate; *ms.'bl.*, mesoblast; × 166⅔ diameters.

The next oldest blastoderm in the collection was sectioned lengthwise. In the stage just described we have not been able to affirm positively that the mesoblast sac does not communicate with the subgerminal space, but in the second stage (Fig. 4) the hypoblast is unbroken. It differs from the younger stage in the absence of the cap-like elevation, in the more compact and mature condition of the primitive plate, and in the absence of the mesoblastic pouches so characteristic of the early stage. They are already flattened and their cavity all but obliterated. The chorda is relatively shorter than in the embryo represented in Figs. 2 and 3, but it is actually of about the same length while the whole blastoderm is about

two and one-half to three times as long as the blastoderm of Figs. 2 and 3. It is very nearly identical to Will's Fig. 36, Taf. 6, which is a section of the embryo represented by him in Fig. 6, Taf. 1, above referred to as equivalent to *Phrynosoma,* Fig. 1. The second stage has three separate solid masses of mesoblast proliferation and ingrowth. Two are from either side of the primitive plate and may be traced forward on either side. The cells become scattered at the edges of the growth. Some of these scattered cells may be seen between the thin epiblast and hypoblast at the anterior margin of the embryonic area, but not in the middle line.

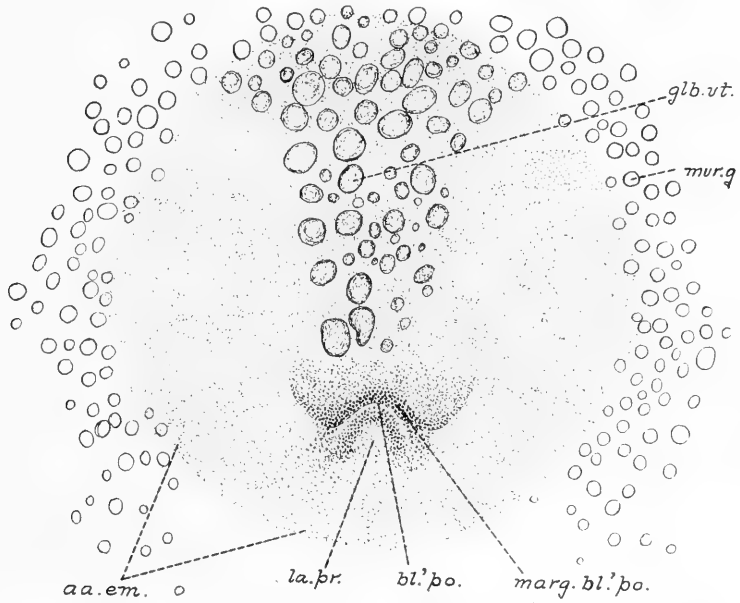

FIG. 5. Dorsal view of a blastoderm older than Fig. 1. The elevation of the embryonic area has given place to a flat plate and the blastopore has changed shape. *aa. em.*, area embryonic; *bl.'po.*, blastopore; *glb. vt.*, yolk spheres; *la. pr.*, primitive plate; *marg. bl.'po.*, margin of blastopore; *mur. g.*, germinal wall; × 74 diameters.

Here the epiblast and hypoblast are closely adherent both in and outside of the embryonic area. The third mass of mesoblast growth is from the ventral wall of the notochord. The primitive plate sends forward a mass of cells (Fig. 4, ms'bl.^{vi}) detached from the hypoblast and extending almost as far as does the chorda. It is comparable to ms'bl.^{vi} of Fig. 2. We do not regard it as a permanent source of anterior mesoblast, rather as evidence of a mesoblast sac. After very careful search it has

been impossible to discover unmistakable mesoblast cells outside the immediate vicinity of that from the embryonic area. On all sides are crowded hypoblast cells derived from the germinal wall, to which the thin epiblast is closely applied. In several instances mesoblast cells have been seen some distance from the embryonic mesoblast yet so similar in position to isolated mesoblast cells that are undoubtedly migrants from the embryonic area, that it seems safe to assume that they, too, have wandered. All cases of this character have been in the vicinity of isolated masses of wandering mesoblast. There is no evidence that up to the age of these two embryos, mesoblast arises elsewhere than around the blastopore. In subsequent stages all mesoblast found in the extra embryonic area must be suspected of having its origin in the detached cells which we have already called attention to some distance from the cell mass to which they originally belonged.

The caplike elevation of the blastoderm of *Phrynosoma* gives place to a flat embryonic area. Fig. 5 is a dorsal view of this stage. In this blastoderm the embryonic area was .95 mm. long and .89 mm. wide. A few large yolk spherules can be seen through the blastoderm in front of the blastopore, whence they extend forward over an ever widening area and may be seen around the rim of the embryonic area, except on the posterior side. They may possibly owe their size and form to the action of reagents, but their pecular distribution is, without doubt, due to some structural condition before fixation. It is a very common thing to find in sections, large yolk laden cells engrossed by the hypoblast adjacent to the germinal wall, and beneath this hypoblast, large yolk masses. Hence it seems natural to regard the yolk masses in Fig. 5 as evidence of the rapid addition of yolk-laden formative cells to the hypoblast from the adjacent yolk mass. From the surface view there is evidence of greater thickness of the blastoderm back of the blastopore than is the case in the youngest stage. This is due rather to the spread of mesoblast than to thickening of the epiblast. The blastopore is less V-shaped than earlier and more like a half-moon. The depression between the primitive plate cells within the blastopore and the epiblast wall which rises in front of it represents the posterior end of the mesoblast sac. It is V-shaped in section (Fig. 10) opening postero-medianly. The inturned edges of the epiblast along the entire length of the blastopore imply a process of ectodermic invagination along the whole margin. The surface view scarcely suggests the part the primitive plate is taking in this process.

This same embryo was subsequently sectioned in a longitudinal plane.

Fig. 6 is a semi-diagrammatic drawing of a sagittal section, and Fig. 7 of the next section to one side. In the latter the continuity of the epiblast and primitive plate mesoblast with the anterior mesoblast confirms what has been seen in the surface view with regard to the infolding epiblast and establishes the lateral growth of the primitive plate mesoblast as well. Hence, both the posterior and anterior lips of the blastopore are undergoing a process of invagination. The yolk masses can be seen adhering to the hypoblast and anteriorly it is receiving additions of large yolk-laden cells from the germinal wall. At this stage the mesoblast

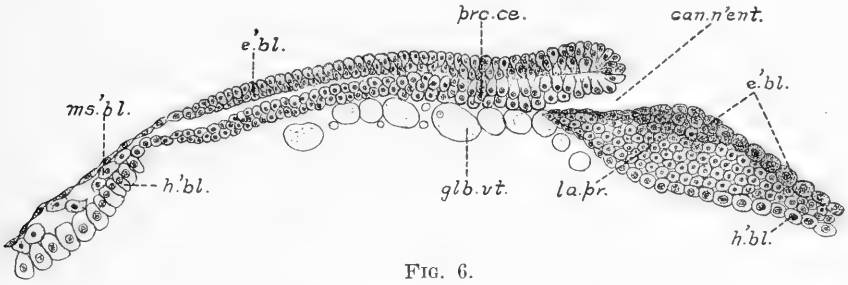

Fig. 6.

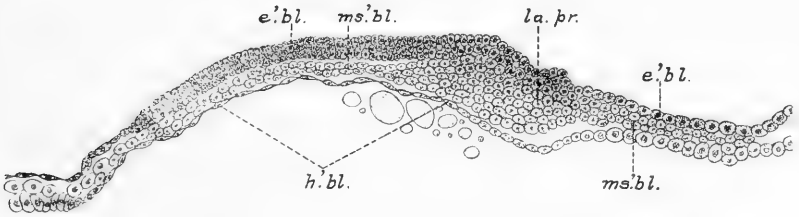

Fig. 7.

Figs. 6 and 7. Longitudinal sections of the embryo represented in Fig. 5. Fig. 6 is sagittal and Fig. 7 is the third section to the right of Fig. 6. *can. n'ent.*, canalis neurentericus; *e.'bl.*, epiblast; *glb. vt.*, yolk spheres; *h.'bl.*, hypoblast; *la. pr.*, primitive plate; *ms.'bl.*, mesoblast; *prc. ce.*, head process; × 109 diameters.

canal is an open passage into the subgerminal cavity, the canalis neurentericus, (cf. Hertwig, 03, pp. 832-4). The head process has become the lower layer along the middle line. This is due, no doubt, to the fact that its elongation has kept pace with the increase in length of the epiblast, hence after fusing with the hypoblast in a position near the middle of the embryonic area at the time, this point is carried forward relatively by the rapid elongation of the head process. The hypoblast has not yet grown together under the head process as it does later. While our ob-

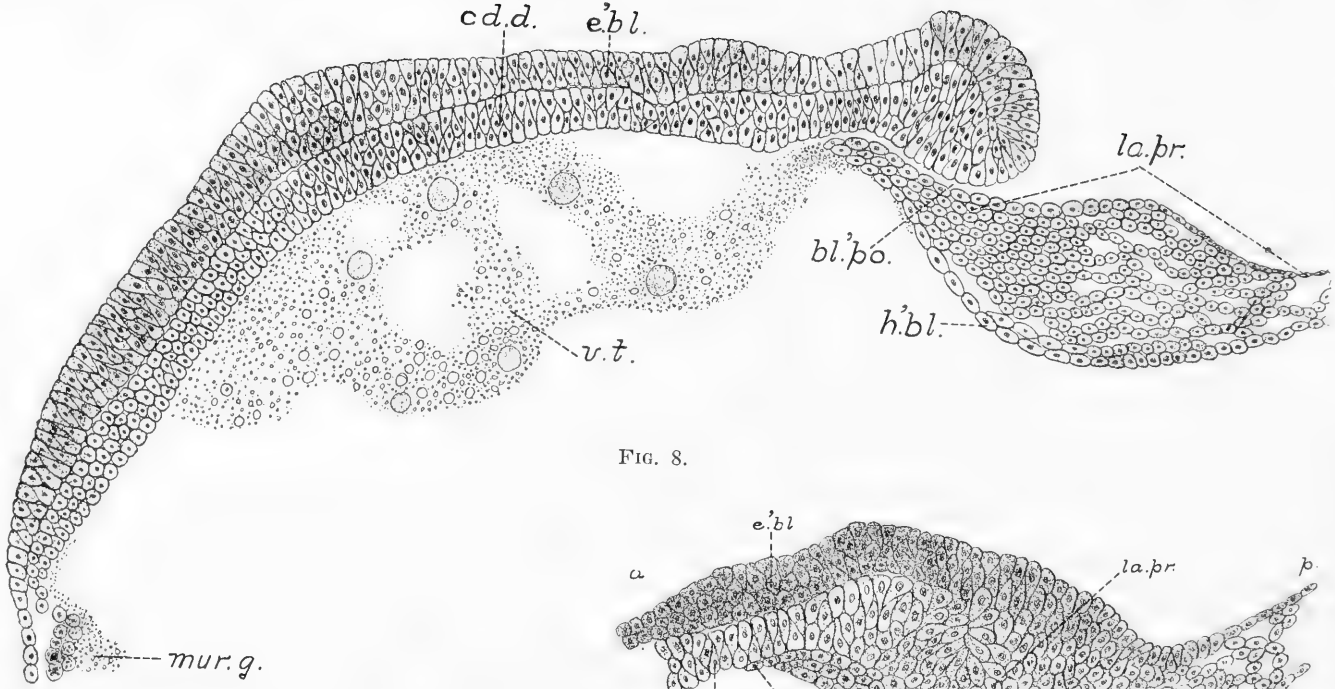

FIG. 8.

FIG. 9.

FIGS. 8 AND 9. Longitudinal sections of a stage still older than Fig. 5. Fig. 8 is median. The embryonic area is still slightly elevated. Fig. 9 is the next section to the right. It shows the epiblast turning in to form mesoblast at the very edge of where it becomes chorda. *a.*, anterior; *bl.'po.*, blastopore; *cd. d.*, chorda dorsalis; *e.'bl.*, epiblast; *h.'bl.*, hypoblast; *la. pr.*, primitive plate; *mur. g.*, germinal wall; *p.*, posterior; *v. t.*, yolk. Fig. 8, × 200 diameters; Fig. 9. × 200 diameters.

servations do not confirm the hypothesis, it seems probable that along with the process just described, the space from the median hypoblast in front of the primitive plate to the point where the chorda is in contact with the hypoblast anteriorly is due at first to the degeneration of the hypoblast and lower layer of the chorda invagination which Will, 93, found in the Gecko and represents in Figs. 57 and 58, Pl. 9. Then the rapid forward growth of the chorda carries its anterior contact still farther from the primitive plate hypoblast. In Fig. 7 one may see the anterior reach of the lateral mesoblast which has established the five or six mesoblast cells that are to be seen in the middle line in front of the embryonic area in Fig. 6.

Longitudinal sections of a stage which is a little younger than this are represented in Figs. 8 and 9. The cap-like form still persists. Other conditions are essentially the same as in the embryo just described. The head process is fused with the hypoblast and extends to the germinal wall, but the histological differentiation has not taken place in the anterior third of its length. In Fig. 9 the continuity of the epiblast and primitive streak mesoblast is not different from conditions described in the older embryo of this stage. The extension of the primitive plate cells under the chorda verifies the assertion previously made that the primitive plate mesoblast proliferates or grows laterally and forward as the ventral wall of the mesoblast sac. By so doing it is the counterpart of the epiblast on the dorsal wall in a single invagination. In this stage the network of mesoblast cells to be seen under the primitive plate in Fig. 2, has been replaced by the solid tissue. The condition persists, however, posterior to this region. Three or four sections from the median line this embryo has the same appearance as in the older specimen represented in Fig. 7. Cross sections of this stage are reproduced in Figs. 10-15

Fig. 10 passes through the blastopore where the V-shaped depressions mentioned in connection with Fig. 5 are prominent. Here the epiblast may be seen turning into these depressions and fusing in this section with the epiblast of the floor of the depression in the formation of a lateral growth of mesoblast along the mesoblast sac. The appearance of the nuclei of the epiblast and of the floor of the mesoblast sac is identical, and in mesoblast cells, which are continuous laterally with each of these tissues, there are here and there small dark nuclei, similar to the epiblast nuclei. These nuclei in mesoblast cells are not met with except in the immediate vicinity of the epiblast and the floor of the mesoblast sac. Under the blastoporic epiblast is a layer of mesoblast of limited lateral extent, which is sharply marked off from the overlying epiblast. This condition prevails in sections back of this point, but three sections an-

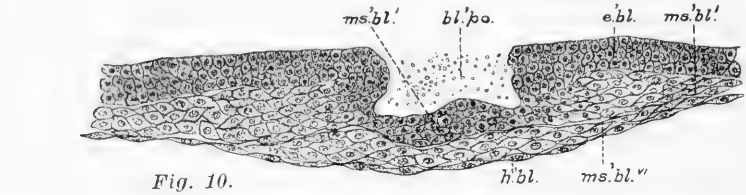

Fig. 10.

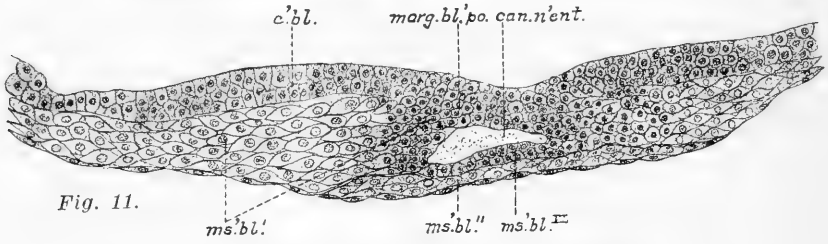

Fig. 11.

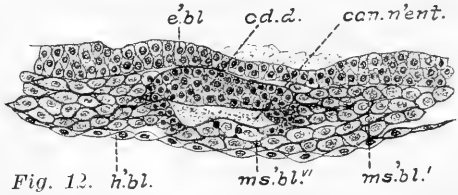

Fig. 12.

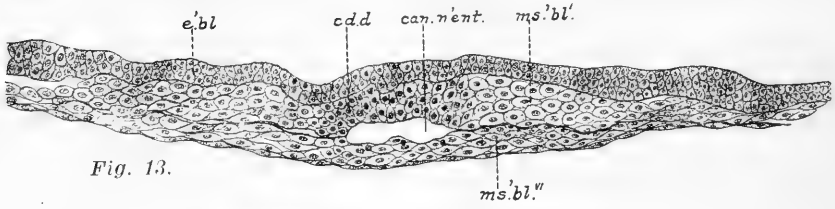

Fig. 13.

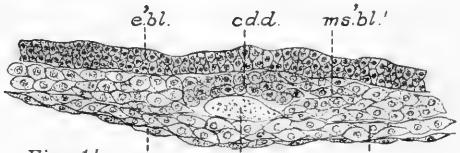

Fig. 14.

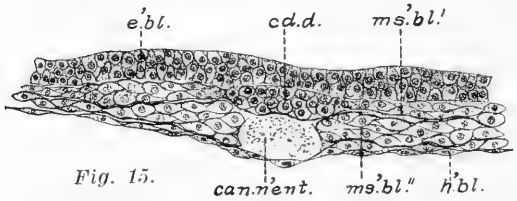

Fig. 15.

FIGS. 10-15. Cross-sections of an embryo of about the same age as Fig. 5, probably a little older. *bl.'po.*, blastopore; *can. n'ent.*, canalis neurentericus; *cd. d.*, chorda dorsalis; *e.'bl.*, epiblast; *h.'bl.*, hypoblast; *marg. bl.'po.*, margin of blastopore; *ms.'bl.*, mesoblast; × 200 diameters.

terior to Fig. 10 the separation cannot be made out (Fig. 12). In the third and fourth section forward (Figs. 12 and 13) several epiblast nuclei are distinguishable in the upper region of this mesoblast layer, otherwise its epiblast character has given place to that of mesoblast. The straggling nuclei indicate that the mesoblast underlying the mesoblast sac is mesoblast derived from the epiblast. Near the anterior end of the neurenteric canal this layer lies on each side of it, Fig. 14. While posteriorly the ventral mesoblast is separate from the epiblast of the floor of the mesoblast sac, it is continuous farther forward with lateral mesoblast which is plainly derived from the epiblast, as shown by the isolated epiblast nuclei above referred to. Hence, we are led to conclude that the lateral is derived mesoblast from two ectodermal sources in the sense just as explained. In Fig. 13 the tissue derived from the upper wall of the mesoblast sac in the middle line is the chorda, that from the upper wall on either side of the middle line is the upper layer of mesoblast (ms'bl.^{i}), that from the lower wall of the mesoblast sac is the lower layer of mesoblast (ms'bl.^{vi}). In Fig. 14 where the mesoblast sac approaches the hypoblast, it divides the mesoblast from the lower wall of the mesoblast sac so that from here forward there is a lateral layer of lower mesoblast (ms'bl.^{ii}) on each side. This condition can be traced forward for several sections. Ectoderm nuclei may be found here and there in the lateral mesoblast also, Figs. 10, 11, 12. In Fig. 11 is seen the line of juncture of chorda and epiblast just in front of the anterior lip of the blastopore. The chorda is well differentiated from the lateral mesoblast immediately in front of its place of origin (Fig. 12), but it becomes less sharply separated from it as one passes forward (Figs. 13, 14), and but a short distance forward it is quite indistinguishable from the lateral mesoblast. No doubt the differentiation of cells proceeds as a process from behind forward. There is no evidence that the chorda becomes separated from the lateral mesoblast by the fusion of the upper mesoblastic layer with the lower mesoblastic layer as in the Chelonia, Mitsukuri, 92, (Figs. 14, 15, 16). The significance of these two layers of mesoblast can be no other than that they are the upper and lower wall of a much compressed hollow pouch such as that described in the youngest embryo here figured (Figs. 1, 2 and 3). One must conclude that from the V-shaped depression seen at the entrance to the mesoblast sac in surface view (Fig. 5), and in section Fig. 10, a pouch highly compressed laterally has grown forward and laterally in a diagonal direction, that the upper wall of this pouch is derived from the epiblast at the sides and in front of the mesoblast sac and that the lower wall is formed by the spreading of a small knot of epiblast tissue in the floor of the meso-

blast sac laterally and forward, that the cavity of the pouch is obliterated to a mere crevice except in the median plane where it remains as the mesoblast sac, in its last condition, the canalis neurentericus, open ventrally where mesoblast and hypoblast come together.

DISCUSSION OF LITERATURE.

While the general process of gastrulation in *Phrynosoma* is similar to that in other reptiles, there are some striking differences. In *Hatteria* (Dendy, 99) there is a stage when the embryonic area very much resembles the cap-like elevation of the *Phrynosoma* embryo represented in Figs. 1-3. The ectoderm is similarly elevated and thickened, beneath is a cellular mass of hypoblast and on the posterior margin of the area is the blastopore, a depression of considerable breadth. From the vicinity of the blastopore, the mesoblast spreads laterally and the head process grows forward. This early growth of mesoblast, when the mesoblast sac is very shallow, exists in *Phrynosoma*. In *Hatteria*, however, the head fold either appears much earlier than in any of the lizards, or Dendy's figure is of a stage in which the blastopore has become closed with age. The lack of information as to further details of the development of the blastopore in *Hatteria* renders the above comparison of little value. Because of the work of Mitsukuri our knowledge of the embryology of the Chelonia is much more complete. In appearance, the blastoderm of *Clemmys Japonica* (Mitsukuri 94, Fig. 1, Pl. VI) as seen in surface view, is much the same as the older stages of *Phrynosoma* after the blastoderm has flattened on the yolk. None of Mitsukuri's figures suggest any elevation of the blastoderm. From his figures (Mitsukuri, 94, Figs. 9, 13, 15, Pl. VIII), it is very apparent that in *Clemmys* there is less cellular differentiation than in *Phrynosoma* at this early stage. The accumulation of yolk in and about the region of the primitive streak obscures the lateral spread of mesoblast. There are certainly no median or lateral cellular pockets at this or later stages. Mitsukuri describes a lateral growth of mesoblast from the primitive streak, but does not call attention to any evidence that it is a hollow evagination of the mesoblast sac. Similarly the growth of the head process anteriorly is *en masse* and gives no evidence of a ventral wall (Mitsukuri, 94, Figs. 15 and 16, Pl. VIII). Only in stages subsequent to the breaking through of the mesoblastic canal and the cellular differentiation of all three germ layers does Mitsukuri find evidence that the lateral mesoblast is an evaginated growth from between the hypoblast and chorda. There is a somatic and splanchnic mesoblast, the former continuous with the chorda, the latter con-

tinuous with the hypoblast which does not pass under the chorda. As we shall point out at another place, the condition here referred to may, and perhaps should, have a slightly different interpretation than that which Mitsukuri has put forward. The spread of mesoblast laterally from the primitive plate begins later in *Clemmys* than in *Phrynosoma* if we may assume that the blastopore appears at the same time in each case.

The early development of the Squamata differs but slightly in the two suborders. The Ophidia are like the Chelonia in having the cellular differentiation less marked than the Lacertilia. The shape of the embryonic area in the Ophidia is not clear from Hertwig's two figures of the earliest stages (Hertwig, 03, Fig. 415, *A* and *B*). The blastopore is more or less posterior to the embryonic area. It is a broad, posteriorly arched depression with no sharp angles. In slightly later stages (Hertwig, 03, Fig. 424, *A* and *B*) the embryonic area becomes narrow posteriorly, a condition not encountered in *Phrynosoma* at any time (Figs. 1 and 5). It is but slightly elevated in the Ophidia and consequently not sharply marked off from the extra embryonic area by a bench (*Phrynosoma,* Fig. 1). The blastopore of both moves, relatively speaking, into the embryonic area as ontogeny progresses. The mesoblast sac in the snake is clearly shown by Hertwig's figures (03, Figs. 427 and 428). It differs from *Phrynosoma* in having the epiblast and chorda anlage less thick and less compact and in having no specially marked lateral pockets from the blastopore. Cell islands in the subgerminal region are characteristic of the snake. At the time the mesoblast sac comes into communication with the subgerminal space, isolated groups of cells remain where the lower wall of the mesoblast sac and the hypoblast broke away, thus establishing the neurenteric canal (Hertwig, 03, Figs. 430, 431). While this condition probably exists in *Phrynosoma,* nothing of the kind has been observed in the stages thus far examined. Hertwig's figures of cross sections (Hertwig, 03, Figs. 443-447) represent a solid outgrowth of mesoblast from the lateral angles of the blastopore and at no place do they reveal a lamination of the mesoblast suggesting a separate origin for somatic and splanchnic layers.

The gastrulation of lizards as worked out by Wenckebach, 91, has nothing of striking contrast to the particular genus we are now studying. In *Lacerta* (Will, 95, Wenckebach, 91) and in *Platydactylus* (Will, 92, Taf. 6), there is not such a marked elevation of the embryonic area. Both have the mesoblast sac developed in a characteristic way, but conditions in the Gecko seem a little more closely allied to those of *Phrynosoma.* In a very early stage there is a much larger space between the

epiblast and hypoblast of the horned toad than in either of the forms just mentioned. This may account for the greater development of the lateral mesoblastic pouches of the horned toad. No figures of cross sections are given by either Wenckebach, or Will, that represent such a lateral extension of the mesoblast sac, yet it seems probable that they may exist. There is in the blastoderm of the Gecko a condition which corroborates the interpretation we have given above of the gastrulation in *Phrynosoma,* namely that there are two paired, and one median, hollow pouches derived from the sides and front of the mesoblast sac respectively. In Will's stage III (Fig. 48 Pl. 7) the mesoblast sac is very shallow, the head process is in evidence and presumably some lateral spreading of mesoblast. In stage IV (Fig 55, Pl. 9) the head process, through the extension of the mesoblast sac has become a long hollow blind sac, flattened considerably between epiblast and hypoblast. By the disintegration of the lower wall of the sac and of the underlying hypoblast (Fig. 57, Pl. 9) the mesoblast sac is placed in communication with the segmentation cavity. It is obvious that this median sac of the Gecko gastrula is homologous with the median sac of the *Phrynosoma* gastrula and that the condition shown in Figs. 2 and 3 is derived in the same manner as that in Will's Fig. 57, Pl. 9. But Will makes no mention of lateral pouches which are doubtless more conspicuous in *Phrynosoma* because as we have already pointed out, in the latter, there is no dorsoventral compression. Will's explanation of the continuity of the chorda with the hypoblast in the anterior median region of the blastoderm is in full accord with conditions found in late gastrula stages of *Phrynosoma.* Will describes a similar median pouch in *Lacerta viridis* (Will, **95b,** Fig. 36, Taf. 6).

In the early development of some lizards one does find a slight elevation of the posterior margin of the blastoderm (Will, **95a,** Fig 36, Taf. 6, *L. viridis;* Will, **93**), but in no sense comparable to the conditions in *Phrynosoma.* In other cases there is no suggestion of it (Weldon, **83,** Wenckebach, **91,** Strahl, **82**). Conditions in the formation of the embryonic area and mesoblastic pouch in *Lacerta* are substantially the same as in the Gecko (Wenckebach, **91**). There are some minor differences in the development of the chorda. The unpaired median invagination even at the very beginning forms a compressed triangular shaped cavity in the lizards, while it is much less compressed at first in *Phrynosoma,* becoming more and more compressed later. As in the Gecko, the *genus Lacerta* does not develop mesoblast by prominent hollow lateral pouches from the antero-lateral sides of the mesoblast sac. In fact, there is some confusion as to the exact origin of the mesoblast. Some authors (Will, **95b**)

state that it arises, in part, from hypoblast budding before invagination and in part grows forward as a solid mass from the anterior region of the mesoblast sac in the middle of which the chorda becomes differentiated (Weldon, **83**, Balfour, **79**, Will, **95**). Other investigators derive all the mesoblast from the sides of the chorda and from a lateral growth of the primitive plate after invagination has begun (Wenckebach, **91**, Strahl, **83**). There is a very close resemblance of the cross sections of *Phrynosoma* embryos in late gastrula stages, to cross sections of *Lacerta* and the Gecko figured by Will (**95b**, Figs. 43[a], 44[c], Taf. 7; **93**, Figs. 59b, Taf. 10-Taf. 36 and of *Clemmys* figured by Mitsukuri, **92**). Mitsukuri interpreted this condition as showing that the lateral mesoblast has been derived by a process of cell budding and an outgrowth from the edges of the chorda and the median margin of the hypoblast. Assuming that the absence of true hollow lateral sacs in *Lacerta* does not forbid one from regarding the lateral solid growth of mesoblast as having its origin in the primitive streak together with epiblast infolded on either side of the chorda anlage, one must regard the two layered mesoblast as having arisen in part from a posterior source and having migrated forward and also from the sides of the mesoblast sac as far forward as it may have extended. This agrees perfectly with the decrease in mesoblast as one passes forward as found by Mitsukuri in Chelonia (Mitsukuri, **92**, Figs. 14, 15 and 16). But from this point of view, one cannot say that the mesoblast is derived from the hypoblast, nor is there any evidence in *Phrynosoma* that such is the case. The close connection of the hypoblast and splanchnic layer of the lateral mesoblast is a necessary consequence of the breaking away of the hypoblast and lower wall of the median invagination, or mesoblast sac, placing the chorda in contact with the hypoblast anteriorly and leaving the broken edges of splanchnic mesoblast and hypoblast on either side to grow together later as Mitsukuri describes when he says the point* moves to the point† (**92**, Fig. 16). According to the interpretation here given the somatic mesoblast will probably receive additions farther anterior than the splanchnic, the latter having a posterior source as its feeder and the former both a posterior source and possibly anteriorly from the chorda anlage. By comparing Fig. 13 with Fig. 15 we find the splanchnic mesoblast decreasing in lateral extent. In fact, there is practically no evidence in *Phrynosoma* that either the chorda or hypoblast contributes anything toward the origin of the mesoblast. Will, in his descriptions of the Gecko, has already presented the view here set forth, but Mitsukuri, **92**, states that in Chelonia the hypoblast turns upward laterally to become the splanchnic layer of mesoblast and that the chorda contributes to the somatic mesoblast.

The egg of *Phrynosoma* stands in closer relation to the lower vertebrates than any other amniote in that the protoplasmic pole of the egg seems less encumbered with yolk, the blastoderm at least is so elevated that processes going on in it are quite as independent as in the Amphibian egg. We may say that the development of this iguanid is a link in the chain of evidence which supports the Weldon, 83,-Will, 93, theory of yolk cleavage wherein the lower layer tends toward the cleavage of the whole mass of the yolk as in the Axolotl, and the mollusc *Bythinia tentaculata,* figured by Weldon.

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SOME RACIAL PECULIARITIES OF THE NEGRO BRAIN.

BY

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WITH 16 FIGURES, 12 CHARTS, AND 7 TABLES.

From time to time in the past hundred years attempts have been made to determine the distinctive points of difference between the Caucasian and the Negro brain. While differences in skull capacity, in brain weight and size—especially of the frontal lobes—or in the gyri have been demonstrated by Gratiolet, Tiedemann, Broca, Manouvrier, Peacock, Marshall, Parker, and others,—more recently by Waldeyer in Germany and by Elliott Smith in Egypt,—yet no exact measurements of the brain, such as we have of the skull, are to be found.[1]

An effort will be made to show by measurement of outline drawings of brains in different positions, by composites of these outlines, and by actual drawings from individual brains that there is a difference in the size and shape of Caucasian and Negro brains, there being a depression of the anterior association center and a relative bulging of the posterior association center in the latter; that the genu of the corpus callosum is smaller in the Negro, both actually and in relation to the size of the splenium; and that the cross section area of the corpus callosum is greater in relation to brain weight in the Caucasian, while the brain weight of Negro brains is actually less. The amount of brain matter anterior and posterior to the fissure of Rolando is roughly estimated, but other points of possible difference, as in the gyri, the insula, the opercula, the "Affenspalte," the proportions of white and gray matter, and the cerebro-cerebellar ratio are necessarily omitted in this study.

In December, 1904, I reported to the Association of American Anatomists the results of the measurements of fifty-four brains, thirty-seven from American Negroes, and seventeen from American Caucasians. Since

[1] The brains measured for this work are in the Wistar Institute under the same numbers given in Table I.

that time I have examined about one hundred additional brains, making in all one hundred and fifty-two, of which one hundred and three are from American Negroes and forty-nine are from American Caucasians.

The work was undertaken at the suggestion of Dr. Mall, as a result of information by Dr. Hrdlička, of the U. S. National Museum, that racial differences exist in the Negro brain. Dr. Hrdlička had observed particularly that the brain of the full-blood Negro has relatively small volume and straighter lines anteriorly to the central fissure, the sides of the Caucasian brain over the same area showing, even in dolichocephals, more mass and arching. I wish here to express my hearty appreciation for the interest Dr. Hrdlička has displayed in my work since its inception and for his generosity in allowing me to make this study. Most of the brains studied are from the collection at the Anatomical Laboratory of the Johns Hopkins University and were placed at my disposal by Dr. Mall who has also controlled the measurements taken. Some of the specimens were obtained through the courtesy of Dr. Page from the Baltimore City Alms House, and some from Dr. W. G. MacCallum, of the Pathological Department of the University

In order to make more exact measurements and comparisons of the brain it is necessary to determine the more fixed points, from which to measure the more variable, and at the suggestion of Dr. Mall the following arbitrary line was passed through the brain as an axis, and its midpoint naturally becomes the brain center. The details regarding these will be discussed later on (p. 404). At this point I wish to state that the axis passes between the hemispheres through the brain stem, passing just above the anterior commissure and just below the splenium (Fig. 2a). The axis usually measures the greatest length of the brain. The position of the brain center is in the middle of the axis and varies but slightly in different specimens. It is seen that the surface of the brain can be measured in great part by extending radii from the center to the surface which may also be marked in degrees,—"of latitude and longitude." The outlines of the brain are generally given in sagittal section (0°), in transverse section (90°, right or left), and by rotating the brain on this axis to a point midway between these two (45°, right or left). "Anterior" has not been separated from "posterior," but the numbers from 0° to 180° are used rather than an "equator" with 0° to 90° for the anterior half of the brain and 90° to 0° for the posterior half.

The first table (Table I) gives a list of the brains from which drawings and measurements were made. The brains are arranged in eight groups, owing to the different methods used in their preservation.

TABLE I.

RECORD OF MATERIAL USED.

Group.	Number of Cadaver.	Age of subject.	Race.	Sex.	Weight of brain in gm. (fresh).	Weight of brain in gm. (when measured.)	Area of corpus callosum in sq. mm.	Length of body in cm.	Weight of body in kilos.	Cause of death.	Color of body (Broca's standard).	Number of days between death and removal of the brain.
1	1189	50	Negro	Male	(1400)	1380	770	...	....	Hanging	..	0
1	1190	23	Negro	Male	(1420)	1400	668	...	....	Hanging	..	0
7	1216	59	Caucasian	Male	....	....	805	...	....	Asthenia	..	1
7	1246	44	Negro	Male	....	....	610	...	....	Asthenia	..	0
1	1405	47	Caucasian	Male	(1430)	1410	700	...	....	Asthenia	..	5
3	1449	20	Negro	Male	....	1157	...	188	Medium	Tuberculosis	..	0
1	1449	28	Negro	Female	(1170)	1150	643	188	37.2	Asthenia	..	1
1	1451	25	Negro	Male	(1390)	1370	710	152	41.2	Pneumonia	..	6
1	1452	45	Negro	Female	(1320)	1300	640	152	52.5	Alcohol	..	12
1	1453	50	Negro	Male	(1245)	1225	492	163	46.7	Frozen	..	18
1	1454	23	Negro	Male	(1135)	1115	605	160	55.8	Tuberculosis	..	1
1	1455	73	Caucasian	Male	(1358)	1330	653	168	47.2	Asthenia	..	1
1	1456	28	Negro	Male	(1150)	1130	706	165	43.1	Pneumonia	..	2
1	1457	79	Caucasian	Male	(1200)	1180	630	173	46.7	Nephritis	..	6
1	1458	43	Caucasian	Male	(1340)	1320	735	173	47.6	Pneumonia	..	1
1	1459	40	Negro	Female	(1080)	1060	602	147	89.0	Pneumonia	..	2
1	1463	60	Caucasian	Male	(1555)	1535	730	188	86.2	Pneumonia	..	13
1	1466	57	Mulatto	Male	(1440)	1420	722	183	70.3	Tuberculosis	..	1
7	1467	27	Negro	Male	....	....	642	...	....	Tuberculosis	34	1
1	1469	81	Caucasian	Male	(1185)	1165	453	178	77.1	Asthenia	..	0
1	1470	70	Negro	Male	(1100)	1080	522	157	61.2	Heart disease	..	0
1	1472	62	Negro	Male	(1060)	1040	438	157	61.2	Tuberculosis	..	0
1	1473	25	Negro	Male	(1135)	1115	525	165	72.6	Tuberculosis	..	1
1	1475	50	Negro	Male	(1355)	1335	...	168	81.6	Heart disease	..	2
1	1476	25	Negro	Male	(1240)	1215	533	163	63.5	Nephritis	..	5
1	1477	54	Negro	Female	(1145)	1125	475	154	47.6	Nephritis	..	2
1	1478	..	Negro	Male	(1200)	1180	663	178	72.6	Tuberculosis	..	7
1	1479	45	Negro	Female	(1130)	1110	603	154	47.6	.....	..	2
1	1480	60	Negro	Male	(1375)	1355	730	176	86.0	Hæmorrhage	..	2
1	1485	62	Caucasian	Female	(1010)	990	463	163	44.2	Asthenia	..	0
1	1486	57	Negro	Male	(1270)	1245	687	186	68.0	Shot	..	7
1	1487	50	Negro	Female	(1025)	1005	368	188	72.6	Heart disease	..	1
1	1489	58	Caucasian	Male	(1280)	1260	773	190	75.0	Pneumonia	..	1
1	1490	40	Caucasian	Male	(1500)	1480	910	193	67.1	Heart disease	..	3
1	1492	12	Negro	Male	(1185)	1165	458	127	27.2	Epilepsy	..	1
1	1493	31	Negro	Female	(1035)	1015	525	157	86.0	Heart disease	..	5
1	1494	44	Negro	Male	(1360)	1340	...	168	77.1	Suffocation	..	20
1	1495	35	Negro	Male	(1245)	1225	568	178	85.0	Heart disease	..	1
1	1496	60	Caucasian	Male	(1390)	1370	742	178	85.5	Pneumonia	..	3
1	1497	19	Negro	Male	(1270)	1245	645	163	48.0	Typhoid	..	4
1	1500	76	Negro	Female	(910)	893	427	176	40.8	Asthenia	..	1
1	1501	30	Negro	Female	(1060)	1040	620	154	46.3	Tuberculosis	..	2
1	1502	23	Negro	Female	(1225)	1205	557	163	72.6	Pneumonia	..	1
1	1506	34	Caucasian	Male	(1300)	1280	...	188	84.0	Pneumonia	..	6
1	1510	36	Caucasian	Female	(1190)	1170	568	171	59.0	Tuberculosis	..	2
1	1511	70	Negro	Male	(1130)	1110	614	157	75.8	Heart disease	..	2
1	1512	74	Caucasian	Male	(1330)	1310	666	163	80.8	Tuberculosis	..	9
1	1514	61	Caucasian	Male	(1480)	1460	722	160	64.0	Heart disease	..	0
1	1515	67	Negro	Female	(1045)	1025	487	163	62.0	Pneumonia	..	0
1	1519	8	Negro	Male	(1120)	1100	500	130	34.4	Pneumonia	..	3

TABLE I.—CONTINUED.

Group.	Number of Cadaver.	Age of subject.	Race.	Sex.	Weight of brain in gm. (fresh).	Weight of brain in gm. (when measured).	Area of corpus callosum in sq. mm.	Length of body in cm.	Weight of body in kilos.	Cause of death.	Color of body (Broca's standard).	Number of days between death and removal of the brain.
1	1520	40	Caucasian	Male	(1540)	1520	832	163	49.0	Tuberculosis	..	3
1	1521	32	Negro	Female	(1230)	1210	683	157	54.0	Asthenia	..	1
1	1522	36	Caucasian	Female	(1065)	1045	617	154	59.2	Tuberculosis	..	1
1	1523	40	Negro	Male	(1145)	1125	...	163	46.4	.....	..	..
1	1524	35	Mulatto	Male	(1180)	1160	590	178	65.3	.....	..	..
3	1526	11	Negro	Male	....	1300	...	69	Light	Tuberculosis	..	0
1	1527	77	Caucasian	Female	(1140)	1120	707	157	44.0	Asthenia	..	0
1	1528	37	Negro	Male	(1410)	1390	913	176	81.6	Pneumonia	..	1
1	1529	42	Caucasian	Male	(1475)	1455	652	170	72.6	Asthenia	..	2
1	1530	70	Negro	Male	(1010)	990	666	163	71.0	Asthenia	..	0
1	1531	25	Mulatto	Female	(1100)	1080	...	157	45.7	Asthenia	..	1
1	1532	65	Mulatto	Male	(1420)	1400	...	178	65.0	Asthenia	..	2
1	1533	50	Negro	Male	(1170)	1150	558	190	81.6	Asthenia	..	1
1	1538	67	Caucasian	Male	(1310)	1290	698	185	77.1	Hæmorrhage	..	0
1	1544	27	Negro	Female	(1160)	1140	658	168	45.0	Tuberculosis	..	1
1	1553	42	Mulatto	Female	(1140)	1120	680	157	65.8	Shock	..	2
7	1582	19	Negro	Male	....	....	555	...	....	Tuberculosis	36	..
1	1583	80	Caucasian	Female	(1275)	1255	733	168	63.5	Asthenia	..	1
1	1591	67	Caucasian	Male	(1235)	1235	672	165	63.5	Heart disease	..	0
1	1593	43	Mulatto	Female	(1000)	1000	...	176	46.3	Tuberculosis	..	1
7	1650	28	Mulatto	Male	....	....	755	...	....	Accident	..	..
7	1653	29	Negro	Female	....	....	655	...	....	Tuberculosis	41	..
2	1659	63	Negro	Female	1050	1050	512	165	59.0	Pneumonia	..	3
2	1660	82	Negro	Male	1560	1520	732	170	....	Nephritis	..	3
2	1661	73	Negro	Male	1040	1000	575	163	....	Pneumonia	22	1
2	1662	45	Negro	Female	1219	1210	713	...	....	.....	43	1
7	1667	31	Negro	Male	....	....	620	...	Heavy	.....	36	..
6	1678	42	Negro	Female	....	1020	600	...	....	.....	..	..
5	1680	62	Negro	Male	1080	1060	438	163	Medium	Nephritis	22	1
7	1681	45	Negro	Male	....	....	...	163	Heavy	.....	36	..
5	1682	35	Caucasian	Male	1380	1290	715	165	Heavy	Accident	..	13
5	1683	42	Caucasian	Male	1320	1265	640	173	Medium	Heart disease	..	4
5	1684	50	Negro	Female	1220	1170	600	163	Light	Tuberculosis	53	3
5	1685	65	Negro	Female	1230	1100	600	150	Heavy	Pneumonia	34	1
5	1686	35	Negro	Female	1090	1040	697	165	Medium	Nephritis	37	1
5	1687	64	Negro	Female	980	925	490	173	Medium	Nephritis	29	2
6	1690	40	Caucasian	Male	1450	1420	773	168	Light	.....	..	..
6	1691	62	Negro	Male	1200	1160	475	152	Light	Pneumonia	43	1
6	1692	24	Caucasian	Female	1250	1235	695	142	Light	Tuberculosis	..	1
6	1693	50	Caucasian	Male	1320	1250	573	173	Heavy	Poison	..	18
6	1695	66	Mulatto	Female	1140	1055	492	150	Heavy	Nephritis	25	2
6	1696	45	Caucasian	Male	1410	1340	757	178	Medium	Tuberculosis	..	1
6	1697	74	Caucasian	Female	980	955	540	157	Light	.....	..	..
6	1699	32	Negro	Male	1200	1205	635	163	59.0	.....	29	..
6	1700	28	Negro	Female	1225	1185	715	160	54.4	.....	40	..
6	1701	39	Negro	Male	1400	1355	715	185	79.4	.....	37	..
6	1702	45	Caucasian	Male	1200	1132	620	163	54.4	.....	..	..
6	1704	50	Negro	Male	1340	1275	710	163	63.5	.....	43	..
6	1706	73	Negro	Male	1335	1255	735	175	63.5	.....	28	..
6	1707	77	Caucasian	Male	1275	1175	465	160	72.6	.....	..	..

TABLE I.—CONTINUED.

Group.	Number of Cadaver.	Age of subject.	Race.	Sex.	Weight of brain in gm. (fresh).	Weight of brain in gm. (when measured).	Area of corpus callosum in sq. mm.	Length of body in cm.	Weight of body in kilos.	Cause of death.	Color of body (Broca's standard).	Number of days between death and removal of the brain.
6	1708	60	Caucasian	Male	1350	1295	610	183	77.1	.....	..	..
6	1709	26	Negro	Male	1475	1410	825	165	77.1	.....	43	..
6	1711	80	Negro	Male	1175	1090	620	173	68.5	.....	29	..
6	1712	70	Caucasian	Male	1325	1210	710	185	76.0	.....	..	..
6	1713	49	Negro	Male	1240	1175	680	175	49.4	Asthenia	29	..
6	1715	20	Negro	Female	950	860	405	137	21.3	.....	53	..
6	1716	48	Caucasian	Male	1265	1238	500	167	57.5	.....	..	..
6	1718	22	Negro	Male	1200	1130	765	170	70.3	Tuberculosis	29	3
6	1719	46	Caucasian	Male	1445	1355	880	193	79.4	Nephritis	..	2
6	1720	53	Caucasian	Male	1430	1245	760	186	59.0	Pneumonia	..	1
6	1722	19	Negro	Female	1050	1010	520	173	39.0	.....	46	4
6	1723	38	Caucasian	Male	1275	1230	545	165	50.3	.....	..	5
6	1727	55	Negro	Male	1265	1227	710	157	Light	Tuberculosis	22	4
6	1728	50	Negro	Male	1330	1270	675	162	56.7	Accident	50	0
6	1729	53	Negro	Male	1410	1460	...	182	Medium	Heart disease	50	5
6	1730	22	Negro	Female	1005	915	525	150	Light	Tuberculosis	36	2
6	1731	70	Negro	Male	1450	1415	820	179	67.0	Nephritis	35	4
6	1734	50	Caucasian	Male	1360	1305	710	172	Heavy	Nephritis	..	2
6	1736	82	Negro	Male	1310	1245	570	162	53.0	Asthenia	28	..
6	1738	22	Negro	Male	1275	1240	535	168	49.9	Tuberculosis	27	5
6	1739	73	Negro	Male	1120	1060	...	168	Light	Tuberculosis	29	5
6	1741	50	Negro	Male	1220	1175	...	163	Heavy	Heart disease	46	3
6	1748	60	Caucasian	Male	1520	1475	820	175	56.0	Tuberculosis	..	4
6	1749	74	Caucasian	Male	1040	1030	750	160	57.6	Nephritis	..	2
3	2469	35	Negro	Male	....	1150	585	180	....	.....	..	0
3	2521	23	Negro	Male	1395	1290	490	172	Heavy	Pneumonia	27	0
3	2522	38	Negro	Male	1350	1270	640	170	Heavy	Pneumonia	42	0
3	2524	45	Negro	Male	1350	1230	660	154	Heavy	Heart disease	36	0
3	2535	24	Negro	Male	....	1065	615	170	Medium	Tuberculosis	34	0
4	87	..	Negro	Male	....	1150	583	...	....	.....	..	0
3	163	48	Negro	Female	1130	920	485	165	Heavy	Cancer	Brown	0
3	164	57	Caucasian	Male	....	1145	575	...	....	Nephritis	..	0
3	169	87	Caucasian	Male	1110	1060	430	...	....	Cancer	..	0
4	172	45	Negro	Male	....	1020	625	...	....	.....	..	0
3	173	45	Negro	Male	....	1245	690	163	Heavy	Nephritis	..	0
3	177	15	Caucasian	Male	....	950	420	...	....	Tuberculosis	..	0
3	193	29	Negro	Male	....	910	430	175	Light	Tuberculosis	Brown	0
9	1G.	58	Caucasian	Male	1240	990	588	...	....	.....	..	..
9	2G.	48	Caucasian	Female	1106	900	566	...	....	.....	..	..
9	3G.	48	Caucasian	Male	1250	1110	790	...	....	.....	..	..
9	4G.	53	Caucasian	Male	1300	990	600	...	....	.....	..	..
9	5G.	16	Caucasian	Female	915	840	613	...	....	.....	..	..
9	6G.	25	Caucasian	Male	1460	1080	520	...	....	.....	..	..
8	105	1	Negro	Male	....	860	445	...	....	.....	..	..
8	107	2	Negro	Male	....	830	540	81	....	.....	39	..
8	108	1*	Negro	Female	....	435	155	53	3.4	.....	53	2
8	109	2*	Negro	Male	....	700	245	58	4.0	.....	52	1
8	110	†	Negro	Male	....	525	190	55	3.4	Birth	53	..
8	111	6*	Negro	Male	....	600?	255	...	....	.....	..	..
8	112	6*	Negro	Male	....	600?	232	...	....	.....	..	..
8	113	†	Negro	Male	....	....	...	...	....	Birth	..	..
8	114	2	Negro	Male	....	800?	390	...	....	.....	..	..

* Months. † Birth.

TABLE I.

RECORD OF MATERIAL TAKEN FROM RETZIUS AND SPITZKA.[1]

Number.	Sex.	Age.	Body length.	Occupation.	Cause of death.	Brain weight.	Area of corpus callosum.	Splenium.	Isthmus.	Body.	Genu.
			cm.			g.	sq. cm.				
1	Female	29	163	Chambermaid	Pul. tuberculosis	1201	8.90	2.10	.90	1.70	3.30
2	Female	63	156	Workwoman	Carcinoma	....	6.70	1.80	1.00	1.60	2.40
3	Male	50	148	Typesetter	Nephritis	1376	7.00	1.70	1.10	1.70	2.60
4	Female	37	156	Workwoman	Pul. tuberculosis	1194	5.40	1.40	.80	1.10	2.20
5	Male	26	179	Laborer	Pul. tuberculosis	1446	6.10	1.60	.70	1.50	2.20
6	Female	29	161	.....	.....	1088	6.40	1.60	.90	1.50	2.40
7	Female	76	151	.....	Arteriosclerosis	1101	6.40	1.80	1.20	1.40	2.10
8	.....	..	...	.....	.....	....	7.10	1.90	.90	1.40	2.80
9	.....	..	...	.....	.....	....	4.00	1.10	.40	.80	1.70
10	.....	..	...	.....	.....	....	6.90	1.80	.90	1.70	2.40
11	.....	..	...	.....	.....	....	6.60	1.70	1.00	1.40	2.50
12	Male	64	167	Builder	Pul. tuberculosis	1179	8.40	2.00	1.30	1.80	3.30
13	Male	44	170	Joiner	Pneumonia	1206	7.70	1.90	1.30	1.80	2.80
14	Male	43	178	.....	Pul. tuberculosis	1465	7.70	2.10	1.00	1.60	3.10
15	Male	58	166	Laborer	Carcinoma	1587	9.80	2.70	1.40	1.90	3.70
16	Male	23	175	Bookbinder	Pul. tuberculosis	1357	6.10	1.40	.90	1.40	2.50
17	Male	52	179	Laborer	Tuberculosis	1518	7.00	1.80	.90	1.40	2.80
18	Male	36	166	Laborer	Tuberculosis	1284	7.30	2.00	.80	1.60	2.80
19	.....	..	...	.....	.....	....	5.30	1.40	.60	1.10	2.20
20	Male	27	163	Shoemaker	Pul. tuberculosis	1383	6.40	1.80	.80	1.10	2.70
21	Male	52	169	Waiter	Nephritis	1346	7.20	1.80	.90	1.40	3.10
22	Male	22	182	Painter	Vit. org. cond.	1351	7.00	1.70	1.10	1.80	2.40
23	Male	55	Medium	Astronomer	Arteriosclerosis	1452	8.40	2.60	1.20	1.70	2.90
24	Female	41	Medium	Mathematician	Pleurisy	1108?	7.00	1.90	1.10	1.50	2.40
25	Male	76	...	Pedagogue	Influenza	1422	6.00	1.70	.90	1.30	2.20
26	Male	..	...	Statesman	.....	1489	7.20	2.20	1.00	1.50	2.50
27	Male	..	...	Morphologist	.....	1545?	10.60	...	...	...	...
28	Male	..	...	Neurologist	.....	....	8.00	...	...	...	...

[1] Nos. 1 to 26 are from cuts by Retzius in Biologische Untersuchungen, Vols. 8, 9, 10, and 11. Nos. 27 and 28 are from cuts by Spitzka in Connecticut Magazine, 1905, and Am. Jour. Anat., Vol. 4.

Group 1 contains brains that were removed from the body after it had been injected in the usual way with carbolic acid, alcohol, and glycerine through the femoral artery under five pounds' pressure, and afterwards with shellac in the same way. The brains were then placed in 10% formalin, vertex down, no weight being taken at the time. Sixty-four brains were treated in this manner.

Group 2 contains brains treated in the same way, except that they were weighed at the time of removal from the body, and placed, vertex up, in 10% formalin.

Brains in Group 3 were weighed when removed from the body, which had not been injected, and placed, vertex up, in 10% formalin.

In Group 4 the brains were weighed a few days after being removed from the body, which had had no previous injection, the brains being placed in 10% formalin, vertex up, after removal.

Brains in Group 5 were weighed at the time of removal from the

body, which had been injected with carbolic acid in the usual way. These brains were *suspended* in a solution of 40% formalin and 6% sodium chloride, vertex up.

In Group 6 the brains were treated in the same way as in Group 5, except that they were *suspended* in 10% formalin and sodium chloride. Thirty-eight brains were preserved in this manner.

Group 7 contains brains that were obtained from frozen and sawed sections of cadavers previously injected with formalin.

Group 8 contains brains of infants preserved in situ by immersion of the head in 10% formalin after opening the membranes so as to allow the fluid to permeate the cerebral structures.

Group 9 contains brains of Germans from Prof. Waldeyer's laboratory in Berlin. The brains were weighed at the time of removal from the body and had been preserved in alcohol several years.

Perfect preservation of the shape of the brain may be obtained by injecting the bodies of fresh cadavers with carbolic acid, alcohol, and glycerine through the femoral arteries under 120 mm. Hg. pressure, leaving the body for 12 hours, then after removing the brain, which is firm and solid, suspending it in 10% formalin and sodium chloride. All brains from No. 1593 onward were suspended base down, thus favoring retention of their shape. The first seventy-three brains, up to No. 1659, were removed prior to the time at which I began the personal supervision of their preservation; those following were personally attended to and all data concerning them is personal. The brain weights given are with the dura mater removed, leaving the pia mater and vessels intact. The brain weights given in parenthesis are estimated from specimens in which the weight, both fresh and after hardening, had been taken. The weight of the hardened brain was taken after it had been thoroughly drained.

The actual weights and areas taken at the time the drainings were made are the ones used in the construction of the tables and charts.

Brain Outlines.

Outline drawings are made of the brains in their normal position, looking from above; lateral and mesial outlines are drawn after the hemispheres have been separated by a sagittal cut through the corpus callosum and brain stem. Outlines are also made of the lateral border of each hemisphere looking from above, the hemispheres being rotated through an angle of 45° on an axis passing beneath the splenium, above the anterior commissure, and through the foramen of Munro. This axis is drawn in every outline and from it all measurements are made; it is dis-

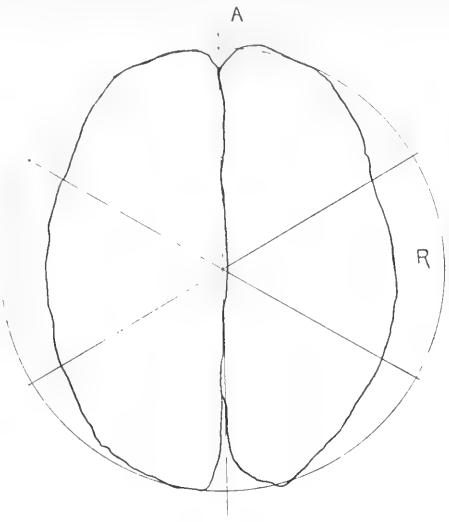


Fig. 1a

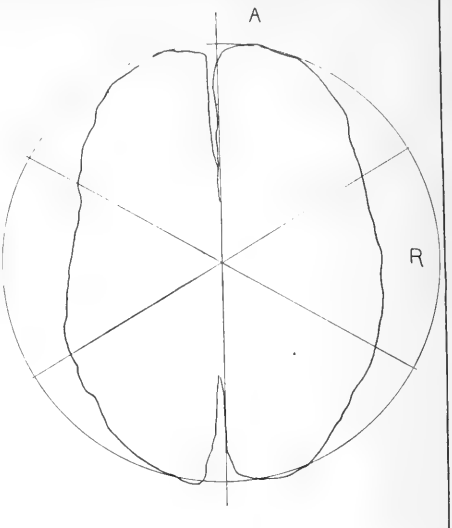


Fig. 1b

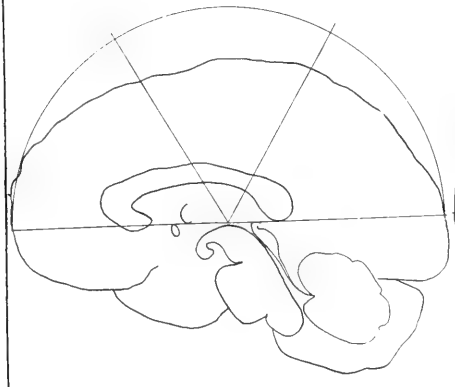


Fig. 2a

Fig. 2b

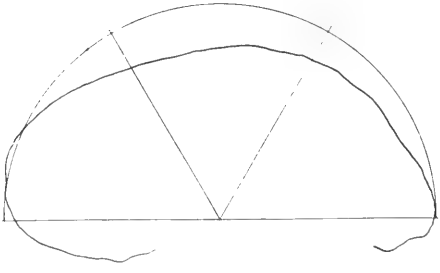


Fig. 3a

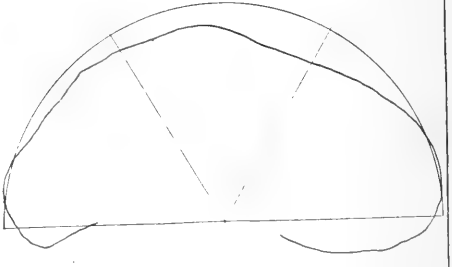


Fig. 3b

FIG. 1a. Caucasian male, age 40, No. 1690, length 168 cm. Brain outline as viewed from above, horizontal plane. *A*, anterior end; *R*, right side. One-third natural size.

FIG. 1b. Negro male, age 37, No. 1528, length 176 cm., weight 81.6 Kg. Brain outline as viewed from above, horizontal plane. *A*, anterior end; *R*, right side. One-third natural size.

FIG. 2a. Caucasian male, age 40, No. 1690, length 168 cm. Brain outline as viewed from within, mesial view, vertical plane. Right hemisphere. One-third natural size.

FIG. 2b. Negro male, age 37, No. 1528, length 176 cm., weight 81.6 Kg. Brain outline viewed from within, mesial view, vertical plane. Right hemisphere. One-third natural size.

FIG. 3a. Caucasian male, age 40, No. 1690, length 168 cm. Brain outline as viewed from above and from the left at an angle of 45°, the outline at 45°. Right hemisphere. One-third natural size.

FIG. 3b. Negro male, age 37, No. 1528, length 176 cm., weight 81.6 Kg. Brain as viewed from above and from the left at an angle of 45°, the outline at 45°. Right hemisphere. One-third natural size.

cussed on page 404 in connection with the brain center. It passes through the longest diameter of the brain between the hemispheres, and its midpoint is taken as the brain center. From this center radii are drawn on all the outlines at 60° and 120°, the anterior end of the axis being marked 0°, the posterior end 180°. The point of contact of the anterior radius (60°) with the brain outline is invariably over the anterior association center (Broca's convolution on the left side), while the point of contact of the posterior radius (120°) is invariably over the posterior association center. These two points are meant whenever the anterior or posterior association centers are referred to unless otherwise expressed or implied.

Outlines with brain axis, and these points located on the brain of an adult male Negro (No. 1528) and of an adult male Caucasian (No. 1690) are seen in figures 1a to 3b, there being semicircles drawn around each hemisphere to facilitate comparison. These two brains are selected because they are nearly alike in many respects, but still show the racial characteristics. They are taken from young adult males of about the same age, the brains being of about the same size and weight. From these outlines it is observed that the Caucasian brain conforms more nearly to a circle in its contour in the different planes than does that of the Negro, which is squared at the ends, and flatter on the sides and above, especially along the frontal lobes, thus exhibiting a distinct box-shaped appearance. This shape of the Negro brain is manifested in the mesial outline by the abrupt rise of the contour from the axis at its posterior end, by the nearly straight line over the anterior association center, by the nearly

Fig 4

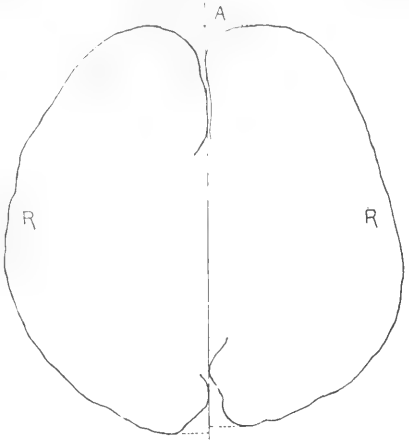


Fig 5

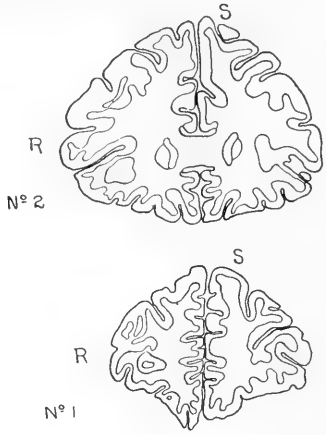


Fig 6

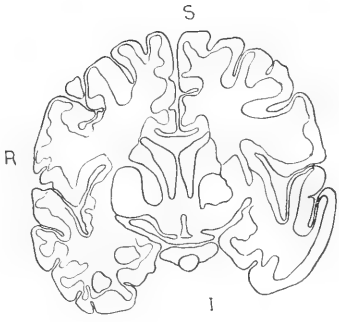


Fig. 7

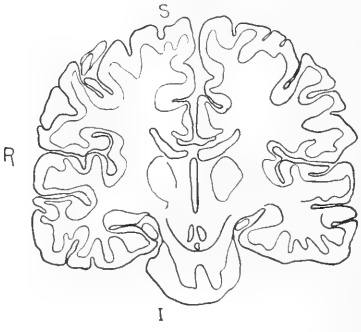


Fig 8ª

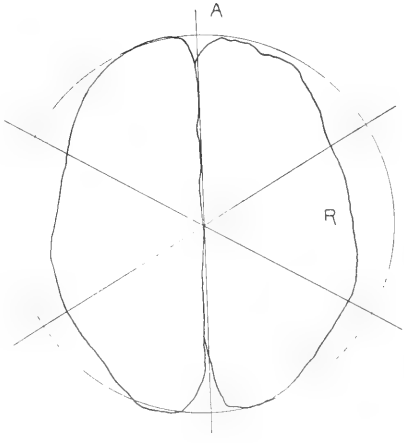
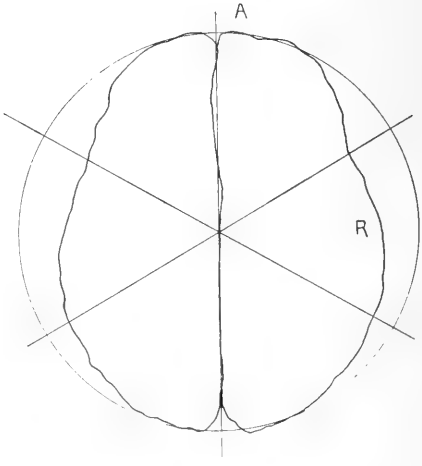


Fig 8ᵇ



FIGURES 4 TO 8b ON PAGE 362.

FIG. 4. Left side of the figure, Caucasian male, age 67, No. 1538, length 185 cm., weight 77.1 Kg. Brain outline as viewed from above and from the left at an angle of 45°. Right hemisphere. (The outline is inverted).

Right side of the Figure, Negro male, age 25, No. 1473, length 165 cm., weight 72.6 Kg. Brain outline as viewed from above and the left at an angle of 45°. Right hemisphere. *A*, anterior end; *R*, right side. One-third natural size.

FIG. 5. Negro male, age 45, No. 1681, length 163 cm., large and fat. Vertical, transverse sections. Section not quite transverse. No. 1 about 15 mm. from anterior end of brain; No. 2, about 45 mm. *S*, superior surface; *R*, right side. One-third natural size.

FIG. 6. Negro male, age 45, No. 1681. Vertical transverse and slightly oblique section. The section is about 75 mm. from the anterior end of the brain. One-third natural size.

FIG. 7. Negro male, age 45, No. 1681. The section is about 105 mm. from the anterior end of the brain, just anterior to external auditory meatus. One-third natural size.

FIG. 8a. Caucasian female, age 36, No. 1522, length 154 cm., weight 59.2 Kg. Brain outline as viewed from above, horizontal plane, 1.2., at 90°. *A*, anterior extremity; *R*, right side. One-third natural size.

FIG. 8b. Negro female, age 27, No. 1544, length 168 cm., weight 45 Kg. Brain outline as viewed from above. One-third natural size.

vertical line along the anterior aspect of the frontal lobe, and by the horizontal line along the inferior border of this lobe; it is manifested in the outline from above by the square front and sides of the outline; and in the outline with the brain rotated laterally 45°, by the more abrupt rise posteriorly, and the depression or apparent flattening over the anterior association center, along with the relative bulging of the posterior association center. These differences are seen more plainly in Figure 4 (brains No. 1473 and 1538) which represents the 45° outlines of a fairly typical adult male Caucasian brain, and of a fairly typical adult male Negro brain of about the same weight and length. It is the straight line seen over the anterior association center in this figure on which especial emphasis is laid as a distinctive characteristic of the Negro brain. Looking at the brain directly from above or from the side one does not so readily notice any apparent flattening, but on rotating the brain on its axis slightly to one side a glance will often bring it out distinctly; or a careful examination, revolving the brain from 10° to 60° from its normal position and looking at it from above, will almost invariably disclose this peculiarity. In some brains it is well marked, in others only slightly so. It usually appears most marked when either hemisphere is rotated through an angle of 30° laterally from its normal position and viewed from above. Viewed from the side the Negro brain appears to be pressed back, while

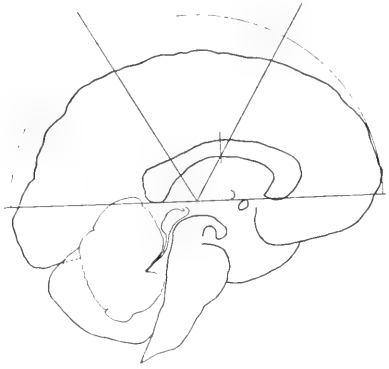


Fig. 9a

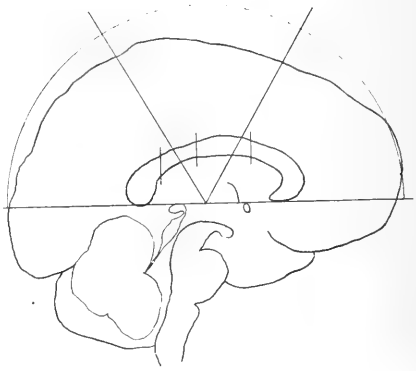


Fig. 9b

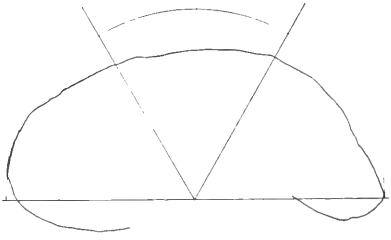


Fig. 10a

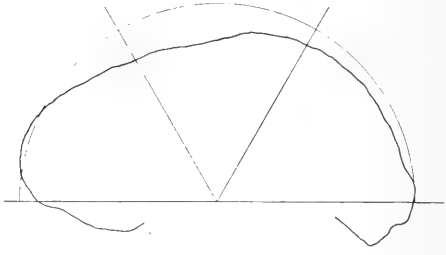


Fig. 10b

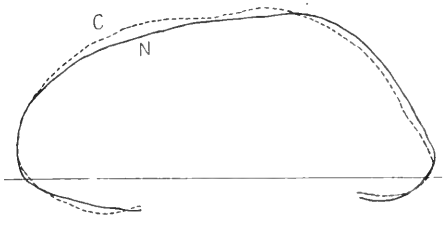


Fig. 11

FIGURES 9a TO 11 ON PAGE 364.

FIG. 9a. Caucasian female, age 36, No. 1522, length 154 cm., weight 59.2 Kg. Brain outline as viewed from the mesial side. Left hemisphere. One-third natural size.

FIG. 9b. Negro female, age 27, No. 1544, length 168 cm., weight 45 Kg. Brain outlines as viewed from the mesial side. Left hemisphere. One-third natural size.

FIG. 10a. Caucasian female, age 36, No. 1522, length 154 cm., weight 59.2 Kg. Brain outline as viewed from above and to the right at an angle of 45°. Left hemisphere. One-third natural size.

FIG. 10b. Negro female, age 27, No. 1544, length 168 cm., weight 45 Kg. Brain as viewed from above and to the right at an angle of 45°. Left hemisphere. One-third natural size.

FIG. 11. Unbroken line represents the composite of 45 Negro male outlines as viewed from above and to the left at an angle of 45°. Right hemisphere. The broken line represents the composite of 45 Caucasian male outlines also viewed from above and to the left at an angle of 45°. Right hemisphere. One-third natural size.

the Caucasian appears to be pushed forward, the result being that the frontal lobe of the Negro brain appears considerably smaller than that of the Caucasian. This difference is greater than is apparent in the outlines, because the gyrus rectus in the Negro brain is low, while the superior orbital plate passes well up into the frontal lobe outside of this, materially diminishing the size of this lobe, the gyrus frontalis superior also projecting upward in Negro brains more than in the Caucasian. This is shown in Figures 5 to 7, brain No. 1681, from a typical adult male Negro. The drawings are made from sawed sections of the frozen head, showing the brain in situ, no distortion of the brain being apparent. In this there may be observed the extremely small frontal lobes; the projection downward of the gyrus rectus; the deep impression of the superior orbital plates; the straight lines along the sides anteriorly, showing the lateral surfaces of the brain to be at an angle of 45° from the vertical plane; the upward projection of the gyrus frontalis superior; the box-like appearance of each outline; and the great bulging in the parietal region. The female Negro brain may differ somewhat from that of the male, but in general the same peculiarities are noticeable in each. Figures 8a to 10b exhibit a characteristic adult female Negro brain and a small adult female Caucasian brain for comparison, the two being selected because they are so nearly alike, yet the racial differences are noticeable. The frontal lobes of the female Negro brain are long and slender, while the parietal region is full and bulging. The peculiaries noted in the other outlines may be seen in these also.

Examination of about fifty Negro skulls, and hundreds of Negro heads has convinced me of a noticeable characteristic: the appearance to be

obtained by a view from behind at an angle of about 30° above the horizontal looking directly forward. The outline of the head or skull seen in this way is pointed anteriorly and broad and flattened posteriorly. This may be seen in the Negro brains under the same conditions. Here we see the small frontal lobes, the large parietal region and the straight, flat sides over the anterior association centers. That this is not only apparent, but real, may be determined by measurements of the radii from the brain center to the outlines of the plane passing through the brain axis at an angle of 45° above the horizontal plane of each hemisphere. Such measurements are found in Table II, which gives the dimensions of this plane in each non-distorted brain. Radii are projected from the brain center for each 10° angle, and perpendiculars are dropped from the brain axis for each centimeter on the axis from either end of the brain, and these radii and perpendiculars are measured from their origin out to the surface of the brain.

From Table II the following summary is given:

TABLE II.a

Averages of the Association Centers.

	Left Side					Right Side				
	Number of brains.	Brain axis.	Anterior association center.	Posterior association center.	Index association centers.	Number of brains.	Brain axis.	Anterior association center.	Posterior association center.	Index association centers.
		mm.	mm.	mm.			mm.	mm.	mm.	
Caucasian male	34	168	70	71	98	34	167	70	72	97
Negro male	43	168	66	73	90	45	168	66	74	89+
Caucasian female	8	161	64	67	96	8	160	65	67	97
Negro female	22	158	62	68	91	22	158	63	69	91

The numbers represent averages in each case for the number of brains given. The fifth column of numbers on each side represents the averages of indices of the association centers. The index of the association centers for each brain is obtained by dividing the length of the radius for each center by the length of one-half the brain axis, and dividing the result obtained for the anterior association center by the result obtained for the posterior association center. The quotient represents the proportion of the size of the anterior association center in terms of the posterior association center, the latter being 100 in each case, the brain axis also en-

tering as an element. For each increase of 20 mm. in the length of the brain axis there is an increase of about one unit in the index. For example, the index for the left hemisphere of the male Caucasian brain is 98, the length of the radius to the anterior association center is 70 mm., that to the posterior association center is 71 mm. 70 : 71 : : 98 : 100 is correct, considering the brain axis element 84 mm. Increase the latter and the index rises, reduce it and the index falls. The index varies, directly with the size of the anterior association center, and inversely with the size of the posterior association center. Increase 70 and the index is increased; diminish 70 and the index is diminished. Increase 71 and the index is diminished; diminish 71 and the index is increased. The index gives a simple numerical expression that may be used to advantage in the comparison of brains, and in the comparison at present in hand it affords an excellent indication of existing differences. It is observed from the table that the index of the male Caucasian brain is the largest; the index of the female Caucasian comes next; with the female Negro third, and the male Negro the lowest. This indicates that the relations of the brain axis and anterior association centers are similar to the index of the association centers, while the posterior association center is dissimilar in the two sexes and races. The index is slightly larger on the left side, except in the female Caucasian. This may be due to the gyrus frontalis inferior, or to a larger motor area on the left side in the males.

The relative differences of the association centers in the males of the two races on the right side are represented in Fig. 11, which is a composite of the 45° outline of the thirty-four male Caucasian and the forty-five male Negro brains The brain axis is practically the same length in each (167-8 mm.). A difference in the size and shape of the two outlines is evident on the inferior surfaces of the frontal and occipital lobes below the axis, as well as above it, the Caucasian brain being further below the axis and more curved along the frontal lobe, while the Negro brain is further below the axis and more curved along the inferior surface of the occipital lobe, a difference which materially diminishes the size of the frontal lobe in the Negro and increases the size of the occipital. The flatness of the anterior association center is seen in the Negro outline, and the actual areas of the parts of these outlines are as follows:

Area of the anterior lineal half of the composite Negro outline...48.4 sq. cm.
Area of the anterior lineal half of the composite Caucasian outline .....51.2 sq. cm.
Area of the posterior lineal half of the composite Negro outline...48.2 sq. cm.
Area of the posterior lineal half of the composite Caucasian outline .....56.2 sq. cm.

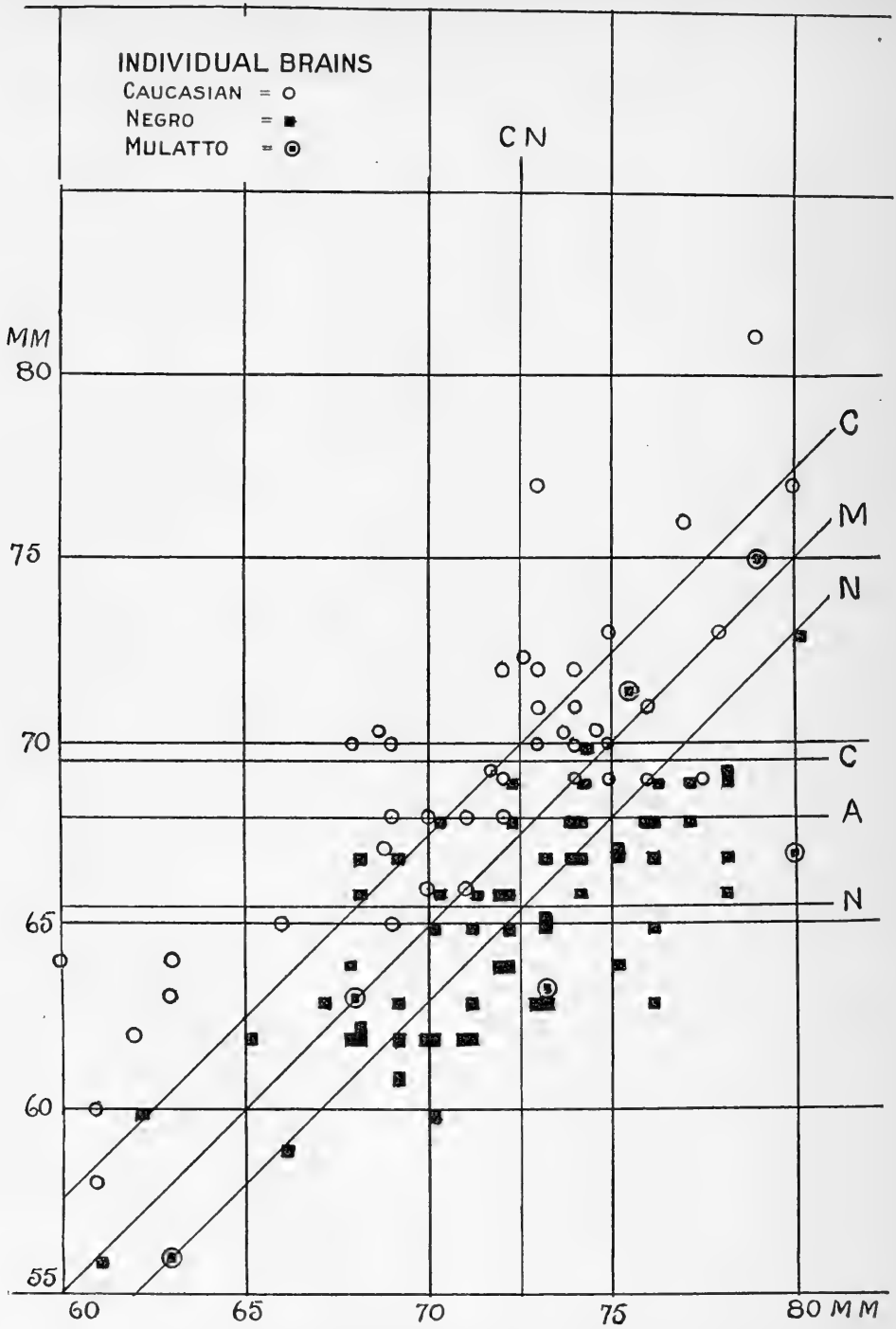


CHART I. *Right Side.*—Relation of the radii of the anterior association center (ordinates) to the radii of the posterior association center (abscissæ); taken with the brain tilted 45°, the former at 60° from the anterior end, the latter 120°, as in Figure 3b. The perpendicular line *C N* gives the mean for the anterior association centers for both races; the horizontal lines *C*, *N*, for the posterior association centers of the Caucasian and Negro respectively; and the diagonal lines are the mean of both centers; for the Caucasian, *C*, for Negroes, *N*, and for both races combined, *M*. This is true of the first four charts.

That these differences are manifested not only in mass, and by averages, but individually, may be determined by examining Table II, and Charts I and II, taken from the numbers in Table II, and giving the relation of the anterior and posterior association centers in each brain. The anterior association center in all cases is represented by the numbers from the column under 60°, the posterior association center by the numbers from the column under 120°. The charts are made up by the use of ordinates and abscissæ, the former representing the length of the radii of the anterior association center, the latter the length of the radii of the posterior association center. An arbitrary line drawn on the charts from the 68-mm. ordinate on each side divides the symbols into racial groups, the Caucasian above the line and the Negro below, indicating a longer radius to the anterior association center in a larger number of brains among Caucasians. This line divides the two sides differently. On the left side a larger number of Caucasian symbols fall below the line and a larger number of Negro symbols fall above the line than on the right side. The symbols that are over the line represent the extremes of each race in relation to the other race. A greater number of Negro extremes have a larger left anterior association center, and conversely, a greater number of Caucasian extremes have a smaller left anterior association center. The extremes may be represented by a table taken from Charts I and II.

TABLE IIb.

Extremes of the Anterior Association Center.

Symbols.	Left Side.		Right Side.	
	Above the arbitrary line.	Below the arbitrary line.	Above the arbitrary line.	Below the arbitrary line.
Caucasian	24	16	27	11
Negro	15	45	10	50

The numbers in this table are of value only in comparing the two sides of the body. On the left side there are 16 Caucasian extremes and 15 Negro extremes. On the right side there are 11 Caucasian extremes and 10 Negro extremes. The deduction from this is that there is greater dissimilarity of the brains of the two races on the right side than on the left side. The majority of the Negro symbols fall below the line, and the majority of the Caucasians fall above on each side, this being the most noticeable difference, that the anterior association is smaller in the Negro than in the Caucasian. The radius to the anterior association center of the left hemisphere invariably passes over the gyrus frontalis inferior, so that this may mean a greater development of the gyrus in the

Negro extremes, and a less development in the Caucasian extremes. It is possible that the size of the motor area may account for this difference on the two sides.

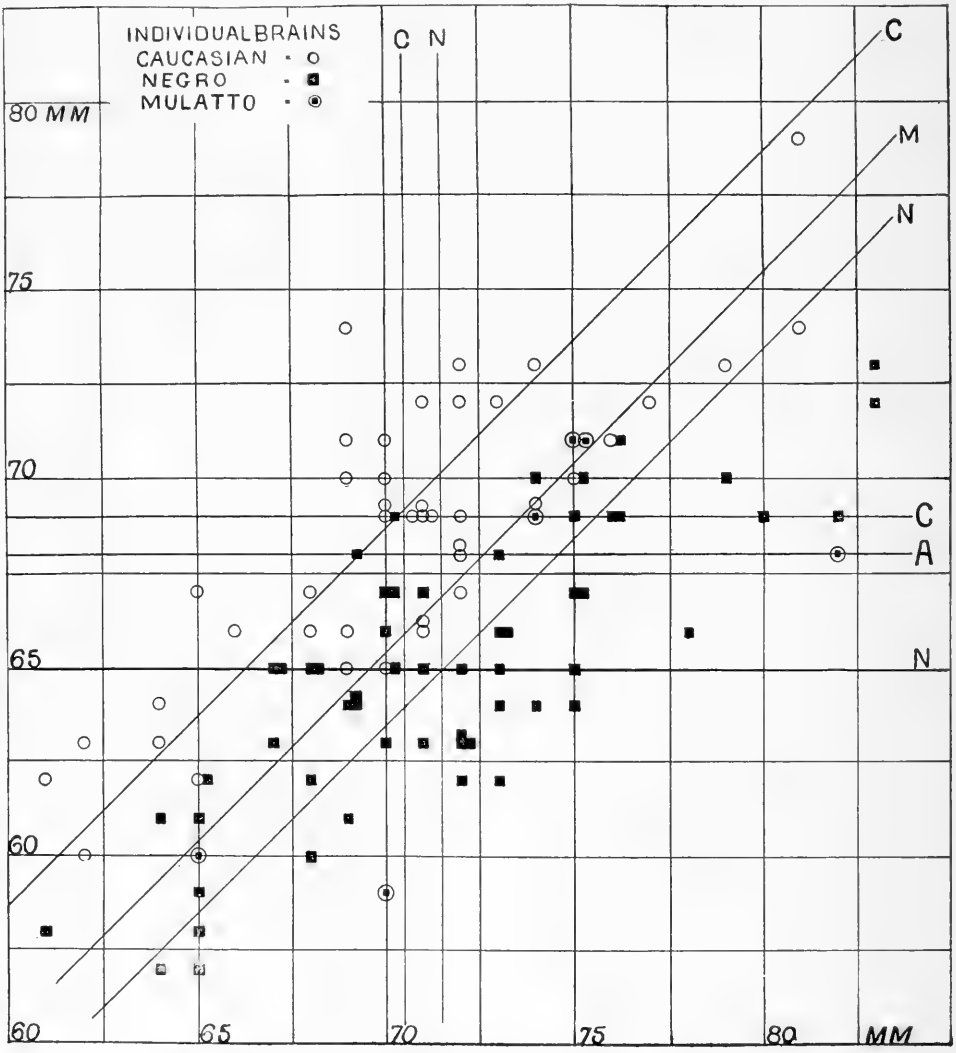


CHART II. *Left Side.*—Relation of the radii of the anterior association center (ordinates) to those of the posterior association center (abscissæ). See Chart I, Legend.

A system of means is adopted for the charts. Extremes are avoided in this way, and a medium for comparison is obtained which is fairer

and more readily visualized on the charts than would be the case with averages or curves. Horizontal lines are drawn on the charts to represent the means of the radii of the anterior association centers (ordinates), vertical lines are drawn to represent the means of the radii of the posterior association centers (abscissæ), and lines are drawn at 45° from these to represent their combined means. In Chart I the Caucasian ordinate mean is 69.5, the Negro ordinate mean is 65.5, *i. e.*, the Caucasian brains have a mean radius to the right anterior association center of 69.5 mm., while the mean radius to this center in the Negro brains is only 65 mm long. The Caucasian and Negro abscissa means are the same, 72.5 mm., therefore the mean radius to the right posterior association center is the same in the two races. A comparison of the means of the two sides taken from Charts I and II is found in the following table:

TABLE IIc.

MEANS OF THE ASSOCIATION CENTERS.

	Left Side.			Right Side.		
	Anterior Ordinate	Posterior Abscissa	Difference of the combined means.	Anterior Ordinate	Posterior Abscissa	Difference of the combined means.
Caucasian ...	69	71.5	71.25 — 70 = 1.25	69.5	72.5	72.5 — 70 = 2.5
Negro	65	72.5	76.5 — 70 = 6.50	65.5	72.5	77 — 70 = 7.0

The ordinate means are slightly larger on the right side than on the left side in the two races, hence the mean anterior association center is larger on the right than on the left. It is demonstrated (Table II[a]) that the averages of the anterior association centers are slightly larger on the right side than on the left in the females of the two races, but the relative difference is in favor of the left side in both male and female. This is evident from the index of the association centers (Table II [a]) and from the differences of the combined means (Table II [c]). The differences of the two sides are slight and may be negligible in the means and the averages. On the other hand the extremes (Table II [b]) present a marked racial difference in relation to the two sides of the brain, the left anterior association center being large in the Negro extremes and small in the Caucasian extremes. The conclusion is that the extremes affect both the means and the averages, explaining the apparent contradiction in each. The abscissa means are the same for all, except on the left side of the Caucasian which is 1 mm. less than the others. This indicates a smaller posterior association center on the left side of the Caucasian. The differences of the com-

bined means for the two races (45° lines) are obtained by subtracting the ordinate 70 mm. from the abscissa of the point at which the 45° line crosses this ordinate. The numbers obtained are purely arbitrary, but afford a basis of comparison for the two sides and the two races. The smaller the number the larger the anterior association center in relation to the posterior association center, and the larger the number the larger the posterior association center in relation to the anterior association center. On comparing this with the index of the association centers (Table II [a]) it will be found that the deductions are the same from each, *i. e.,* the anterior association center is larger relatively to the posterior association center in the Caucasian than in the Negro, and larger on the left side in each than on the right side, although the latter difference is slight. Or the converse of this proposition may be taken. The posterior association center is relatively larger in the Negro than in the Caucasian and larger on the right side than on the left. A line on the charts at 45° representing the mean of all brains separates the races in much the same way as the arbitrary line before described. A table presents this figuratively:

TABLE II[d].

EXTREMES OF THE COMBINED MEANS OF THE ASSOCIATION CENTERS.

Symbols.	Left Side.		Right Side.	
	Above the line.	Below the line.	Above the line.	Below the line.
Caucasian	32	10	33	3
Negro	18	45	14	48

In this table, as in others, a more marked racial difference is found on the right side than on the left; fewer brains being over the line on the right side. It is interesting to find all of the perfect adult male mulattoes in white territory on the charts, each one being near the line representing the mean of all brains. Examination of the charts will reveal the fact that all the symbols range along this line or in the direction of it from left to right, and from below upwards as the size of the brains are shown to be larger, the Negro symbols being below and to the right of the line, while the Caucasian symbols are above and to the left, except those represented in heavy type in the above table as the "Extremes of the Combined Means of the Association Centers."

To summarize:

An attempt is made to demonstrate that the anterior association center is relatively smaller in the Negro brain than in the Caucasian; that the

left anterior association center of Negro brains resembling the Caucasian brain in shape is larger than the right, while the left anterior association center of Caucasian brains resembling the Negro brain in shape is smaller than the right, although this difference may be in the gyrus frontalis

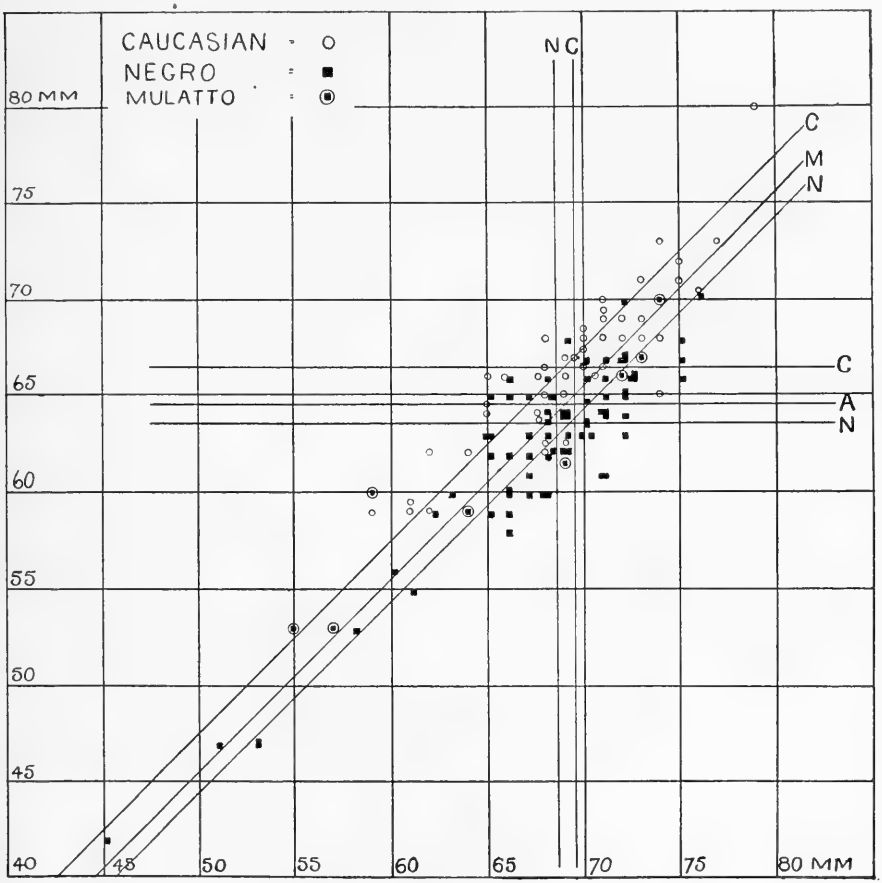


CHART III. *Right Hemisphere.*—Relation of the average length of radii at 60° in the sagittal plane (0°, Fig. 2a), in the horizontal plane (90°, Fig. 1a), and in the plane with the brain tilted at 45° (Fig. 3a); to the average of the radi at 120° in the same plane. The average is obtained by adding the length of the radii in these three positions and dividing by three.

inferior or the motor area, instead of in the anterior association center; and an attempt is also made to point out minor racial differences in individual brains. To accomplish this, outline drawings of individual

brains in various positions are presented; composites are constructed based upon actual measurements; a table of actual measurements is compiled from which an index of the association centers is worked out; and charts and tables are produced to determine the averages, the means, the

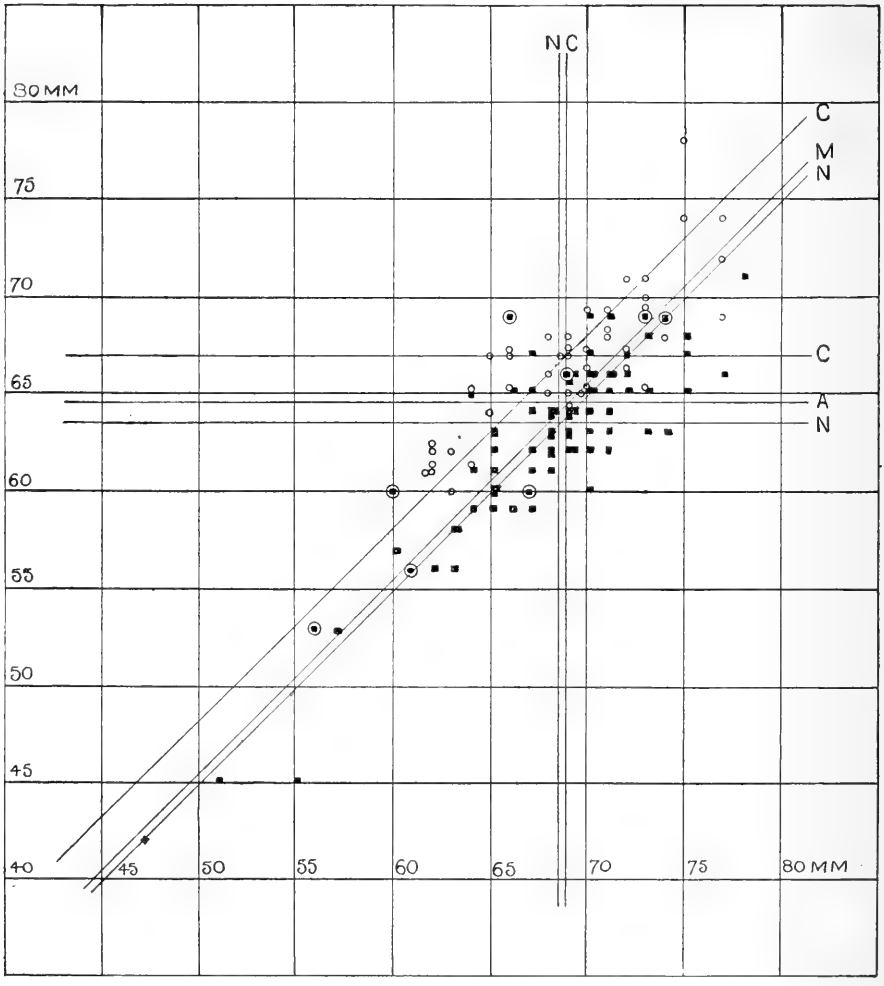


CHART IV. *Left Hemisphere.*—See legends of Charts III and I.

extremes, and the extremes of the combined means, of the association centers.

Not only is the anterior association center smaller in the Negro than in the Caucasian, but the whole frontal lobe of the Negro is smaller, as

may be determined by examining Charts III and IV, constructed from the numbers in Table III, and also from the position of the fissure of Rolando, to be discussed further on, and the areas of the brain outlines anterior to this fissure. The numbers in Table III are obtained by measuring the radii at 60° and 120° of the three outlines of the horizontal, vertical and 45° planes intersecting the brain axis, the numbers representing the average length of these three radii in each instance. Charts III and IV are constructed in a manner similar to that described for Charts I and II, and they are treated throughout in the same way. The *arbitrary line* is found to separate the races similarly, but it passes through the 64.5 mm. ordinate instead of through the 68 mm., which means that the average length of the 45° radii to the frontal lobes for the three planes is less than the average length of the radii to the anterior association center. The arbitrary line is an approximate compound ordinate mean in this table as well as in Table II^{b}, representing the ordinate mean for all brains on Charts I and II, and III and IV respectively.

A table showing the comparison of the frontal lobes in the two races is as follows:

TABLE III^{a}.

EXTREMES OF THE FRONTAL LOBES.

Symbols.	Left Side.		Right Side.	
	Above the arbitrary line.	Below the arbitrary line.	Above the arbitrary line.	Below the arbitrary line.
Caucasian	34	10	32	12
Negro	30	52	29	54

This presents the fact that there is a greater number of large frontal lobes among the Caucasian brains (66 large, 22 small), and a greater number of small frontal lobes among the Negro brains (106 small, 59 large) the relations being nearly proportional, and practically the same on the two sides of the brain in each race. The difference between the two sides found in Tables II^{a} and II^{b} evidently lies about a point on the 45° plane where the 60° radius intersects the outline of this plane. This point lies over the anterior association center on the right side, and over the gyrus frontalis inferior on the left side. From Table II^{a} it is determined that the average for this side is relatively greater on the left side in each race. From Table II^{c} it is determined that the mean for this point is relatively greater on the left side in each race. From Table II^{b} it is determined that the extreme for this point is greater in the Negro brain and less in the Caucasian. We may conclude that in general the gyrus frontalis inferior is well developed in the two races, causing the

left side to be more prominent at this point, but extreme Negro brains that approach the Caucasian brain in type have a larger gyrus frontalis inferior and extreme Caucasian brains that approach the Negro brain in type have a smaller gyrus frontalis inferior. Of course this difference may be due to the anterior association center or to the motor area, increase in the size of either causing the gyrus frontalis inferior to bulge.

It is interesting to note in this connection relatively to the arbitrary line in Charts III and IV, that all the adult male mulattoes (3) are above the line on each side, while all the female mulattoes (4) are below, except on the left side. Only three (of 26) female Negroes are above the line on the left side, and five on the right side, and all of these are close to the line. Only four (of 35) male Caucasians are below the line on the right side, and three on the left side, and these are all near the line. This indicates a divergence in the males of the two races and a convergence in the females. Evidence of the same relation is obtained from Table II[a] in the index of the association centers, the Caucasian male being 98-97; the Negro male, 90-89; the Caucasian female, 96-97; and the Negro female, 91-91, for the left and right sides, respectively. This fulfills the biological law that the females are more homogeneous, the males more heterogeneous, the latter being more apt to vary from the type, or to be extreme.

A slight difference from that found in the association centers is found in the frontal and parietal lobes of the brain in relation to the means. A table is given for comparison, which is derived from Charts III and IV in the same way that Table II[c] is derived from Charts I and II.

TABLE IIIb.

Means of the Frontal and Parietal Lobes.

Symbols.	Left Side.			Right Side.		
	Frontal Ordinate	Parietal Abscissa	Difference of the combined means.	Frontal Ordinate	Parietal Abscissa	Difference of the combined means.
Caucasian	67	69	72 — 70 = 2	66.5	69.5	72.5 — 70 = 2.5
Negro	63.5	68.5	75 — 70 = 5	63.5	68.5	75.5 — 70 = 5.5

On comparing this table with Table II[c], it is found that the differences are similar, but not so great. The inferences are that the frontal lobes are smaller in the Negro than in the Caucasian, but practically the same size on the two sides in each race; that the parietal lobe is slightly larger in the Caucasian than in the Negro, but practically the same size on the

two sides in each race; and that the left frontal lobe is relatively larger than the right in each race, this difference being very slight.

The extremes of the combined means of the two lobes may be represented in a table prepared in the same way as Table II[d], and with like results, except that the differences are not so marked in this table as in Table II[d].

TABLE III[c].

EXTREMES OF THE COMBINED MEANS OF THE FRONTAL AND PARIETAL LOBES.

Symbols	Left Side.		Right Side.	
	Above the line.	Below the line.	Above the line.	Below the line.
Caucasian	35	9	36	9
Negro	26	55	25	56

A greater racial difference exists on the right side than on the left side, *i. e.*, more Negro brains have a relatively large frontal lobe, and a relatively small parietal lobe on the left side than on the right side; and more Caucasian brains have a relatively small frontal lobe and a relatively large parietal lobe on the left side than on the right side, although this difference is manifested in two Negro brains and one Caucasian brain only. The racial separation of the races by the 45° line representing the mean for all brains is presented in this table by the fifty-five Negro symbols below the line and the thirty-five Caucasian symbols above the line, on the left side, and by the fifty-six Negro symbols below the line, and the thirty-six Caucasian symbols above the line, on the right side.

It is evident that the frontal lobe of the Negro brain is smaller than the frontal lobe of the Caucasian brain, as demonstrated in Charts III and IV, and Tables III[a], III[b] and III[c]. This racial difference has been recognized by anatomists heretofore, but in only a few individual instances has it been emphasized.[1]

Even Tiedemann [65 66] that eminent continental champion of the Negro, although recognizing few differences between the brains of the Negro and the European, does admit that the frontal lobes of the Negro brain are smaller than those of the European. This difference is not so great, however, as the difference demonstrated between the anterior association centers of the two races, as represented in outlines, tables, and charts.

Flechsig,[2] in his masterly work on the development of the fiber tracts and cortical areas as represented by myelinization, throws some light on the connections of the great association areas, and on their probable func-

[1] Reference Nos. 1, 2, 3, 8, 10, 17, 20, 23, 24, 32, 33, 35, 36, 39, 52, 59, 62, 65, 66, 68, 79, 82.

[2] 1, 18, 19, 56.

tion. The cortex may be divided into three grand areas representing the sequence in development. First the primary sensory areas develop, representing the area for smell in the lamina perforata anterior and extending through the septum pellucidum and the fornix to the uncus and cornu ammonis; the area for touch and muscle sense, and the motor area, in the gyrus centralis posterior and anterior, and the gyrus frontalis superior, the sequence for the types of fibers for this area being sensory, motor, callosal, horizontal and arcuate, and association bands; the area for sight around the fissura calcarina, the gyrus descendens and the occipital pole; the area for taste possibly just posterior to the splenium and connected with the subiculum cornu ammonis; and the area for hearing in the gyrus temporalis superior. Next there develop several centers of unknown meaning in the cuneus, the anterior extremity of the temporal lobe, the posterior extremity of the gyrus frontalis inferior, the gyrus subangularis and suprangularis, their positions being near the primary sense areas but not touching them.

All the areas so far mentioned develop before birth, except the gyrus superangularis, while the remaining areas develop after birth. They make up the third grand division composed of the three association centers, anterior, posterior and temporal, and include the border zones to the areas already developed, these having short fibers, and the terminal or central zones of the association centers with long fibers. The central zones are the last to develop. The anterior association center is in close relation to the areas representing the body, and in slight relation to the olfactory area, while the others are in close relation to the areas of special sense. In his earlier works Flechsig [18] determined that lesions of the anterior association center caused alteration, or loss, of ideas regarding personality, the ego, the relations of self subjectively and objectively; a diminution in capacity for ethical and æsthetic judgment; a loss of self-control, of the powers of inhibition, of will power; and in fact all the symptoms which Bianchi observed on higher apes in which the fore brain on both sides had been extirpated. In simple lesions or in the early stages of the lesion, when the person is "subjected to unaccustomed stimuli, especially to sexual excitement, anger, or vexation, he may lose all control of his movements and acts, so that simple influence may lead him to try to satisfy his desires without any regard to custom or good taste. In later stages of the disease imbecility may appear, with entire loss of the mental pictures regarding his personality" (Barker [1]). The individual may distort his own personality, and be unable to distinguish the imagined from the real; thus he may think himself of enormous dignity, of great importance, or that he is possessed of great wealth, or that

he is a genius. Lesions of the posterior association center do not present so clear a picture, and naturally so because of its more intimate connection with the special senses. It is generally understood that the posterior association center is objective, while the anterior is subjective, the one representing the powers of conception in the concrete, the other, the powers of thought in the abstract. The relative differences found in the association centers of the two races is suggestive in relation to the known characteristics of the two, in view of Flechsig's work. The Caucasian is subjective, the Negro objective. The Caucasian—more particularly the Anglo-Saxon, which was derived from the Primitives of Europe, is dominant and domineering, and possessed primarily with determination, will power, self-control, self-government, and all the attributes of the subjective self, with a high development of the ethical and æsthetic faculties. The Negro is in direct contrast by reason of a certain lack of these powers, and a great development of the objective qualities. The Negro is primarily affectionate, immensely emotional, then sensual and under stimulation passionate. There is love of ostentation, of outward show, of approbation; there is love of music, and capacity for melodious articulation; there is undeveloped artistic power and taste—Negroes make good artisans, handicraftsmen—and there is instability of character incident to lack of self-control, especially in connection with the sexual relation; and there is lack of *orientation,* or recognition of position and condition of self and environment, evidenced by a peculiar bumptiousness, so called, that is particularly noticeable. One would naturally expect some such character for the Negro, because the whole posterior part of the brain is large, and the whole anterior portion small, this being especially true in regard to the anterior and posterior association centers. Flechsig's work favors the conclusion that the gyrus rectus may have a definite relation to smell, and the gyrus frontalis superior to muscle, and as both of these gyri are well developed in the Negro, and the motor area and Broca's convolution also being large, the presumption is that the anterior association center is exceedingly small in the Negro. The findings in regard to the relative size of the anterior and posterior portions of the Negro brain correspond to those of Broca[7] on the Negro cranium. His conclusions are as follows:

1. That the face of the Negro occupies the greater portion of the total length of the head.

2. That his anterior cranium is less developed than his posterior, relatively to that of the white.

3. That his occipital foramen is situated more backwards in relation to the total projection of the head, but more forward in relation to the

cranium only. Topinard [68] corroborates these statements, and concludes that the Negro has the cerebral cranium less developed than the white, but its posterior portion is more developed than the anterior. It falls within the occipital races of Gratiolet [20 21] and the Caucasian in his frontal races. Barnard Davis [13 14] demonstrated practically the same in relation to the radii from the external auditory meatus to the three regions of the skull, frontal, parietal and occipital. The white and the black races are evidently opposites in cardinal points. The one is subjective, the other objective; the one frontal, the other occipital or parietal; the one a great reasoner, the other emotional; the one domineering, but having great self-control, the other meek and submissive, but violent and lacking self-control, especially when the passions are aroused, or any sudden danger appears; the one a greyhound, the other a bulldog.

Spitzka [63] emphasizes the differences of the two parts of the brain, anterior and posterior, in comparing the brains of Prof. Joseph Leidy, Maj. J. W. Powell and Prof. Cope, by contrasting the characteristics of these eminent men, and in so doing corroborates Flechsig's work and lends plausibility to the generalizations given above.

Wagner [68 72 73] gives some interesting figures in relation to the relative size of the various lobes in man and the ourang-outang which may be appropriately presented here.

	Man.	Ourang.
Frontal lobes	43.6	36.8
Parietal and Occipital lobes	34.6	43.6

The Negro evidently stands in an intermediate position in this relation, which becomes more evident when the areas anterior and posterior to the fissure of Rolando are considered.

Sulcus Centralis. Fissure of Rolando.

The racial difference found in the lobes of the brain and in the association centers is also observable in the position of the sulcus centralis and the relation of the amount of brain matter anterior and posterior to it. The position of the fissure is practically the same in the two races in relation to the brain axis and the brain center, but the amount of brain matter anterior to the fissure is less in the Negro, while the amount posterior to it is more than is to be found in the Caucasian. The inferior end, central part, and superior end of the fissure of Rolando are located on the brain outlines of sixty-three brains, in degrees from the anterior end of the brain axis, as in other measurements, with the radii extending from the brain center. The superior terminal point of the fissure is also lo-

cated by direct measurement from the brain center on the horizontal planes. Table IV shows the individual measurements taken in this manner. Table IV[a] presents the averages.

TABLE IV[a].

AVERAGE POSITION OF THE FISSURE OF ROLANDO.

	No. of Brains.	Left Side.			Right Side.		
		Inferior.	Middle.	Superior.	Inferior.	Middle.	Superior.
Caucasian male	22	73°	88°	107°	77°	88°	106°
Negro male	27	76	88	108	77	87	108
Caucasian female	3	68	83	107	74	82	107
Negro female	11	80	90	111	81	86	112

A difference of 1° may be allowed for the personal equation in these measurements, and the female Caucasian measurements may be eliminated in the discussion, because only three brains of this kind were measured. The male Caucasian and the male Negro fissure of Rolando have practically identical relative positions, while the female Negro fissure is located nearer the posterior end of the brain than is that of the male in either race. This would seem to indicate that more of the brain lies anterior to the fissure of Rolando in the female Negro than in the males, but by actual measurements of the parts there is less (Table V[a]). This apparent discrepancy is due to the fact that the frontal lobes of the female Negro are comparatively longer, but narrower transversely, and from above downward, than those of the males of the two races. The areas of individual brain outlines are found in Table V, and the averages for these are in the following table.

TABLE V[a].

AVERAGES OF AREAS OF THE BRAIN OUTLINES IN RELATION TO THE FISSURE OF ROLANDO. AREAS IN SQUARE CENTIMETERS.

	No. of Brains.	Left Side.		Right Side.	
		Anterior.	Posterior.	Anterior.	Posterior.
Caucasian male	22	146.2	140.5	148.3	139.9
Negro male	22	146.6	145.0	146.7	145.7
Caucasian female	3	118.1	116.6	119.1	115.7
Negro female	10	125.4	122.3	125.1	123.9

To compile these tables the three outlines, such as are taken for each hemisphere shown in Figures 1[a] to 3[b], the sulcus centralis was located on each of the three outlines, radii were projected on the horizontal plane to the inferior end of the fissure, on the vertical, or mesial plane, to the

superior end of the fissure, and on the 45° plane to the middle part of the fissure, and lines were drawn from the brain center to the inferior surface of the occipital and frontal lobes, striking them tangentially. These lines are taken as limits of the outlines, because no lines are shown in the drawings. The radius to the sulcus centralis is taken as the dividing line between the anterior and posterior parts of each outline. The temporal lobe is not included in the drawings. The area of each hemisphere, in three planes, both anterior and posterior to the sulcus centralis is determined by means of the planimeter. The results are found in Table V. These results are averaged, the averages for the anterior part of each outline being added to one another, the same being done for the posterior part, and the sums placed together for comparison in Table V[a].

The anterior part of the Negro brain outline is the same size as the anterior part of the Caucasian brain on the left side; the anterior part of the Caucasian brain is larger than the anterior part of the Negro brain on the right side; while the posterior part of the Negro brain is larger than the posterior part of the Caucasian brain on each side. In the right hemisphere the racial distinction is considerable; in the left it is not so great. The similarity of the two races in the apparent size of the frontal lobes on the left side may be due to the greater size of the left motor area and of the left gyrus frontalis inferior in the male negro, as heretofore pointed out. The areas of the female Caucasian brain need not be considered, because only three are given. The areas of the female Negro brains are less than the areas of the males in either race and the racial distinctions are relatively the same as in the male Negro. The distinctions throughout may be expressed in ratios of the anterior to the posterior parts of the brain representing the posterior part by 100 in each case (Table V[b]).

TABLE V[b].

RATIO OF THE ANTERIOR TO THE POSTERIOR PARTS OF THE BRAIN.

	Left Side.	Right Side.
Caucasian male	104 : 100	106 : 100
Negro male	101 : 100	100+ : 100
Caucasian female	101 : 100	103 : 100
Negro female	102 : 100	101 : 100

This table brings into clearer view the differences mentioned above. The frontal lobe of the male Caucasian is relatively larger than that of the Negro, and the right frontal lobe is both relatively and absolutely larger than the left. The right frontal lobe of the female Negro is rela-

tively smaller than the Caucasian, and the left is relatively and absolutely larger than the right. The female Caucasian is similar to the male Caucasian and the male Negro is similar to the female Negro, but in a less degree. It might have been supposed that the fissure of Rolando is further posterior in the Negro brain than in the Caucasian, and that the small size of the frontal lobe in the Negro is an apparent and not a real deficiency of brain matter, but the above measurements indicate that the frontal lobe and all the brain matter anterior to the fissure of Rolando is less in the Negro than in the Caucasian. As the gyrus rectus is apparently larger in the Negro than in the Caucasian, and the gyrus frontalis inferior is larger in the Negro than in the Caucasian, and as the frontal lobes in the Negro appear larger than they really are, owing to the projection downward of the convolution just mentioned, as well as to the projection upward of the superior orbital plates and the gyrus frontalis superior, if it be true that the motor area and the left gyrus frontalis inferior are larger in the Negro, then it must be true that the anterior association center is considerably smaller in his case than in the Caucasian, because even the apparent size of the whole frontal lobe is smaller in the Negro. That the anterior association center is smaller in the Negro seems plausible when the corpus callosum is examined, in which the racial distinction is more pronounced than in the brain outlines, the anterior end (genu) being distinctly smaller in the Negro.

Corpus Callosum.

The cross section area of the corpus callosum is measured with the planimeter from outlines made directly on glass, and from other outlines made on paper by projection. These areas are given in Table I, with the brain weights taken at the time the outlines were drawn. Measurements made from Retzius [55] photographs and drawings of brains by others are given in Table I', with brain weights, when possible, for comparison. Chart V is made up from these two tables, the brain weights (abscissæ) being given in grams, and the areas of the corpora callosa (ordinates) in square centimeters. There is in general an increase in area of the corpus callosum with each increment of brain weight. There are, however, many individual exceptions. For instance, one Caucasian brain weighing about 1100 grams has a corpus callosum of about 8 square centimeters area, while another brain weighing about 1400 grams has a corpus callosum of about 6 square centimeters area. These are extreme instances,

but there are other similar ones. Spitzka has measured the cross section area of the corpus callosum in the brains of ten eminent men, and he finds the average area higher than in ordinary men. Their average brain weight was also greater than in ordinary men. The weight of Prof. Joseph Leidy's brain was estimated to be 1545 grams or possibly more, and the corpus callosum measured 10.6 sq. cm. in sectional area. The symbol representing this brain may be found in Chart V and its unusual

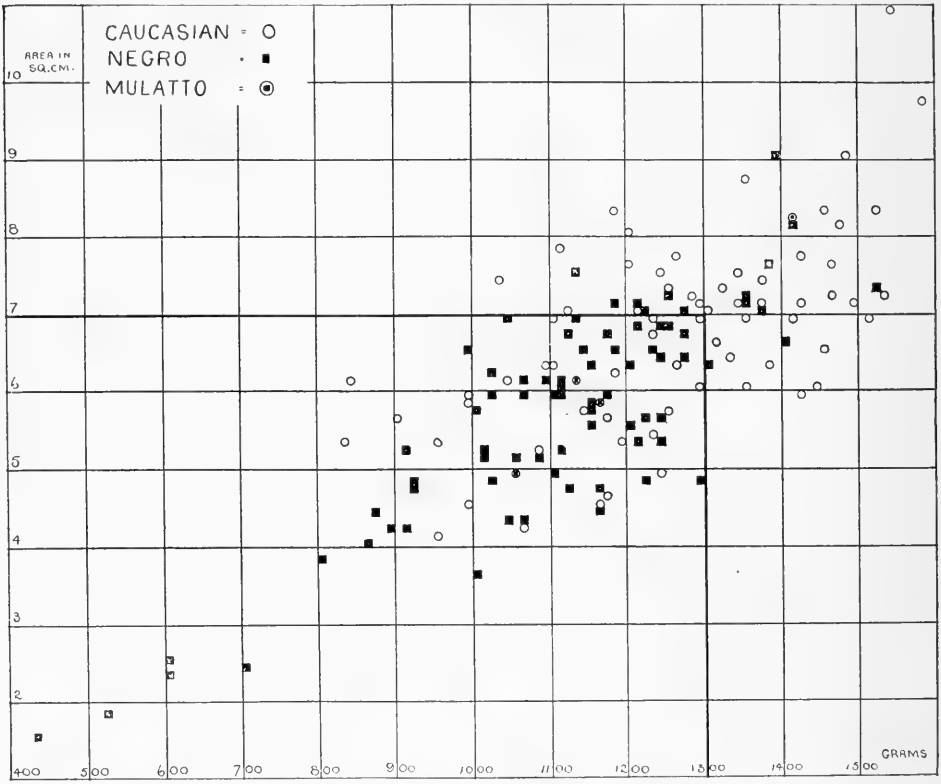


CHART V.—Relation of the area of the cross section of the corpus callosum (ordinates) to brain weight (abscissæ). The heavy black lines enclose the majority of the Negro symbols and exclude the majority of the Caucasian.

position attracts immediate attention. This may be an exception to the rule that the cross section area of the corpus callosum varies directly with brain weight and at a proportionate rate, and exceptional size of the corpus callosum may mean exceptional intellectual activity. One of the Negro brains, however, had a corpus callosum with a cross section area of 9.1 square centimeters, which is nearly 2 square centimeters

above the average Spitzka gives for the ten eminent men, and there is no reason to believe that this Negro had greater mental powers than any one of those eminent men, although he may have been an obscure genius. One Caucasian male brain in my series had a corpus callosum of 9.1 square centimeters cross section area, and eight other brains, six Caucasian male, one mulatto male, and one Negro male had areas between 8 and 9 square centimeters, and there is nothing to indicate that these brains were from exceptional men, although they may have been. The brain of a laboring man pictured by Retzius had a corpus callosum which measured 9.8 square centimeters in area. The brain weight was 1587 grams. The brains in my series with large callosa are invariably large. Of the ten brains mentioned above with large callosa each one weighed about 1500 grams (Table I). The racial distinction in the relation of brain weight to the area of the corpus callosum is not marked, but it is noticeable. To show this, lines are drawn on Chart V through the 7 square centimeter ordinate and through the 1300 gram abscissa, these lines being extended in a horizontal and in a vertical direction respectively, until they intersect. One-third of the brains represented below the horizontal line and to the left of the vertical line are Caucasian, and two-thirds are Negro. Two-thirds of the brains represented above the horizontal line and to the right of the vertical line are Caucasian and one-third are Negro. A majority of the Negro brains are thus represented within the lines and a majority of the Caucasian brains are represented without the lines. It is a noteworthy fact that about half of the Caucasian brains represented within the lines are from women, or from the inmates of Bay View Pauper Asylum, a great many of whom are known to have had dementia—alcoholic, syphilitic, or senile. With them the brains of such noted men as Gyldens[55] (No. 23), Siljeström[55] (No. 25), a statesman[55] (No. 26), and Prof. Leidy[63] (No. 27), are found.

These men each had a large brain, or a large callosum, or both. Thirteen Negro brains are found without the lines having a corpus callosum of more than 7 square centimeters area, and only eight have a brain weight of more than 1300 grams. These invariably give evidence of Caucasian characteristics. To be found outside of the lines are a mulatto; a Negro who had been instrumental in at least three, and possibly five, murders; a Negro accomplice of the latter; a Negro laborer from North Carolina; a Negro killed in a railroad wreck; and another the victim of a third-rail accident. The racial difference is really more marked than is apparent in the chart (V) because the class of Negroes from which bodies are obtained is comparatively better than the class from which Caucasian bodies are obtained, this being especially marked in the females of the two races.

In dealing with the corpus callosum as a whole, it is found to be smaller in the Negro than in the Caucasian, just as the brain of the Negro is smaller than that of the Caucasian, and in about the same degree. The averages of brain weights and areas of the corpora callosa reveal interesting racial and sexual differences. They are given in Table I[a], with ratios made up from Table I.

TABLE I[a].

THE RELATION OF THE AREA OF THE CORPUS CALLOSUM TO BRAIN WEIGHT. AVERAGES AND RATIOS.

	No. of Brain.	Area of the Corpus Callosum. sq. cm.	Brain Weight. gm.	Ratio.
Caucasian male	54	7.02	1302	54
Negro male	50	6.27	1208	52
Caucasian female	14	6.40	1087	59
Negro female	26	5.68	1064	53

The average brain weight is greatest in the Caucasian male, least in the Negro female, and intermediate in the Negro male and the Caucasian female. The average cross section area of the corpus callosum is relatively the same, with the Negro male and the Caucasian female transposed in relation to each other. The ratio of area to weight is greatest in the Caucasian female, least in the Negro male, with the Negro female and the Caucasian male respectively a little higher than the Negro male; but the ratio of the Caucasian female is hardly a fair one, because so few brains of this kind are examined, and they are from such varied sources, and with so many methods of preservation. The relation of the anterior and posterior lineal halves of the corpus callosum exhibits a greater racial difference. This is perceived by a glance at Chart VI, compiled from Table VI in a manner similar to that of the charts previously presented. The corpus callosum is divided into halves of equal length by a line perpendicular to the brain axis, at a point intermediate between two lines perpendicular to the brain axis, dropped from each end of the corpus callosum. It is hardly necessary to do more than point out the racial difference indicated in the chart, because it is so plain, even to a casual observer. There is not an absolute separation of the races, but there is a decided difference. In general, as the area of one end of the corpus callosum increases, the other increases also, but the increase in area of the anterior end is greater in the Caucasian than in the Negro, while the increase in the area of the posterior end is greater in the Negro than in the

Caucasian. The relative difference is noticed throughout. The anterior end is relatively larger in the Caucasian, the posterior end is relatively

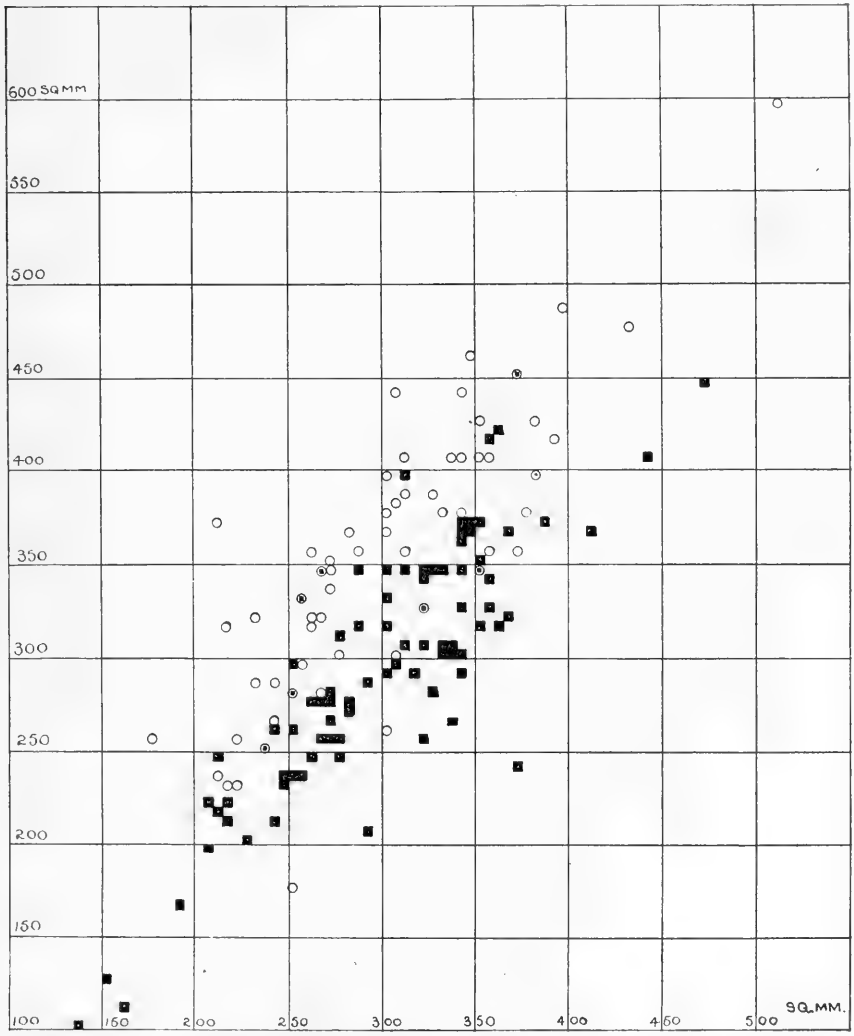


CHART VI.—Relation of the anterior lineal half of the corpus callosum (ordinates) to the posterior lineal half (abscissæ). The races are separated.

larger in the Negro. This may be expressed in averages in a table made up from Table VI.

TABLE VIa.

Relation of the Averages of the Areas of the Anterior to the Posterior Lineal Half of the Corpus Callosum.

	No. of Brains.	Anterior. sq. cm.	Posterior. sq. cm.	Ratio.
Caucasian male	42	3.70	3.04	122 : 100
Negro male	62	3.06	3.02	101 : 100
Caucasian female	9	3.17	2.87	110 : 100
Negro female	25	2.86	2.86	100 : 100

Each end of the corpus callosum is larger in the Caucasian male than in the Negro male or in the others. Likewise the Caucasian female is larger than the Negro female, the anterior end is larger than the Negro male, the posterior end being smaller than the Negro male and about the same size as the Negro female. The anterior end of the corpus callosum is small in the Negro male, and smaller in the Negro female. It is large in the Caucasian female and larger in the Caucasian male. The posterior end is about the same size in each sex, but smaller in the female than in the male, so that the anterior end shows a racial and sexual difference, while the posterior end shows a sexual difference only. This can be located more definitely than in the two lineal halves of the corpus callosum. Comparing the genu and the splenium, leaving aside the intermediate portion of the corpus callosum, a distinct racial difference is found similar to that just discussed. Chart VII taken from Table VII gives a graphic picture of the essential differences, which are about the same as those found in Chart VI. To prepare this chart, the corpus callosum is divided into four parts, six-tenths (.6) anteriorly being separated from four-tenths (.4) posteriorly, and each of these two parts being divided in half. This is done by using lines perpendicular to the brain axis, and parallel to lines used in preparing for measurements for Table VI. This gives the splenium two-tenths of the total lineal length of the corpus callosum anterior to the splenium a narrow part, which I call the isthmus, two-tenths of the total length; anterior to this the body, three-tenths of the total length. These divisions are shown in Figures 9a and 9b. Several brains are broken through the fissure of Rolando and the break invariably passes through the isthmus. The conclusion is that the body of the corpus callosum contains the fibers connecting the motor areas of the two hemispheres, and the isthmus and splenium contain the fibers connecting the sensory areas of the two hemispheres, and all areas posterior to these. Eliminating the isthmus and body must leave the fibers that more definitely connect the association centers and

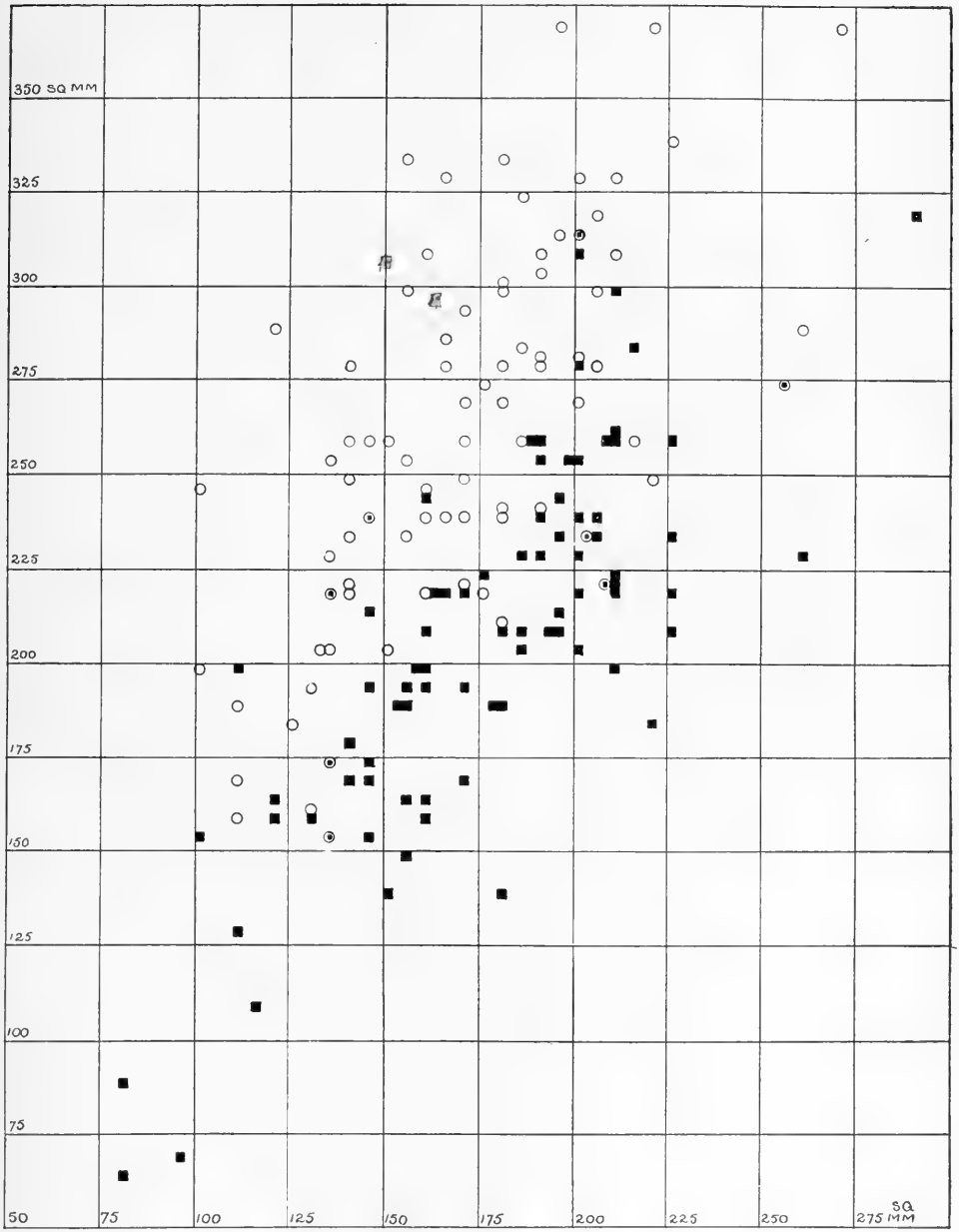


CHART VII.—Relation of the area of the genu (ordinates) to the area of the Splenium (abscissæ). The races are further separated.

to special sense centers in the two hemispheres. Flechsig [19] indicates that part of the centers for smell may lie in the gyri recti, which are larger in the Negro than in the Caucasian. The latter is evidently true from what we know about the sense of smell in the Negro, and the size of the olfactory apparatus in this race. If the fibers connecting the frontal lobes anterior to the motor area are contained in the genu, and a greater number of the fibers in the genu connect the olfactory lobes in the Negro than in the Caucasian, then the genu of the Negro should be larger. But it is really smaller. Consequently the fibers connecting the anterior association centers must be less in the Negro than is indicated by the size of the genu. Comparing the areas of the genu and splenium must give an approximate comparison of the anterior and posterior association centers. They are compared in the two races in Chart VII, made up from Table VII. A more definite racial difference is seen in this chart than in Charts I and II where the association centers are contrasted from brain outlines. A glance at Chart VII convinces that the genu is relatively and absolutely larger in the Caucasian than in the Negro. This may also be expressed in a table of averages taken from Table VII.

TABLE VII[a].

The Relation of the Averages of the Areas of the Genu and Splenium, Etc., in sq. cm.

	Number of Brains.	Genu.	Body.	Isthmus.	Splenium.	Ratio Genu to Splenium.	Ratio Body to Isthmus.
Caucasian male	57	2.72	1.49	.94	1.72	158 : 100	160 : 100
Negro male	60	2.12	1.33	.81	1.76	120 : 100	164 : 100
Caucasian female	17	2.41	1.36	.88	1.61	150 : 100	155 : 100
Negro female	25	1.98	1.27	.79	1.73	115 : 100	160 : 100

The genu is absolutely and relatively largest in the Caucasian male, absolutely and relatively smaller in the Caucasian female, absolutely and relatively smaller still in the Negro male, and absolutely and relatively smallest in the Negro female. The relations of the splenium are the converse of this. The relation of the isthmus to the body is similar, but with less marked racial difference. Compare the relation of the ratios of the genu to the splenium in the males of the two races (158 : 120 = 131), with the relation of the ratios of the body to the isthmus (160 : 164 = 97), and a greater racial difference is evident in the former (131) than in the latter (97). This difference is also evident in the females of the two races (150 : 115 = 130. 155 : 160 = 97). The relation of the ratios of the two-lineal halves of the corpus callosum (Table VI[a]) is 122 : 102 = 119 in the males of the two races, and 110 : 100 = 110 in

the females of the two races. Compare these results with the results obtained above and there appears a greater racial difference in the relation of the genu to the splenium than in the relation of the body to the isthmus, or in the relation of the anterior to the posterior lineal halves of the corpus callosum. This may be expressed in a table.

TABLE VIIb.

THE RELATION OF THE RATIOS OF THE PARTS OF THE CORPUS CALLOSUM.

	Genu to Splenium.			Body to Isthmus.			Lineal Halves.		
Negro and Caucasian.	Caucasian Ratio.	Negro Ratio.	Relation of Ratio.	Caucasian. Ratio.	Negro. Ratio.	Relation of Ratio.	Caucasian Ratio.	Negro Ratio.	Relation of Ratio.
Males	158	120	131	160	164	97	122	102	119
Females	150	115	130	155	160	97	110	100	110

The racial difference is greater in the "relation of the ratios" of the genu and the splenium (130) than it is in the "relation of the ratios" of the body and the isthmus (97), or of the lineal halves (119, 110). The sexual difference is slight in the relation of the ratios of the genu to the splenium (131 : 130 = 101); it is more marked in the "relation of the ratios" of the anterior lineal half to the posterior lineal half (119 : 110 = 108); and it is least marked in the "relation of the ratios" of the body to the isthmus (97 : 97 = 100). In other words the above table may be interpreted as follows: The genu of the Caucasian female is larger in proportion to the size of the splenium than it is in the Negro female, and this difference is greater than the racial difference in the females in the proportion of the body to the isthmus, or the anterior lineal half to the posterior lineal half of the corpus callosum, the same difference being noticed in the relative sizes of these, but in a lesser degree. The same racial differences are found in the males, but they are not so marked. The splenium and genu, then, exhibit the most noticeable racial differences. The most striking sexual differences are found in the anterior and posterior lineal halves, the anterior in proportion to the posterior being larger in the males than in the females. The ratio of the body to the isthmus is greatest in the Negro male, least in the Caucasian female and intermediate in the Caucasian male and Negro female. This may be explained by the relative muscular power of the four classes, the commissural fibers of the motor areas forming the body of the corpus callosum. The greatest racial differences being found outside of the motor areas and their commissural fibers gives strong presumptive evidence that the great racial difference lies in the relation of the anterior to the posterior association center.

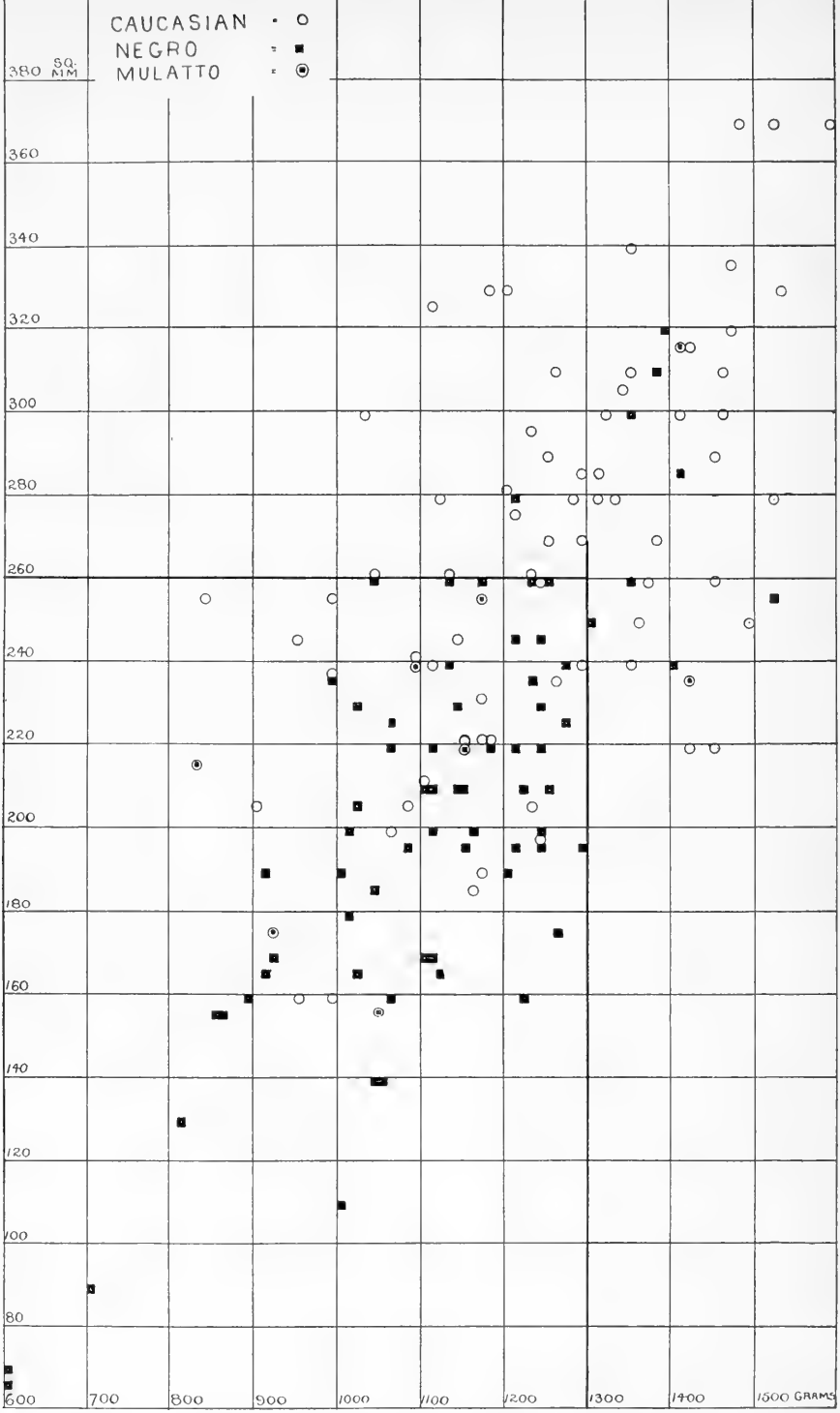


CHART VIII.—Relation of the area of the genu (ordinates) to brain weight (abscissæ). The heavy black lines include the majority of the Negro symbols, and exclude the majority of the Caucasian. Cf. Chart V. With equal increments of brain weight there is a proportionate increase in area of the genu.

The genu is not only larger in the Caucasian than in the Negro, but the size of the genu bears a more or less definite relation to brain weight in both races, an increase in brain weight being accompanied by a corresponding increase in the size of the genu. The splenium does not bear so definite a relation to brain weight, although there may be a slight increase in the size of the splenium with increase in brain weight. These statements may be verified by examining Charts VIII and IX, compiled

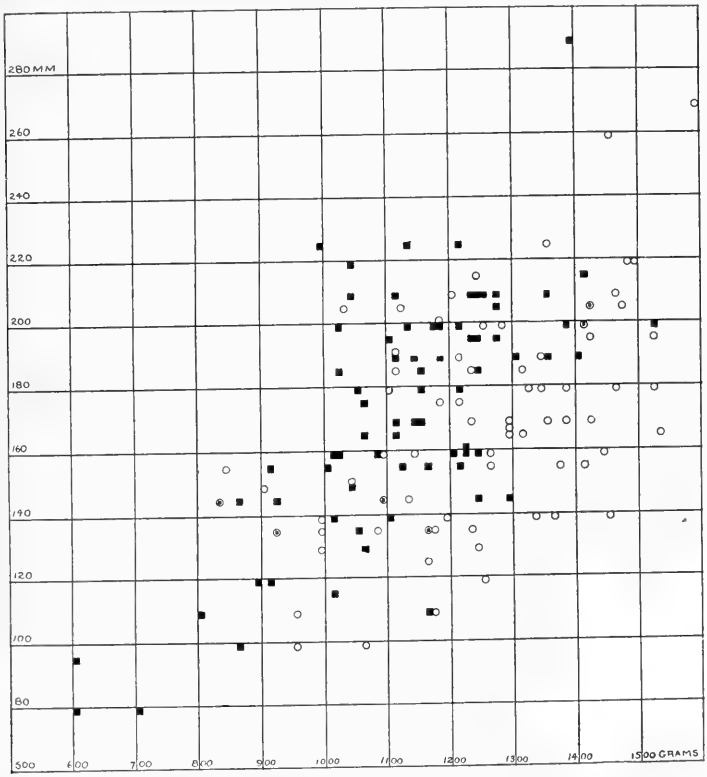


CHART IX.—Relation of the area of the splenium (ordinates) to brain weight (abscissæ). With equal increments of brain weight there is not a proportionate increase in the area of the splenium.

from Tables I, I′ and VII. A more or less definite racial difference is noted in the charts, but it is not marked. In Chart VIII draw a line horizontally through the 2.60 square centimeter ordinate, and draw another line vertically through the 1300-gram abscissa until these two lines intersect, and continue them to the limits of the charts. Very few symbols representing Negro brains are found above and to the right of these

Fig. 12

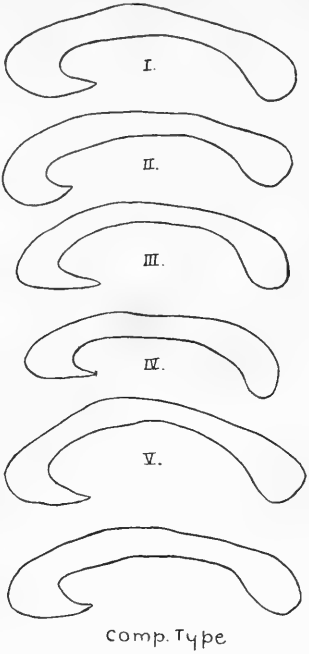


Fig. 13.

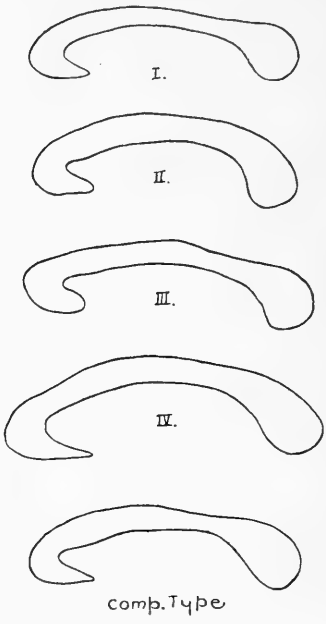


Fig 14

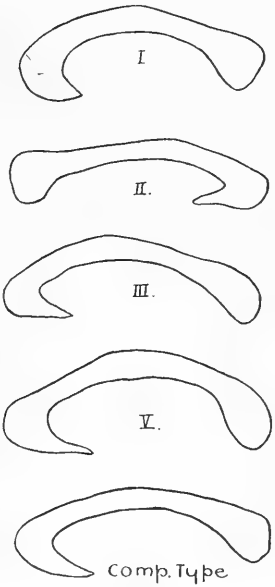


Fig. 15

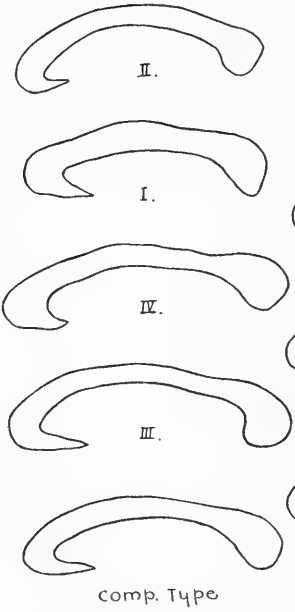


Fig. 16

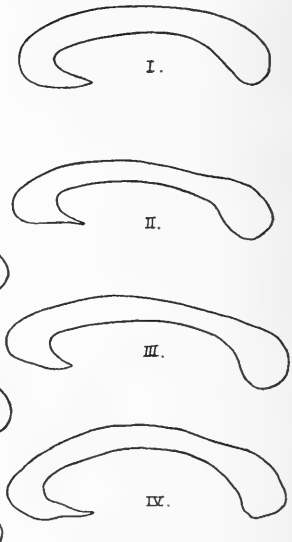


Fig. 12. Types of the corpus callosum in the Caucasian male. Type I, 8 subjects; Type II, 7 subjects; Type III, 7 subjects; Type IV, 6 subjects; Type V, 4 subjects; composite Type made up of the others, 32 in all. One-half natural size.

Fig. 13. Types of the corpus callosum in the Negro male. Type I, 18 subjects; Type II, 8 subjects; Type III, 10 subjects; Type IV, 9 subjects; Composite Type made up of the others, 45 in all. One-half natural size.

Fig. 14. Types of the corpus callosum in the Caucasian female. Type I, 1 subject; Type II. 2 subjects; Type III, 1 subject; Type V, 1 subject; Composite Type made up of the others, 5 in all. One-half natural size.

Fig. 15. Types of the corpus callosum in the Negro female. Type I, 5 subjects; Type II, 3 subjects; Type III, 6 subjects; Type IV, 5 subjects; Composite Type made up of the others, 19 in all. One-half natural size.

Fig. 16. Composite types of both races and sexes. Type I, 22 subjects, 13 Negro and 9 Caucasian. Type II, 29 subjects, 21 Negro and 8 Caucasian. Type III, 22 subjects, 15 Negro and 7 Caucasian. Type IV, 28 subjects, 15 Negro and 13 Caucasian. One-half natural size.

lines, which signifies that very few Negro brains are found with a brain weight of more than 1300 grams, or an area of the genu of more than 2.60 square centimeters. A majority of the symbols representing Caucasian brains are found above and to the right of the lines, signifying that the majority of the Caucasian brains have a brain weight of more than 1300 grams, and an area of the genu exceeding 2.60 square centimeters. The converse of these propositions is true. Few Caucasian brains have a brain weight of less than 1300 grams or an area of the genu less than 2.60 square centimeters, while the majority of the Negro brains are in this class. Compare Chart V with Chart VIII and a similarity is noticed, in fact they are nearly identical, but there is a more decided identity between the area of the genu and brain weight, than between the area of the entire corpus callosum and brain weight. This is due to the less decided identity between the area of the splenium and brain weight. That the size of the genu and brain weight are closely related may be significant in the relation of brain weights in races and in distinguished individuals.

Composite Types.

The corpus callosum may be classified in types racially and sexually according to the size and shape of the outline of its cross section. One hundred and one selected cases are taken and composite outlines are made of each racial and sexual type, as, Caucasian male, 5 types; Negro male, 4 types; Caucasian female, 4 types; and Negro female, 4 types. Composites are made by selecting outlines similar in size and shape, and

placing them over each other so that they coincide throughout as much as possible. The heaviest resulting outline is taken as the composite. Then the types are combined for each race in the same way, and finally the race types are combined. The types are represented in Figures 12 to 16. The type of brain varies with the type of corpus callosum, and the type of individual varies likewise.

Caucasian male type.—There are five types of the corpus callosum in the Caucasian male, but these may be brought together into two groups. Types I, II and IV belong to the primary group, and Types III and V to the secondary group. The primary group represents the young and vigorous, the secondary represents the old and infirm.

The corpus callosum representing Type I is a composite of eight cases. It is large in cross section, and every part is full and well developed. The splenium is of moderate size, the isthmus is not small, the body and genu are large and heavy. The type of brain to which this belongs is large, heavy (1400-1500 grams), and well rounded in all its outlines, approaching the dolichocephalic in shape. The frontal and temporal regions are large, the parietal and occipital regions are relatively not so large. The bodies from which these brains are taken are of men in the prime of life, from 40 to 50 years of age, and in apparently good physical condition, death coming rapidly or suddenly (pneumonia, heart disease, nephritis, galloping consumption, or accident), without great emaciation. The average height is 184 cm. (6 feet, $\frac{1}{2}$ inch), and the average weight is 73 kilo. (161 pounds). There is evidence of average intelligence and individuality among these men. One was manager of a livery stable, another was an eccentric man who became alienated from his family on Long Island and wandered off with considerable money, drifted to Baltimore and died in the Bay View Pauper Asylum, while a third was the victim of a third-rail accident, and apparently a man of affairs. Two are noted as "blonde." The others are not described as to color.

Type II is a composite of seven cases. The cross section of the corpus callosum is longer and narrower than in Type I. The splenium is large, the isthmus is small, the body is of medium size, and the genu is large. The brains representing this type are of medium size (1300-1500 grams), high and narrow (dolichocephalic), and the outlines are squared—not so rounded as in Type I. The frontal and parietal regions are large, the temporal is of fair size, and the occipital hangs low and is long. The bodies from which these brains were removed were in a well nourished condition, death having resulted rapidly (pneumonia, nephritis, etc.) The men were in the prime of life—approaching old age, 40 to 60 years old, with an average height of 172 cm. (5 feet 8 inches) and an average

weight of 75 kilo (165 pounds). Two are noted as "brunette." One is a dark Scandinavian. No records are made of the intellectual condition, or anything that would give a clue to it.

Type III is a composite of seven cases. The cross section of the corpus callosum is long, narrow, and highly arched. The splenium is large, the isthmus and body small, and the genu large with a long beak. The brains of this type are small (1200 to 1350 grams), high, long (dolichocephalic), and oval in shape from the side and from above. The ventricles are large and full of fluid. The bodies from which the brains are obtained are emaciated, the majority weighing little more than 45 kilo. (100 pounds), death being the result of lingering disease (senility, asthenia, etc.). The men were old (60 to 80 years), with an average height of 168 cm. (5 feet 6 inches). There was evidence of dementia in two or three.

Type IV is a composite of six cases. The cross section of the corpus callosum is short, of medium size, and not large anteriorly. The splenium is large, the isthmus is large, the body and genu are relatively small. The brains of this type are of medium size (1200 to 1500 grams), high, short, narrow, and boxlike in appearance, with full frontal and temporoparietal regions. The men were of average height, 165 cm. (5 feet 5 inches), of ages ranging from 15 to 75 years, and in weight varying from 50 to 80 kilo. (111 to 178 pounds). Two were Germans from Berlin.

Type V is a composite of four cases. The cross section of the corpus callosum is long, and the arch is high and more curved than in any other type. The splenium is large, the isthmus thin, the body of medium size and the genu not large, but having a long pointed beak. The brains vary in weight from 1040 to 1520 grams. They are high, long and rounded in all outlines. The ventricles are large and distended as if by pressure from within. The bodies were in a fair state of nourishment. The men were old (60 to 75 years), and ranged in height from 157 to 186 cm. (5 feet 2 inches to 6 feet 1 inch).

Caucasian female types.—In general the female types are similar to the male types of the same number. So few cases are given that generalization is inadmissible.

Composite Caucasian types.—The composite types are composites of all the Caucasian male types and of all the Caucasian female types. The most noticeable features of the corpus callosum of the Caucasian in comparison with that of the Negro are the high arch, and the greater size of the anterior half of the corpus callosum in the Caucasian. The splenium is of good size in the Caucasian, but not so large as in the Negro, while the isthmus, body, and genu are larger than the same parts in the Negro.

The sexual differences are slight. The cross section area is larger in the male than in the female Caucasian, but the splenium of the female is relatively larger than that of the male, the isthmus likewise, while the body is relatively smaller in the female, and the genu is relatively about the same size. (cf. Table VIII[a] et seq.)

Negro male types.—There are four types of the cross section outlines of the corpus callosum in the Negro male.

Type I is a composite of eighteen cases. This type is representative and characteristic of the Negro race. The cross section of the corpus callosum is small. The splenium is large and club shaped, the remainder of the corpus callosum is small, narrow, long, and slender. The brain weight is from 1000 to 1200 grams. The brains are short, with narrow frontal lobes, and wide, bulging parietal region. The mesial outline is oval. The bodies from which the brains are removed are well nourished and muscular. The average height is 162 cm. (5 feet 4 inches), and the average weight is 67 kilo (148 pounds). The age limit is 20 to 40 years. This represents a familiar type of Negro, the low, heavy set, muscular, dark-skinned young Negro, with small head, having the parietal bosses prominent and the frontal region low, narrow and receding. This is the lowest order and most prevalent type of Negro. There is evidence of little foreign blood. This type represents the Guinea Coast Negro, from which the subjects are probably derived. A few may be representative of the Hottentot Negro type.

Type II is a composite of eight cases. The cross section of the corpus callosum is larger than Type I and the anterior end is better developed. The splenium is also large. This may be considered as a sub-type of the one above, with evidence of more mixture with a foreign element. The brains are larger, weighing from 1100 to 1300 grams. The characteristics of the type are otherwise similar to those of Type I.

Type III is a composite of ten cases. The cross section of the corpus callosum is long and large. The splenium is large and club-shaped; the genu is large and round; the isthmus and body are long and narrow. The brains are long (dolichocephalic), high, and narrow in front, wide and bulging in the parietal region. The weight is from 1200 to 1400 grams. The bodies are in a fairly well nourished condition, death being rapid or sudden (accident, pneumonia, heart disease, etc.) The height averages 162 cm. (5 feet 4 inches), and the weight averages 63 kilo. (140 pounds). The men of this type are lighter skinned than those of Type I, and are built on broad lines in general. These are long armed, flat-footed, and loose-jointed individuals, not so compactly built or well knit as those of the previous types, and having long heads and faces, with high foreheads.

Unmistakable evidences of a previous mixture of other races with the Negro exist. Three are mulattoes. Three are accident cases. The majority are between the ages of 50 and 80 years. This type represents the higher and better class of the Guinea Coast Negro.

Type IV is a composite of nine cases. The cross section of the corpus callosum is long, large, and highly arched, resembling Type V in the male Caucasian. The splenium is large and regular in outline, tapering off gradually in the isthmus and body, which are long, curved, and smaller than the splenium. The genu is of medium size and has a long pointed beak. The brains are large, heavy (1300 to 1500 grams), long (dolichocephalic), and high in the frontal region. The frontal lobes are comparatively large and the parietal region is massive and bulging. The bodies are in a well nourished condition. The average weight is 72 kilo. (158 pounds), the average height is 175 cm. (5 feet 9 inches), and the age varies from about 40 to 70 years. This represents the tall, fair-skinned Negro (or mulatto), of the enterprising nature, but the most dangerous of all characters to human society. Rape and murder attach themselves here. Two of them were murderers, four Mulattoes, and the others exhibit traits of considerable Caucasian intermixture. This type represents the Kaffir Negro, probably a mixture of Semitic (Arab), Hamitic, and Negro at a remote period of time, the Zulus being the characteristic tribe of the Kaffir Negro.

Negro female types.—There are four female Negro types, which correspond in general to the four male Negro types. These may be combined into two groups for the two sexes alike. The primary group, composed of Types I and II, is the prevalent Negro type, being purer Negro than the secondary group, composed of Types III and IV, which is largely mixed with Caucasian.

Type I is a composite of five cases. The cross section of the corpus callosum is short, wide, and compact. The splenium, isthmus, body and genu are relatively of good size. The brain is small, short, and boxlike in appearance. The brain weight is from 1000 to 1100 grams. The frontal lobes are small, narrow from side to side and from above downward. The parietal region is large, full, and bulging. The subjects are about 160 cm. (5 feet 3 inches) average height, 50 to 54 kilo (110 to 120 pounds) average weight, and the age is from 20 to 30 years. They represent a class of young, stocky built, dark-skinned Negro women of the Guinea Coast Negro type. There is a trace of racial intermixture in some of them.

Type II is a composite of three cases. The cross section of the corpus callosum is long, arched, and narrow. The splenium and genu are of

good size, the isthmus is not well marked, and the body is slender. The brain is slightly longer than in Type I, but is smaller, the smallest of all brains being in this type. The average weight is 995 grams. The subjects are taller (168 cm.—5 feet 6 inches), and weigh less (45 kilo.—100 pounds or less) than those in Type I. These are probably of the Hottentot or Bosjeswoman type.

Type IV is a composite of five cases. The cross section of the corpus callosum is long, straight and slender. The splenium is large and club-shaped, the isthmus is narrow, the body is long and narrow, and the genu is of good size. The brains are long and narrow (dolichocephalic). The frontal lobes are narrow, low, and long, the parietal lobes are large and prominent. The brain weight ranges from 1000 to 1200 grams. The subjects are in a fairly well nourished condition, weighing from 54 to 59 kilo. (120 to 130 pounds), and having a height of 165 cm. (5 feet 5 inches) average. These are the old women from 60 to 70 years of age, of medium height and weight and light-brown skin. There is evidence of a little white blood. This type is probably of Kaffir origin.

Type III is a composite of six cases. The cross section of the corpus callosum is long, extremely thin and curved. The splenium is large and knob-shaped, the isthmus is narrow, the body long, narrow, and curved, and the genu small, with a long pointed beak. The brains are exceedingly long and narrow, and somewhat high in front. The frontal lobes are long, narrow, and thin, but high, the parietal lobes are full and bulging. The brain weight is from 1000 to 1100 grams. The subjects are low, fat and heavy. The average height is about 155 cm. (5 feet 1 inch), the average weight is 68 kilo. (150 pounds), and the age is from 30 to 50 years. This is the Negro "mammy," who is so well known. We have here a fat, fair-skinned Negro woman, not tall, but of a voluptuous type. There is evidence of white intermixture. This type probably represents the better class of the Guinea Coast Negro. The female types conform to the type of the race more nearly than do the males. The latter show more markedly the traces of racial intermixture.

The composite types for the Negro are made in the same manner as those for the Caucasian. The composite male and female are almost identical in shape, except that the splenium of the male is relatively larger than that of the female, just the opposite of what was found in the Caucasian. The cross section area in the male is altogether larger than in the female. Racial differences are more marked. The cross section area of the corpus callosum is less in the Negro than in the Caucasian.

The area of the posterior lineal half is relatively larger in the Negro, while the area of the anterior lineal half is relatively smaller. The

splenium is absolutely and relatively larger in the Negro than in the Caucasian, while the genu is relatively and absolutely smaller. The isthmus and body are relatively about the same size in the males of the two races, but in the females the isthmus is relatively smaller in the Negro, while the body is relatively larger. The hooked beak of the genu is larger in any case in the Caucasian, especially in the female.

Composite types of both races and sexes.—There are four of these types made up as follows: Type I is a composite of Type II Negro male, and Type I of the others, twenty-two individual cases in all, thirteen Negro and nine Caucasian. The Caucasian traits predominate. This type represents the young, active, vigorous individuals. Type II is a composite of Types I Negro male, II Negro female and Caucasian female, and Type IV Caucasian male, twenty-nine individual cases in all, twenty-one Negro and eight Caucasian. The Negro traits predominate. This type represents the old and the passionate. Type III is a composite of Type II Caucasian male, and Type III Negro male and Type IV Negro female, twenty-one individual cases in all, fifteen Negro and seven Caucasian. The Negro and Caucasian traits are well mixed. This is a Mulatto type. Type IV is a composite of the remaining types, twenty-eight individual cases in all, fifteen Negro and thirteen Caucasian. This type represents the mentally dull, the demented, and the degraded.

Whenever the number of Caucasian exceeds one-third of the whole number of cases in any type the Caucasian traits predominate. This may indicate a certain amount of Caucasian mixtures among the Negroes.

The American Negro may be divided into two groups, each with subdivisions.[68] The first group comprising the greater number of blacks, being represented by the Negro types I, II and III, and the second group, including only a comparatively small number, being represented by the Negro Type IV. The first group includes the Guinea Coast Negro and may be the few Hottentots in America, and is divided into three classes. First the Hottentot, or Bosjesman, having gray or old yellow skin resembling dirty varnished oak; low, dwarfed stature, either weak, or squat and muscular; long, woolly hair, in small obliquely inserted tufts; very dark eyes, wide apart; extraordinarily broad, flat nose; large mouth, with thick, projecting, turned-out lips; enormous prognathism; heads extremely dolichocephalic; the smallest brains (900-1000 grams) of any human beings probably; and lastly, having the distinctive steatopyga and the tablier which are not always present. This class is comparatively rare. Secondly, the low class Guinea Coast Negro, the most ancient and most classical Negro type, having a cool, velvety skin, glossy, and varying from a reddish, yellowish, or bluish black to jet black; low stature, well knit and

muscular; black hair and eyes; platyrrhine nose; thick lips; prognathous face; beautifully white, sound teeth; small square ears (Hrdlička[27]); long upper and short lower extremities; flat feet; heads dolichocephalic, or even approaching subbrachycephaly; and brains weighing from 1000 to 1200 grams,—possibly more. This is the most prevalent class of Negro in the South. Thirdly, the high class Guinea Negro, similar to the low class, but developed along broader lines, and instead of being ugly, diminutive, with large and squat limbs, and a round or short face, they are comparatively handsome, taller, with well-proportioned limbs and a long face. They exist in fairly large numbers in certain localities, but are much less prevalent than the low-class Guinea Negro. The second group is made up of Kaffirs and other Mulattoes, and Mulattoids, or Mulatto-like individuals. The Kaffirs are represented by the Zulus in Virginia and North Carolina, being particularly noted for their height and intelligence. They have various shades of dark brown skin; very high stature, slim and well made; thick, woolly hair, and dark brown eyes; broad, flat nose, sometimes highly arched, Romanesque, or Arablike; thick lips; long, oval face; slight prognathism and platyrrhiny; long, high heads, with narrow foreheads, and median frontal protuberances; and large brains, weighing from 1300 to 1500 grams. They do not exist in great numbers except in certain sections, as in Virginia and North Carolina where they are fairly prevalent. The Mulattoes are such a heterogeneous conglomeration as to beggar description. Three classes do stand out distinctly though. One is the large, yellow Mulatto with every feature magnified and like the Negro, tremendous frame, sometimes veritable giants, and a conspicuous bumptiousness and volubility. Another is the small, almost white Mulatto, with Caucasian features, neat, compact frame, and partaking of the qualities of the Caucasian mentally. A third is that peculiar mottled Mulatto or Mulattoid mentioned by Shaler.[58] There are all sorts of mixtures of all the classes mentioned above forming a not inconsiderable part of the Negro population. There may be a few other types of Negroes here and there, such as the Ethiopians, Papuans, Negritos, and perhaps Australians, and one occasionally sees a red Negro, probably a Foulah from the heart of Africa in the region of the Soudan, or a Dahomian from near there, but these are so rare as to be inconsiderable. A few mixed bloods with Indian characteristics are occasionally observed. This classification is slightly different from that given by Prof. Shaler,[58] but only in minor points. It does not differ materially from Tobinard's[68] classification of the Negro in the West and South of Africa, from which sections nearly all of the Negroes of America are supposed to have been brought.

Foramen of Munro.

The position of the foramen of Munro bears an interesting relation to the two ends of the corpus callosum and to the brain center, sexually and racially. Measurements are made on the brain axis, all points not on the axis being projected to it by lines perpendicular to the axis. The average of all measurements is represented in Table VIII in millimeters. In this table "Genu" and "Splenium" mean the anterior and posterior ends, respectively, of the corpus callosum. The "Ratio" is the number preceding it divided by the length of the brain axis for that race and sex. The two hemispheres measure alike practically. A difference of one millimeter in the numbers in the table is to be ignored.

TABLE VIII.

Relative Positions of the Genu, Splenium, Foramen of Munro, and Brain Center. Averages and Ratios.

	Center to Splenium.	Ratio.	Center to For. Munro.	Ratio.	Center to Genu.	Ratio.	For. Munro to Splenium.	Ratio.	For Munro to Genu.	Ratio.	Brain axis.
Caucasian male	26	155	18	107	50	300	44	262	32	190	168
Negro male	28	170	16	95	48	285	44	262	32	190	168
Caucasian female	25	155	17	105	47	292	42	261	30	186	161
Negro female	29	186	14	90	43	270	43	276	29	186	156

The splenium is further from the brain center in the Negro than in the Caucasian, and it is further posterior in the female Negro than in the male. The foramen of Munro is nearer the brain center in the Negro than in the Caucasian and it is nearest in the female Negro. The genu is nearer the center in the Negro, and nearest in the female Negro. The splenium is further removed from the foramen of Munro in the female Negro than in any of the others. The genu is nearer the foramen of Munro in the females than in the males, there being no racial difference in either sex here. The corpus callosum and the foramen of Munro are both placed further posterior in the female Negro, indicating that more brain substance lies anterior to these structures in the female Negro than in the others. This corresponds to the findings in relation to the position of the fissure of Rolando. There is less brain substance anteriorly in the female Negro, the apparent discrepancy being due to the fact that the frontal lobes of the female Negro are long and slender. The male Negro has an intermediate position between the female Negro and the Caucasian in regard to the location of the corpus callosum and the foramen of Munro.

Brain Axis.

The brain axis used in the measurements for this study is determined and located by three points, the inferior border of the splenium, the superior border of the anterior commissure and the foramen of Munro. A line is drawn on each outline of the mesial surface of each hemisphere

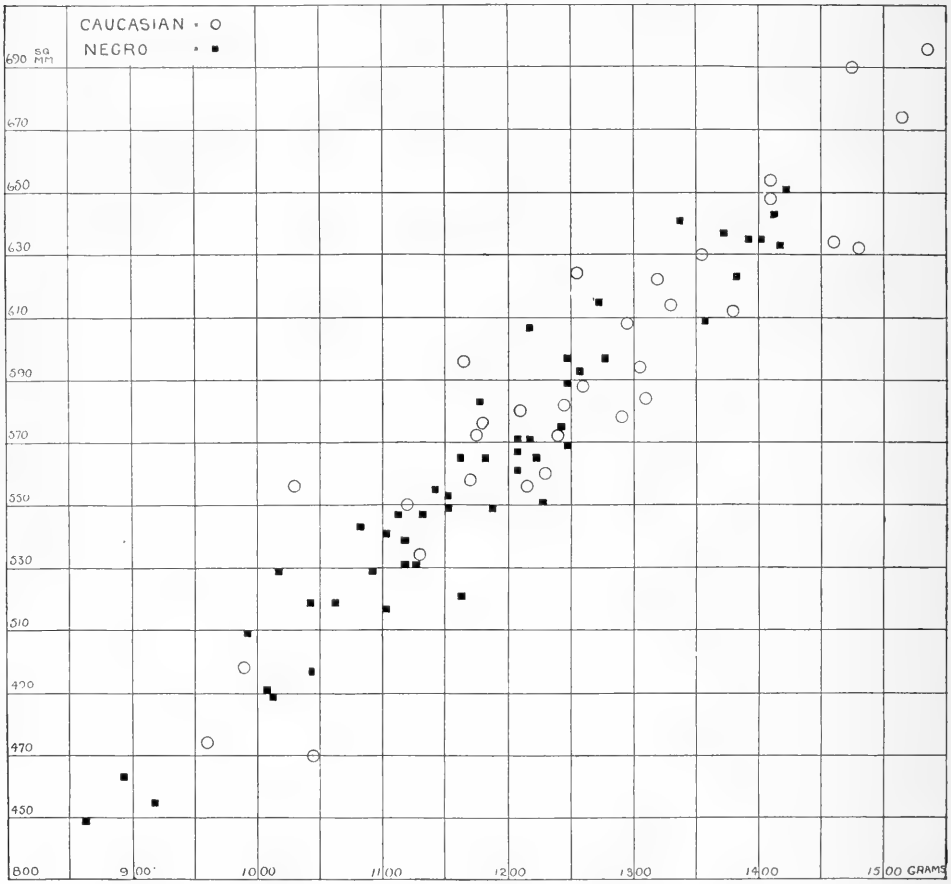


Chart X.—Relation of the area of the brain outlines (ordinates) to brain weight (abscissæ). The Caucasian is more variable, the Negro more constant.

through these three points, to each extremity of the brain. This line is arbitrarily made to touch the lower border of the splenium and the upper border of the anterior commissure, and it passes through the foramen of Munro in 90% of the cases, and falls from 1 to 3 mm. below it in 10%.

The line passes through the longest diameter of the brain in 68% of the male Caucasian brains; 70% of the adult male Negro; none of the infant male Negro; 60% of the female Caucasian, and 33⅓% of the female Negro. The line passes near the longest diameter, below in front and above behind, in 32% of the male Caucasian brains, 30% of the adult male Negro, 40% of the female Caucasian, 66⅔% of the female Negro, and 100% of the infant male Negro. This gives a distinct gradation from the male Caucasian to the infant male Negro, the female Negro resembling most closely the infant type. In relation to the brain axis the infant has a larger amount of brain substance below the axis posteriorly, and a smaller amount below anteriorly, than is found in any of the others. When the brain axis does not exactly coincide with the longest diameter of the brain, lines are drawn from the ends of the brain perpendicular to the axis, and in all cases the length of the axis between these lines coincides in length, practically, with the longest diameter of the brain. Refer to Figures 1 to 4 and 8 to 12 for evidence of these facts. The average distance between the lower border of the genu and the lower border of the frontal lobe is 22 mm. in the male Caucasian, 21 mm. in the male Negro, and 20 mm. in the female Negro. This difference, in connection with the extreme thinness of this part of the frontal lobe in the Negro, especially the Negro woman, indicates the frontal lobes to be even smaller than is apparent in the outlines, and by measurements taken from them.

The brain axis is used because it is located definitely by three points that seem to be fairly constant in position, relatively; because it passes through the longest diameter of the brain in the majority of cases; because it is a convenient line for measuring all parts of the brain in any position, thus facilitating speed and accuracy in brain measurements, and affording a just basis for comparison of any brain in the relation of its parts to each other and to other brains. By means of the brain axis a brain center is established which is constant within a small circle, and by a composite is shown to retain its position, relatively, in the brains measured. It is located just above and anterior to the opening of the aqueduct of Sylvius into the third ventricle, a line drawn at an angle of 45° above the anterior end of the brain axis through the aqueduct of Sylvius passes through the brain center. It is just posterior to the gray commissure in the sulcus of Munro separating the alar from the basal lamina of the embryonic brain tube at a point that is perhaps as constant in position as any other during development. Shifting of the brain axis by rotation, antero-posteriorly or infero-superiorly, its usual variation when it changes, does not alter the position of the brain center. If its position

is slightly altered by shifting the axis up and down, the relations to different points remain the same. Shifting the position of the brain center forwards or backwards indicates altered relations of the anterior or posterior extremities of the brain outlines and is something to be desired as an indication of existing conditions. The brain center, then, is a comparatively constant point and is a good one to use as a basis for all measurements of the brain. By means of the brain axis and the brain center, racial and sexual differences are demonstrated in the size and shape of the corpus callosum; in the position of the fissure of Rolando; in the amount of brain substance anterior and posterior to this fissure; in the relations of the foramen of Munro to the whole brain, to the whole corpus callosum, to the genu and the splenium, and the relation of these parts to one another and to other parts of the brain; and by means of the brain axis and the brain center a system of notation is devised whereby any point anywhere about the brain may be located definitely and accurately. This may be done by representing the brain as a sphere, and using degrees of latitude and longitude, in this way bringing everything to the brain center as a basis. The degrees of longitude may be represented by semicircles connecting the extremitis of the brain axis and extending over the surface of the brain in the direction of its long diameter. Degrees of latitude may be represented by lines joining the terminal points of radii drawn from the brain center to these semicircles. The anterior end of the brain axis represents the north pole, and the posterior end the south pole. The equator is represented by the outline of a vertical plane passing through the vertex of the brain and through the brain center at right angles to the brain axis, supposing the brain to be in its normal position with the body standing erect in all this description. The brain axis will be horizontal under such conditions. A horizontal plane passing through the axis and the right hemisphere will cut a semicircle around the side of this hemisphere and this semicircle represents 0° longitude, which will be called L. 0°. Revolve this plane to the left and upward through a distance of 360° to its original position, and as it traverses the circle its different positions in the course of its transit will vary from 0° to 360°, any one of which may be located on the brain surface. Thus the mesial surface of each hemisphere above the axis will be L. 90°, the mesial surface below the axis will be L. 270°, the horizontal plane of the left hemisphere opposite L. 0° and similar to it will be L. 180°, and intermediate planes likewise according to position. In like manner the planes of the outlines represented in Table II will be L. 45° for the right hemisphere and L. 135° for the left. The degrees of latitude as radii from the brain center begin at the north pole and pass towards the vertex of

the brain through the equator above the axis, through the south pole and through the equator below the axis to the original position, describing a circle of 360°, the north pole being R. 0°, the south pole R. 180°, and intermediate points likewise. These radii are to be represented on any plane of longitude, and they may be placed so close together as to form a plane which will coincide with the anterior halves of L. 0° and L. 180° when the radii are R. 0°, with the equator above the axis when the radii are R. 90°, with the posterior halves of L. 0° and L. 180° when the radii are 180°, and with the equator below the axis when the radii are 270°. L. 0° and L. 360° are identical. R. 0° and R. 360° are identical. By combining the degrees of latitude and of longitude definite points may be located. For example, the vertex of the brain being at the central point of the equator above the axis will be L. 90° R. 90°, and the bifurcation (or junction) of the Crura cerebri will be about L. 270° R. 270°. The point representing the right anterior association center as used in Table II would be L. 45° R. 45°, and a similar point in the left hemisphere would be L. 135° R. 45°. In this way any other point may be determined. The brain center being located, the distance of any point from the brain center may be determined. Degrees of latitude are used instead of parallels of latitude in order to bring everything to the brain center as a basis. To sum up these: There is a north pole, the anterior end of the brain axis (R. 0°); there is a south pole, the posterior end of the brain axis (R. 180°); there is an equator circumscribing a plane which passes through the vertex of the brain and through the brain center at right angles to the brain axis; there are planes of latitude cutting sections of the brain from the periphery to the center beginning at the north pole and completing a circle by passing upward and backward to the south pole, and downward and forward from this point to the original position, the planes being represented by R. 0° to R. 360°; and there are planes of longitude cutting longitudinal sections of the brain, the planes passing from the horizontal plane of the right hemisphere upwards and to the left through a circle of 360° to the original position and being represented by L. 0° to L. 360° in their course.

ADDENDA.

Certain relevant subjects are not treated at length for various reasons, but are simply added as an appendix that anyone who is interested may examine, and take for what it is worth. Not much value is attached to these subjects, but there may be something of value and interest in them as discussed below.

Brain Weight.

So many factors enter into brain weight that it is questionable whether discussion of the subject is profitable here. A few points will be touched on, however. The brain weight (Chart XI), actual or approximate, of seventy-nine Negro brains in the fresh state is given. The average for

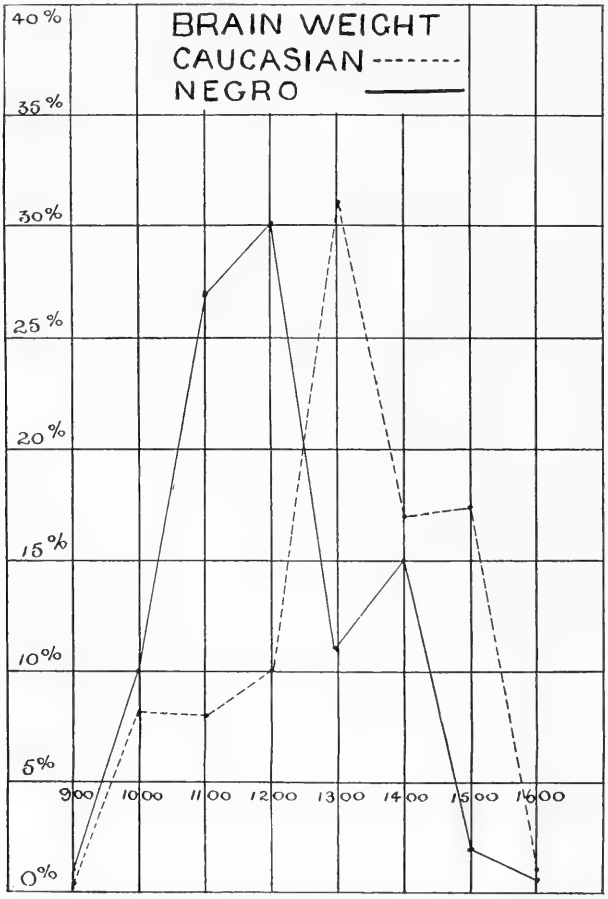


Chart XI.—Percentages of brain weight in the two races.

fifty-one males is 1292 grams; the largest, 1560 grams; the smallest, 1010 grams. The average for twenty-eight females is 1108 grams; the largest, 1320 grams; the smallest, 910 grams. The brain weight, actual or approximate, of forty-six Caucasian brains in the fresh state is given. The average for thirty-seven males is 1341 grams; the largest, 1555 grams; the smallest, 1040 grams. The average for nine females is 1103

grams; the largest, 1275 grams; the smallest, 915 grams. The lot of brains includes a larger number from high-class Negroes than from high-class Caucasians, and a larger number from low-class Caucasians than from low-class Negroes, this being especially true in regard to the Negro males and the Caucasian females. This statement is based on the following facts:

1. There is a larger number of deaths resulting from acute illnesses and from accidents among the Negroes, giving a larger number of brains from normal individuals.[31 68]

2. That a larger number of Negro bodies are regularly disposed of to anatomists indicates less respect for the dead among Negroes, and it follows that more of the better class of Negroes would be received, since the whites greatly outnumber the blacks in Baltimore.

3. It is well known that only the lowest classes of whites are unclaimed, especially among the women, who are apt to be prostitutes, or depraved, or the like, while among Negroes it is known that even the better class neglect their dead unless provision has been made for their care after death.

4. It is a well attested fact that the Negroes are at present roaming over the country without fixed abode in greater numbers than the whites and this might result in many stray unclaimed bodies of the better class of Negroes being turned over to the anatomists, and finally,

5. Many Mulattoes and mixed bloods are included among the Negroes.

So then the brain weights do not really represent the exact racial difference between the Negro and the Caucasian, but do perhaps show that the low class Caucasian has a larger brain than a better class Negro. Many of the brains are from the senile, the demented, or those dying of wasting diseases, which would tend to make the average weight lower than among normal individuals. The total stature of the Caucasian exceeds that of the Negro, and the total body weight is slightly greater in the Caucasian, the stature and body weight being greater in the males than in the females. The majority of the Caucasian males and Negro females were between the ages of 30 and 50, the majority of the others under 35 or over 45. The percentage curve of brain weight for the two races shows the greater number of Negro brains to be about 1100 to 1200 grams, the greater number of the Caucasian brains being 1300 grams and over, with a drop in the number of Negro brains at 1300 grams and an increase at 1400 grams, indicating a mixture of Caucasian and Negro in the largest brains. There are on record the weights of less than 100 Negro brains,[80] perhaps, with the exception of 380 weighed by Hunt and Russell,[30] who include Mulattoes and mixed bloods, as I have done. The

average weight of twenty-two male Negro brains weighed by sundry men, at various times, in divers places with different systems of weights and under dissimilar conditions is 1256 grams; the largest, 1458 grams; the smallest, 1100 grams. The average weight of 10 female Negro brains under similar conditions is 980 grams; the largest, 1325 grams; the smallest, 738 grams. Waldeyer [74] gives the average weight of twelve Negro brains in the fresh state as 1148 grams; the largest, 1450 grams; the smallest 780 grams. Sandford B. Hunt [28 29] gives the average weight of 140 male Negro brains as 1331 grams; the largest, 1585 grams; the smallest, 1010 grams; the average of 240 male mixed bloods, Negro and white, 1285 grams; the largest, 1736 grams; the smallest, 980 grams. Hunt concludes by grouping the brains according to the estimated amount of white blood, that the weight varies directly in proportion to the amount of white blood. The mulattoes and those more than one-half white have brains nearly as large as the pure white and larger than the Negro, while those less than one-half white have smaller brains, those with the least amount of white blood having smaller brains than the pure Negro. Practically the same conclusion is reached by a similar classification of the male Negro brains I weighed. The average for the mulattoes is 1347 grams; for those one-fourth white, 1340 grams; for the one-eighth white, 1335 grams; for the one-sixteenth white, 1191 grams; but for the pure Negro 1157 grams. The difficulty about any such classification is that no two individuals may agree as to what constitutes the exact markings of the different grades. Only those Negroes should be considered pure that show no evidence of any previous crossing with another race at a previous time, perhaps the low-class Guinea Coast Negro representing this type in the brains studied. Certainly the high-class Guinea Coast Negro and the Kaffir (Zulu) show unmistakable evidence of a previous mingling of races. (Topinard).[68]

The conclusion is that the brain of the Negro is smaller than the brain of the white, the stature is also lower, and the body weight is less, and any crossing of the two races results in a brain weight relative to the proportion of white blood in the individual.

The skull capacity of the Negro has been repeatedly demonstrated to be less than that of the Caucasian.[51]

Test to Determine Race and Sex of Brains.

When this work was undertaken I had handled comparatively few brains, so I examined about twenty and measured them in various ways before attempting to differentiate the Negro from the Caucasian brain,

or the male from the female. After that a record was kept of the guess made on each brain, except those I could recognize from previous handling, before the race or sex was known, these being looked up afterwards, to determine the degree of accuracy possible in such a guess. The race was determined correctly 70 times, doubtfully 5 times, and incorrectly 5 times in 80 brains. The sex was determined correctly 69 times, doubtfully once, and incorrectly 10 times. The race and sex were determined correctly 60 times, one or the other correctly 15 times, and incorrectly 5 times. Of the 5 incorrect guesses a Caucasian female brain was taken to

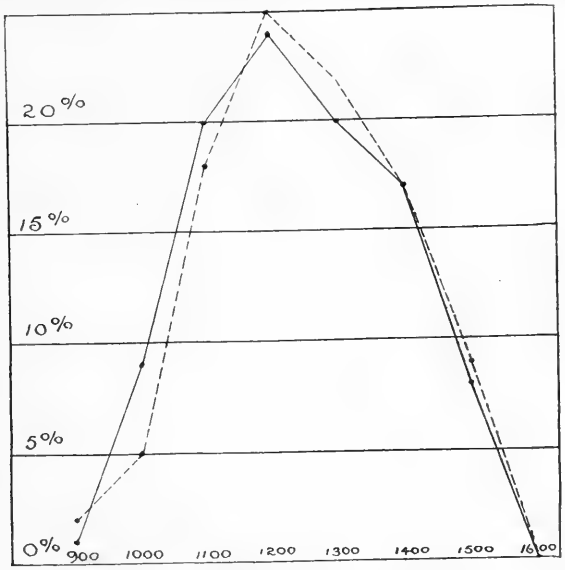


CHART XII.—Percentages of brain weight, in relation to stature and body weight combined.

be a Negro male in one case (No. 1583), a Negro female in another (No. 1527); a Caucasian male brain was taken to be a Negro male in two cases (Nos. 1716 and 1749), but with one of these there was some doubt; and a Negro male was taken to be a Caucasian male in one case (No. 1707). Mulattoes partook of one type or the other as a rule, sometimes resembling the Negro and sometimes the Caucasian more closely.

CONCLUSIONS.

1. The brain of the American Negro is smaller than that of the American Caucasian, the difference being primarily in the frontal lobe, and it follows that the anterior association center is relatively and absolutely smaller.

2. The Negro brain can be distinguished from the Caucasian with a varying degree of accuracy according to the amount of admixture of white blood.

3. The area of the cross section of the corpus callosum varies with the brain weight. However, in the Negro its anterior half is relatively smaller than in the Caucasian, to correspond with the smaller anterior association center; the genu is relatively larger and the splenium relatively smaller.

4. From the deduced difference between the functions of the anterior and posterior association centers and from the known characteristics of the two races the conclusion is that the Negro is more objective and the Caucasian more subjective. The Negro has the lower mental faculties (smell, sight, handicraftsmanship, body-sense, melody) well developed. the Caucasian the higher (self-control, will power, ethical and æsthetic senses and reason).

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83. Articles on Head Size and Brain Weight. Nos. 3, 8, 9, 14, 16, 21, 22, 23, 26, 27, 31, 36, 37, 41, 42, 50, 68, 69, 70, 76 and 77.
84. Articles on Classes of Negroes. Nos. 5, 6, 7, 11, 23, 24, 27, 28, 29, 30, 32, 58, 62 and 68.

APPENDIX.

TABLES II TO VII.

TABLE II.—LENGTH OF THE RADII IN MILLIMETERS FROM THE DEGREE OUTLINE. ALSO DISTANCE OF FRONTAL AND OCCIPI CENTIMETER FROM EACH END OF THE BRAIN. DEGREES BE

NEGRO MALE. LEFT SIDE.

No.	Brain axis.	0°	10°	20°	30°	40°	50°	60°	70°	80°	90°	100°	110°	120°
1189	**176**	86	89	87	**84**	80	75	**71**	70	68	68	**72**	75	**76**
1190	**184**	89	92	89	82	77	70	**66**	64	63	66	68	72	**73**
1456	**164**	75	82	81	77	73	70	**69**	65	65	67	71	74	**76**
1466	**180**	89	90	89	**84**	78	73	**71**	69	69	69	71	73	**75**
1470	**164**	78	84	86	80	74	65	**61**	57	57	57	59	62	**64**
1472	**154**	62	78	76	75	71	66	**62**	60	59	61	64	69	**72**
1473	**160**	77	81	82	78	71	64	**62**	61	61	62	68	71	**73**
1476	**164**	79	84	83	78	75	70	**67**	65	65	67	70	73	**75**
1478	**166**	80	83	81	75	71	68	**65**	62	64	63	67	69	**72**
1480	**180**	86	91	90	85	79	74	**73**	73	73	76	80	80	**83**
1486	**174**	85	88	87	82	75	69	**67**	65	63	63	67	69	**71**
1492	**164**	83	85	82	78	71	68	**64**	63	62	63	66	70	**74**
1495	**170**	83	87	85	81	75	69	**65**	64	63	64	66	72	**73**
1497	**170**	79	85	85	82	76	70	**63**	63	61	60	63	66	**71**
1502	**164**	76	84	86	84	80	74	**69**	66	65	64	65	67	**70**
1511	**158**	78	83	83	82	75	71	**65**	64	62	62	64	66	**68**
1519	**160**	74	78	79	75	70	66	**64**	64	63	65	69	73	**75**
1528	**176**	84	87	85	79	76	72	**70**	67	69	71	74	76	**79**
1530	**158**	75	78	77	74	68	64	**61**	61	61	62	65	67	**69**
1532	**188**	92	95	88	81	74	70	**68**	67	68	70	76	79	**82**
1533	**160**	77	82	84	80	76	69	**65**	62	61	61	63	65	**68**
1661	**156**	69	76	77	75	72	66	**62**	60	59	59	60	62	**65**
1680	**166**	78	84	84	80	73	67	**65**	62	60	61	62	63	**67**
1691	**172**	80	90	86	80	74	69	**65**	63	63	62	63	65	**67**
1699	**166**	75	83	85	83	78	71	**67**	63	62	62	65	68	**70**
1701	**167**	80	84	83	81	76	69	**67**	65	67	68	70	73	**75**
1704	**177**	85	92	90	84	78	72	**69**	67	68	69	71	73	**75**
1706	**173**	83	89	89	85	78	74	**70**	69	69	71	73	75	**74**
1709	**181**	88	92	89	84	77	73	**71**	71	68	70	71	74	**75**
1711	**159**	76	80	80	74	68	66	**64**	62	62	64	67	72	**73**
1713	**171**	75	86	87	83	80	73	**69**	67	67	69	73	77	**80**
1718	**156**	72	79	77	75	72	67	**65**	64	65	67	69	72	**75**
1727	**180**	86	91	91	84	79	74	**67**	64	62	61	62	66	**70**
1728	**182**	81	90	91	86	78	72	**69**	68	68	70	74	78	**82**
1731	**176**	80	88	88	84	78	74	**72**	72	71	75	78	81	**83**
1736	**164**	78	82	80	80	75	70	**66**	66	65	67	71	75	**78**
1738	**173**	78	84	86	85	78	73	**68**	64	61	62	65	67	**69**
2521	**171**	84	87	86	82	77	71	**69**	68	67	71	73	74	**76**
2522	**171**	86	87	85	82	77	72	**70**	67	67	69	71	73	**75**
2524	**165**	81	82	83	79	73	69	**68**	68	68	70	72	73	**73**
2535	**160**	75	82	81	78	71	67	**63**	62	63	65	68	70	**72**
105	**148**	70	75	75	73	68	64	**62**	60	60	60	62	65	**68**
173	**171**	83	87	84	78	72	66	**63**	62	61	62	64	65	**67**
Avs. 43	**168**	80	85	84	80	73	70	**66**	65	65	65	68	71	**73**

NEGRO FEMALE. LEFT SIDE.

1449	**164**	76	83	84	80	74	69	**64**	62	62	61	62	66	**69**
1459	**162**	81	83	78	74	67	64	**60**	59	57	58	60	65	**68**
1477	**160**	78	80	81	76	71	68	**65**	64	63	63	67	68	**70**
1479	**162**	82	82	82	77	72	67	**64**	60	61	63	65	65	**69**
1487	**150**	74	75	73	68	63	60	**58**	57	56	57	59	63	**65**
1493	**166**	80	83	83	78	71	66	**61**	60	59	58	61	63	**65**
1500	**154**	77	78	75	72	66	62	**57**	57	57	59	61	62	**64**
1501	**144**	71	79	82	74	69	65	**63**	62	61	63	67	69	**70**
1515	**164**	80	80	78	74	71	66	**64**	62	62	63	66	70	**69**
1544	**160**	80	81	81	78	72	68	**66**	64	63	64	68	70	**72**

BRAIN CENTER FOR EACH TEN DEGREES ON THE FORTY-FIVE-
TAL LOBES BELOW THE AXIS OF THE BRAIN, MEASURED AT EACH
GIN WITH 0° AT ANTERIOR END OF BRAIN AXIS.

NEGRO MALE. LEFT SIDE.—(Continued across.)

130°	140°	150°	160°	170°	180°	From anterior end. 1cm.	2cm.	3cm.	4cm.	5cm.	From posterior end. 1cm.	2cm.	3cm.	Index. association centers.
76	77	77	79	84	88	8	11	13	14	12	11	5	..	93
75	76	77	79	85	92	5	8	10	11	11	12	12	..	89
77	79	79	80	82	80	7	11	12	13	10	6	3	..	90
76	77	80	86	89	88	11	12	13	12	10	9	11	6	94
66	70	72	75	80	82	5	10	12	12	..	22	15	..	95
75	76	76	76	79	69	—2	1	5	8	..	1	10	6	86
74	76	75	76	79	79	5	8	9	11	10	12	14	4	84
76	77	78	79	82	83	7	11	13	14	12	8	9	5	89
74	77	79	81	84	83	11	10	12	14	11	8	11	5	90
84	85	85	89	93	85	5	9	9	9	9	1	2	0	88
73	75	80	81	85	86	7	10	12	14	13	10	8	..	94
76	77	78	79	80	82	8	13	15	16	14	18	18	..	86
74	76	77	78	79	84	5	8	10	9	5	9	10	..	89
73	76	79	83	83	84	3	13	12	13	14	7	14	6	88
72	73	73	74	79	80	1	5	9	10	10	15	12	7	98
70	70	72	73	76	80	4	8	9	9	8	19	16	12	95
77	78	78	78	81	75	5	8	10	9	8	0	1	—2	85
82	84	84	85	89	70	8	12	16	17	15	—2	1	—4	88
71	72	75	75	78	77	5	8	9	10	10	5	6	1	88
83	84	87	91	95	88	15	18	17	15	14	5	9	1	83
70	72	75	76	78	79	4	7	10	10	..	14	..	..	95
66	68	69	71	74	76	4	9	11	10	..	5	8	..	95
70	73	75	77	81	84	5	7	10	11	10	7	10	..	96
71	77	79	81	83	86	5	8	9	15	9	11	12	12	97
73	75	76	77	80	81	1	5	7	9	6	9	10	5	95
76	77	78	80	83	82	6	11	14	14	14	9	3	..	89
77	78	82	84	87	85	5	8	9	10	1	1	5	0	90
75	77	77	80	86	85	5	8	10	11	9	4	4	..	94
79	79	81	83	87	91	8	11	12	13	14	16	14	..	94
76	79	78	80	78	68	6	11	12	12	10	—2	5	0	87
81	82	83	85	84	75	0	2	5	6	4	0	6	2	86
77	78	79	82	78	65	3	9	10	12	12	—2	1	..	86
74	77	80	84	88	90	5	7	9	9	9	7	12	6	95
84	83	84	85	89	86	1	4	6	7	8	2	1	..	84
84	84	85	87	88	71	6	9	9	10	10	—1	0	—1	86
80	80	82	81	83	73	5	6	6	6	4	0	5	0	84
73	75	75	77	82	83	2	6	9	11	11	14	11	..	98
78	81	81	85	86	72	4	9	12	13	9	—4	3	..	90
77	78	78	79	84	80	10	11	11	11	10	5	6	1	93
76	76	76	78	82	79	10	11	12	14	13	5	6	..	93
74	76	77	82	78	78	3	6	7	8	9	4	5	..	87
70	70	70	69	72	71	3	5	7	7	..	5	..	..	91
69	73	76	81	85	83	10	14	15	16	17	6	4	..	94
75	76	78	80	83	80	5	9	10	11	10	6	8	3	90

NEGRO FEMALE. LEFT SIDE.—(Continued across.)

71	75	77	78	79	82	3	7	10	11	7	17	18	10	92
70	74	75	77	80	81	7	12	14	17	17	2	3	..	88
69	69	70	75	80	75	8	9	13	14	11	10	5	..	92
72	74	76	77	79	82	2	7	10	10	10	12	11	..	92
67	69	70	71	74	74	9	12	14	16	15	8	9	..	89
68	72	76	79	82	78	8	11	12	12	10	4	5	..	93
68	72	72	73	77	77	9	14	14	11	7	3	1	..	86
70	70	69	67	68	71	8	11	11	10	..	16	10	..	90
69	70	73	77	83	80	8	9	12	11	6	7	4	..	92
74	75	75	76	77	80	1	4	9	11	8	11	15	7	91

TABLE II.—Continued.

NEGRO FEMALE. LEFT SIDE.

No.	Brain axis.	0°	10°	20°	30°	40°	50°	60°	70°	80°	90°	100°	110°	120°
1593	**148**	72	75	74	70	65	62	**60**	60	59	61	63	65	**65**
1659	**168**	80	85	83	78	72	68	**66**	64	63	65	65	66	**70**
1678	**158**	68	79	80	79	74	71	**66**	63	61	61	64	65	**70**
1684	**176**	80	90	87	84	77	72	**69**	68	65	66	68	70	**74**
1685	**160**	70	80	82	79	74	69	**63**	61	61	61	64	68	**72**
1686	**160**	80	81	80	75	70	68	**66**	65	64	66	69	72	**73**
1695	**158**	79	80	76	71	66	62	**59**	59	58	59	63	68	**70**
1700	**161**	74	81	81	80	75	70	**66**	65	63	65	68	70	**73**
1715	**145**	62	73	74	74	68	61	**58**	57	56	56	57	58	**61**
1722	**149**	59	78	82	75	71	68	**65**	65	62	62	66	70	**71**
1730	**146**	57	79	74	72	68	63	**59**	58	58	58	60	64	**65**
163	**153**	69	77	76	73	67	62	**57**	55	54	57	58	62	**65**
Avs. 22	**158**	74	80	79	75	70	66	**62**	61	60	61	63	66	**68**

NEGRO MALE. RIGHT SIDE.

1189	**176**	84	87	86	81	78	73	**69**	66	67	69	72	74	**76**
1190	**180**	85	90	90	85	75	68	**65**	63	61	63	67	70	**73**
1453	**168**	70	86	85	78	74	70	**67**	66	67	69	71	72	**75**
1456	**164**	75	81	79	75	71	68	**64**	63	64	65	70	71	**75**
1466	**180**	89	92	89	85	81	77	**75**	72	71	74	76	77	**79**
1470	**160**	76	83	83	80	73	67	**62**	60	59	59	62	65	**69**
1472	**158**	71	81	81	78	72	67	**62**	59	58	58	62	67	**71**
1473	**160**	77	81	80	76	70	64	**63**	61	63	65	68	71	**76**
1476	**162**	78	82	82	79	73	68	**65**	65	64	64	67	69	**72**
1478	**170**	85	85	83	75	70	61	**63**	63	62	64	66	70	**73**
1480	**180**	87	90	88	81	76	70	**67**	66	66	67	70	73	**77**
1486	**176**	82	86	86	82	75	71	**67**	65	62	64	68	72	**76**
1492	**162**	77	80	79	74	69	64	**63**	61	61	63	66	69	**71**
1495	**166**	80	84	85	83	76	71	**67**	66	65	65	66	71	**73**
1497	**170**	77	87	87	83	77	72	**69**	68	66	66	68	72	**74**
1502	**166**	80	84	85	80	75	67	**64**	63	61	62	64	68	**72**
1511	**160**	79	81	82	78	73	67	**65**	64	64	64	65	68	**71**
1519	**160**	75	80	81	79	74	68	**65**	64	64	67	70	74	**76**
1528	**176**	83	89	87	83	80	76	**73**	72	72	77	78	78	**80**
1530	**158**	75	80	80	76	74	70	**66**	65	65	65	68	69	**72**
1532	**180**	86	91	88	81	74	70	**67**	66	65	67	72	76	**80**
1533	**160**	74	79	81	80	77	70	**66**	62	63	63	64	66	**70**
1661	**156**	75	79	78	75	71	61	**62**	62	64	65	67	69	**71**
1680	**164**	80	82	80	75	70	65	**62**	57	57	57	60	64	**67**
1691	**170**	81	87	85	76	70	65	**61**	60	59	60	62	64	**69**
1699	**168**	77	84	86	83	77	72	**68**	65	65	66	68	71	**74**
1701	**169**	82	86	86	82	77	72	**69**	68	69	71	71	74	**77**
1704	**174**	84	89	87	81	77	72	**68**	66	65	68	70	72	**76**
1706	**169**	85	86	84	80	73	70	**68**	67	69	70	72	74	**76**
1709	**179**	86	89	88	82	79	74	**71**	69	68	68	71	74	**76**
1711	**160**	78	81	81	77	74	70	**67**	66	68	68	71	72	**74**
1713	**174**	83	87	86	81	75	70	**66**	65	64	66	69	73	**78**
1718	**157**	75	80	77	72	69	65	**63**	62	63	65	68	69	**73**
1727	**182**	73	91	93	88	80	74	**67**	62	59	58	60	65	**68**
1728	**180**	70	92	95	89	80	74	**68**	64	64	63	64	69	**72**
1731	**176**	82	87	87	82	78	73	**69**	67	66	67	71	76	**78**
1736	**167**	76	78	83	81	76	71	**69**	68	68	69	73	76	**78**
1738	**174**	76	87	87	83	76	73	**68**	65	63	63	64	68	**70**
2521	**173**	83	86	85	81	76	71	**69**	68	66	68	71	71	**72**
2522	**169**	80	84	84	82	75	70	**68**	67	66	68	71	73	**77**
2524	**163**	77	82	81	78	74	71	**70**	68	68	68	71	71	**74**
2535	**159**	75	81	81	78	72	68	**65**	63	63	64	68	71	**73**
105	**148**	63	75	75	74	70	62	**63**	60	59	60	62	66	**67**
173	**173**	82	87	86	80	74	71	**67**	66	64	66	69	72	**75**
B.V.87	**162**	66	81	83	80	75	71	**68**	66	65	65	68	72	**74**
Avs. 45	**168**	79	84	84	80	75	70	**66**	64	64	63	66	70	**74**

NEGRO FEMALE. LEFT SIDE.—(Continued across.)

130°	140°	150°	160°	170°	180°	From anterior end. 1cm.	2cm.	3cm.	4cm.	5cm.	From posterior end. 1cm.	2cm.	3cm.	Index association centers.
65	65	66	71	75	70	12	13	12	10	..	6	2	..	92
71	74	76	79	83	82	7	12	12	13	11	8	13	5	94
73	74	77	77	75	76	0	3	6	7	..	13	11	5	94
77	79	81	83	85	88	0	5	9	9	7	8	12	..	93
74	76	75	74	75	79	1	5	7	8	11	11	15	9	87
72	74	76	78	82	69	9	13	12	11	7	—2	4	—1	90
70	72	74	75	77	79	11	14	16	15	14	5	10	12	84
75	76	76	78	81	70	3	9	12	12	8	0	0	..	98
62	64	66	69	71	72	0	4	7	7	..	12	7	..	95
72	73	72	72	78	74	—3	2	6	8	6	6	7	..	95
67	69	72	74	72	69	—2	1	4	6	4	3	—1	..	90
66	68	70	71	71	76	2	8	10	11	7	20	19	..	87
70	72	73	75	77	77	5	8	10	10	9	9	9	7	91

NEGRO MALE. RIGHT SIDE.—(Continued across.)

76	76	77	80	86	88	9	14	16	18	17	4	11	9	90
75	78	80	81	85	88	3	6	10	11	11	8	17	17	88
77	77	76	83	85	80	—2	3	7	8	8	4	3	..	88
77	78	79	82	84	73	4	10	13	14	12	0	3	..	85
82	81	83	87	91	85	8	13	15	16	16	2	9	8	94
72	74	77	77	80	75	1	7	11	9	..	3	7	..	89
74	77	77	74	78	79	1	7	11	10	..	13	15	8	87
77	78	78	77	80	78	8	10	12	12	10	3	10	5	82
75	76	78	78	81	82	9	12	15	16	16	5	5	2	90
74	76	78	79	82	85	11	14	15	13	10	9	11	10	86
80	82	88	90	91	86	8	12	14	15	11	1	2	0	87
79	81	83	84	87	85	7	11	11	12	13	3	9	6	88
74	75	75	77	80	76	8	15	17	17	16	3	11	11	88
75	76	79	80	84	84	4	7	10	8	7	10	11	..	91
77	79	81	81	85	85	1	6	9	10	8	12	14	..	93
74	74	75	75	81	81	8	14	15	15	13	4	9	5	88
72	72	73	74	78	80	6	10	11	11	11	7	9	5	91
76	76	77	79	81	..	6	8	8	7	7	—6	—4	—8	85
82	82	83	86	88	82	6	11	12	13	11	6	7	2	91
74	76	75	76	80	73	6	7	10	9	8	2	7	1	91
82	83	84	88	92	87	8	13	13	..	..	3	2	5	85
72	75	78	79	81	78	4	7	9	8	..	14	15	..	94
72	74	77	80	71	..	5	9	10	9	..	—9	—5	..	87
70	72	76	77	80	80	7	10	12	12	12	14	12	..	92
70	75	79	80	80	85	6	10	19	19	19	7	20	17	88
77	77	78	79	81	81	4	8	11	11	8	6	8	..	89
78	78	78	82	84	83	4	9	11	12	11	6	14	..	91
77	79	82	88	89	76	5	9	10	10	10	—2	0	..	88
76	76	78	82	87	76	8	10	14	15	14	0	5	..	89
80	82	84	86	91	86	8	11	15	14	13	2	7	..	94
74	75	76	77	80	67	6	11	12	10	..	—2	3	..	90
80	81	82	82	84	86	2	5	8	10	10	13	9	..	84
75	77	78	80	80	70	7	11	14	16	15	0	4	..	85
71	75	79	82	89	90	—2	1	5	10	11	7	15	15	98
74	76	79	82	85	91	—6	0	3	6	7	20	18	..	94
80	82	84	84	89	83	7	10	13	13	12	3	14	..	88
79	80	80	80	83	80	2	9	12	13	12	4	2	..	88
72	73	75	77	83	80	1	6	11	13	11	4	..	..	97
74	75	76	80	84	83	8	12	15	15	12	9	12	..	95
79	80	82	82	84	80	8	10	12	13	12	0	3	..	88
75	76	76	79	82	71	7	10	11	13	14	3	10	..	94
73	73	76	77	80	76	2	4	8	10	8	7	13	..	89
69	70	71	70	73	66	—1	2	6	7	..	3	5	..	94
78	80	80	85	87	76	7	11	13	15	16	0	6	..	89
76	77	77	78	81	76	—4	2	6	9	7	6	7	..	90
76	77	78	80	83	80	5	8	11	12	12	5	8	7	89

TABLE II.—Continued.

NEGRO FEMALE. RIGHT SIDE.

No.	Brain axis.	0°	10°	20°	30°	40°	50°	60°	70°	80°	90°	100°	110°	120°
1449	**162**	72	79	80	81	77	73	**66**	66	63	62	62	64	**68**
1459	**164**	81	82	79	73	68	66	**62**	61	60	61	64	66	**70**
1477	**160**	78	80	81	76	70	64	**62**	61	61	60	63	65	**68**
1479	**162**	78	81	82	79	73	69	**66**	64	62	63	66	68	**71**
1487	**152**	68	76	78	77	74	70	**67**	66	65	65	67	68	**69**
1493	**166**	79	81	77	75	69	65	**63**	62	61	62	63	66	**69**
1500	**154**	75	75	74	70	66	61	**56**	57	58	57	59	59	**61**
1501	**142**	71	72	74	75	72	68	**65**	63	63	65	67	69	**70**
1515	**160**	76	76	74	71	66	65	**62**	60	62	65	69	69	**70**
1544	**160**	74	81	81	79	74	69	**67**	66	65	67	70	73	**74**
1593	**148**	74	73	71	66	61	58	**56**	56	55	55	60	61	**63**
1659	**168**	81	82	81	74	68	63	**60**	60	59	61	63	65	**70**
1678	**160**	70	78	80	79	75	69	**64**	62	60	60	62	65	**68**
1684	**176**	82	90	88	84	76	69	**63**	60	60	60	61	65	**68**
1685	**158**	72	79	82	77	71	67	**62**	59	58	59	59	62	**65**
1686	**160**	79	80	82	78	72	69	**66**	66	67	69	70	72	**74**
1695	**160**	76	81	81	72	71	66	**63**	63	65	66	69	71	**73**
1700	**161**	72	82	83	80	75	70	**66**	65	65	67	69	70	**72**
1715	**147**	67	74	75	71	65	62	**60**	59	57	56	58	60	**62**
1722	**151**	65	78	82	75	72	66	**64**	63	62	62	67	71	**72**
1730	**149**	65	75	75	72	67	62	**59**	59	58	59	63	65	**66**
163	**148**	69	79	82	76	71	66	**62**	60	61	61	65	66	**68**
Avs. 22	**158**	74	79	79	75	71	66	**63**	62	61	62	64	66	**69**

CAUCASIAN MALE. LEFT SIDE.

1406	**172**	82	88	88	86	82	75	**72**	72	71	70	72	75	**77**
1455	**172**	86	86	82	78	75	73	**69**	68	68	69	68	70	**70**
1457	**165**	85	85	85	81	74	69	**66**	65	64	65	67	67	**68**
1458	**174**	85	87	85	82	77	73	**71**	70	70	70	73	75	**76**
1463	**184**	88	92	92	87	81	78	**74**	73	72	73	77	79	**81**
1469	**172**	85	86	85	81	78	73	**69**	67	66	66	68	71	**71**
1489	**168**	84	85	84	80	76	72	**69**	68	66	67	69	70	**71**
1490	**173**	87	88	85	81	77	74	**73**	74	74	74	74	75	**74**
1496	**174**	82	88	88	84	79	73	**70**	67	65	65	65	68	**70**
1512	**166**	74	84	86	85	81	75	**71**	69	68	66	66	68	**70**
1514	**170**	85	86	84	80	75	74	**72**	70	70	71	72	72	**73**
1529	**172**	81	87	90	89	85	81	**79**	78	77	77	80	81	**81**
1538	**162**	81	80	79	73	68	66	**65**	62	62	63	64	66	**69**
1591	**164**	81	82	85	83	77	71	**69**	68	67	67	70	69	**71**
1682	**170**	75	86	89	85	82	76	**72**	69	67	65	67	69	**71**
1683	**164**	81	82	78	77	74	72	**69**	68	69	70	71	70	**72**
1690	**170**	82	87	87	83	77	74	**72**	70	69	69	70	72	**72**
1693	**166**	63	85	88	89	84	77	**74**	71	67	66	66	67	**69**
1696	**168**	81	85	82	78	75	72	**69**	67	67	68	69	70	**71**
1702	**164**	73	82	84	82	76	72	**70**	66	64	64	66	68	**69**
1707	**177**	84	89	90	82	76	72	**67**	62	61	61	67	66	**68**
1708	**169**	82	85	83	80	75	72	**71**	68	66	66	67	68	**69**
1712	**167**	82	81	82	73	69	68	**67**	66	67	69	70	71	**72**
1716	**165**	81	85	82	76	71	69	**66**	65	67	69	71	70	**71**
1719	**173**	86	86	86	81	77	72	**70**	68	70	71	75	75	**75**
1723	**161**	72	81	81	77	75	70	**69**	68	68	68	69	70	**74**
1734	**166**	75	84	86	82	79	73	**69**	67	65	67	67	68	**70**
1748	**185**	89	93	92	90	84	82	**77**	75	77	77	79	78	**79**
1749	**164**	73	84	85	85	82	77	**73**	68	68	69	69	72	**72**
177	**159**	75	79	80	77	70	66	**64**	60	59	61	63	61	**64**
1G.	**160**	78	80	79	74	69	65	**66**	62	61	62	62	63	**66**
3G.	**162**	81	84	82	78	73	69	**67**	65	64	64	65	65	**65**
4G.	**156**	76	79	77	73	69	65	**62**	58	57	57	58	60	**61**
6G.	**156**	70	78	80	78	73	71	**68**	66	66	67	69	71	**72**
Avs. 34	**168**	80	85	85	81	76	72	**70**	68	67	65	66	70	**71**

NEGRO FEMALE. RIGHT SIDE.—(Continued across.)

130°	140°	150°	160°	170°	180°	From anterior end. 1cm.	2cm.	3cm.	4cm.	5cm.	From posterior end. 1cm.	2cm.	3cm.	Index association centers.
69	74	75	78	80	80	2	7	9	9	8	14	14	..	96
72	74	75	78	81	73	11	14	16	15	9	2	4	0	88
70	71	73	73	77	78	7	8	12	12	10	12	13	10	91
75	76	76	76	81	77	5	9	11	9	..	3	7	7	92
70	72	73	73	75	72	3	7	11	13	14	5	10	5	97
71	75	77	81	83	77	9	11	14	13	14	6	6	3	91
64	66	67	69	74	73	7	9	9	9	..	7	7	..	91
71	73	72	71	71	70	8	8	7	..	..	11	15	12	92
70	70	72	77	78	..	10	12	13	10	7	—1	—1	..	88
75	75	76	76	78	80	3	9	12	14	12	10	16	7	90
64	66	65	65	73	74	14	15	..	..	..	7	12	9	88
72	77	78	80	84	76	12	15	17	17	16	9	7	4	88
71	72	73	73	76	78	0	4	5	5	..	14	10	..	94
73	75	79	81	85	82	3	8	10	13	12	3	9	..	92
68	70	72	73	74	78	3	6	10	11	11	10	19	16	95
76	77	78	80	82	71	4	5	7	8	7	1	7	1	89
75	77	77	77	79	79	4	7	9	11	8	7	7	..	86
74	76	76	76	81	79	0	5	8	11	12	8	9	..	91
64	66	68	70	72	73	2	5	7	9	..	17	14	..	97
73	73	71	69	74	73	0	5	8	9	4	4	9	..	89
68	70	72	73	74	75	1	7	10	11	..	8	..	..	89
70	70	70	71	73	71	2	6	9	9	7	12	16	..	91
74	73	74	75	78	76	5	9	10	10	7	8	10	7	91

CAUCASIAN MALE. LEFT SIDE.—(Continued across.)

80	85	85	85	84	86	4	7	12	12	10	8	9	5	93
72	71	75	79	84	86	10	19	19	19	15	13	11	4	98
71	72	72	78	83	85	6	8	11	11	10	13	12	..	97
77	78	78	82	87	86	9	14	17	18	17	7	8	7	93
82	83	83	87	90	90	9	14	15	16	14	10	10	..	90
73	76	78	81	84	85	3	7	9	11	11	6	8	5	97
73	74	76	80	84	84	8	13	15	15	11	12	10	..	97
74	74	76	79	84	87	11	15	18	18	15	7	6	3	98
72	74	76	78	84	86	7	11	15	15	14	9	11	12	100
73	74	75	77	83	78	2	6	9	10	10	4	8	6	101
76	76	76	78	82	86	14	17	19	19	15	10	14	..	98
81	80	79	82	87	86	4	9	10	10	10	7	3	1	97
70	73	74	75	79	81	11	16	17	15	15	13	20	..	94
70	71	70	72	79	81	1	5	9	8	..	8	..	..	97
74	74	75	78	86	73	0	4	8	10	7	0	1	1	101
73	74	75	79	83	75	11	17	18	16	13	4	6	..	96
74	75	78	83	85	87	9	19	14	15	14	13	15	..	100
69	71	74	77	82	78	—5	0	4	5	5	2	2	1	107
73	76	77	81	84	82	8	14	16	16	16	12	10	..	97
71	72	73	77	80	78	1	6	10	11	9	7	8	5	101
70	72	73	79	84	86	5	8	11	10	9	7	9	7	98
73	75	78	83	85	80	9	13	16	17	15	1	3	1	103
74	78	83	86	84	..	16	21	22	21	20	—8	—10	..	93
70	71	71	72	78	83	13	16	15	15	14	10	11	6	93
76	80	83	88	87	76	11	14	15	15	16	0	2	..	93
75	75	74	76	79	80	2	7	9	11	9	9	7	..	93
72	74	76	79	81	83	2	5	8	10	9	15	23	..	98
80	80	84	88	93	72	6	10	10	11	10	—4	0	—1	98
75	76	77	80	85	71	0	6	7	7	4	—1	2	..	101
64	66	67	71	75	79	7	10	12	12	11	6	5	3	100
67	69	72	76	82	79	5	10	13	13	11	4	..	..	100
68	70	74	75	80	83	12	16	17	16	14	8	8	..	103
63	65	67	68	74	79	11	16	16	16	11	9	5	..	102
74	74	75	76	79	71	2	9	12	11	8	2	5	..	94
73	74	76	79	83	81	7	11	13	14	12	6	7	4	98

TABLE II.—Continued.

CAUCASIAN FEMALE. LEFT SIDE.

No.	Brain axis.	0°	10°	20°	30°	40°	50°	60°	70°	80°	90°	100°	110°	120°
1510	**170**	80	85	84	79	72	67	**66**	65	63	65	67	70	**71**
1522	**150**	70	76	76	72	68	65	**62**	61	59	60	60	62	**65**
1527	**164**	73	82	82	80	74	68	**66**	63	63	64	66	68	**69**
1583	**180**	85	91	91	85	78	72	**68**	66	65	65	67	70	**72**
1692	**162**	78	81	80	79	73	69	**65**	63	62	64	66	69	**70**
1697	**156**	75	78	77	72	68	63	**60**	58	58	59	60	61	**62**
2G.	**156**	75	78	77	74	69	64	**63**	62	61	62	62	63	**64**
5G.	**150**	67	75	76	71	67	64	**63**	59	58	58	59	61	**62**
Avs. 8	**161**	75	81	80	77	71	67	**64**	62	61	62	63	66	**67**

CAUCASIAN MALE. RIGHT SIDE.

1406	**174**	74	88	90	88	80	75	**72**	70	69	69	69	71	**73**
1455	**172**	86	86	81	78	75	74	**71**	70	71	73	75	77	**76**
1457	**165**	75	85	86	78	72	68	**67**	66	65	65	67	68	**69**
1458	**172**	81	85	81	81	74	70	**69**	68	69	70	72	75	**77**
1463	**182**	84	91	93	89	86	83	**81**	78	73	74	76	79	**79**
1469	**172**	80	87	82	79	75	71	**68**	66	63	64	66	68	**70**
1489	**166**	78	84	83	80	75	72	**69**	67	66	67	68	70	**74**
1490	**170**	83	86	87	80	78	73	**72**	70	69	71	73	74	**74**
1496	**174**	82	88	91	88	84	81	**77**	73	70	70	71	72	**73**
1512	**170**	81	86	91	87	82	74	**68**	66	65	64	67	68	**71**
1514	**168**	84	84	83	78	74	73	**71**	70	69	70	73	74	**74**
1529	**172**	82	88	85	84	78	75	**73**	72	71	71	75	76	**78**
1538	**162**	79	81	78	74	70	68	**68**	66	67	68	70	71	**72**
1591	**162**	69	82	85	83	79	74	**72**	72	69	68	71	71	**72**
1682	**170**	77	88	89	84	78	75	**73**	71	71	71	73	72	**75**
1683	**164**	80	82	79	76	71	67	**66**	63	65	64	66	69	**70**
1690	**170**	82	86	87	83	79	73	**69**	66	66	68	69	71	**72**
1693	**166**	65	82	87	87	84	78	**76**	74	74	73	75	76	**77**
1696	**170**	81	86	85	81	78	73	**70**	68	67	68	71	71	**73**
1702	**160**	70	80	81	79	75	72	**70**	68	65	65	66	68	**69**
1707	**174**	75	85	88	83	79	75	**70**	67	68	67	70	73	**74**
1708	**170**	80	84	85	81	77	74	**72**	71	70	70	71	72	**73**
1712	**168**	73	83	85	84	78	74	**70**	70	69	71	73	74	**74**
1716	**162**	77	81	81	78	74	70	**69**	68	68	69	72	74	**75**
1719	**176**	86	88	84	82	75	72	**70**	68	70	71	73	75	**75**
1723	**163**	75	81	82	80	75	73	**71**	71	69	69	72	73	**73**
1734	**168**	69	83	86	83	78	75	**70**	67	67	65	67	68	**69**
1748	**183**	90	92	91	88	84	80	**77**	75	76	75	76	78	**80**
1749	**159**	73	79	82	80	75	72	**70**	68	68	70	72	72	**75**
177	**158**	74	80	83	79	72	66	**64**	60	59	59	61	63	**63**
1G.	**160**	79	81	79	72	66	63	**58**	55	55	56	56	59	**61**
3G.	**160**	76	81	80	77	72	68	**65**	61	61	62	63	65	**66**
4G.	**152**	72	75	76	76	73	71	**70**	68	67	67	67	67	**68**
6G.	**156**	72	78	81	79	76	72	**68**	64	63	65	67	67	**69**
Avs. 34	**167**	78	84	84	81	77	73	**70**	68	65	65	69	71	**72**

CAUCASIAN FEMALE. RIGHT SIDE.

1510	**168**	84	85	84	81	73	71	**69**	69	66	67	70	72	**72**
1522	**148**	68	73	76	72	64	60	**63**	61	60	59	61	62	**63**
1527	**160**	75	81	81	80	74	68	**65**	62	62	65	66	70	**69**
1583	**176**	85	88	88	82	76	72	**69**	66	66	62	69	73	**76**
1692	**164**	80	83	83	79	73	69	**66**	64	62	62	65	68	**71**
1697	**157**	75	78	76	64	67	62	**60**	58	58	57	58	60	**61**
2G.	**152**	72	77	79	76	71	66	**62**	58	57	56	58	59	**62**
5G.	**150**	64	72	77	72	69	66	**64**	60	58	56	58	59	**60**
Avs. 8	**160**	75	80	81	76	71	67	**65**	62	61	61	63	65	**67**

CAUCASIAN FEMALE. LEFT SIDE.—(Continued across.)

130°	140°	150°	160°	170°	180°	From anterior end. 1cm.	2cm.	3cm.	4cm.	5cm.	From posterior end. 1cm.	2cm.	3cm.	Index association centers.
72	73	74	77	82	85	3	9	11	13	13	8	9	4	**93**
66	67	69	69	72	75	6	10	12	12	7	8	3	..	**95**
72	72	72	75	79	82	0	6	10	10	7	12	14	..	**95**
76	79	80	84	89	85	5	8	11	11	7	2	14	10	**93**
72	73	74	74	77	80	10	14	16	17	16	6	10	6	**93**
64	68	71	77	79	50	9	12	13	13	11	—2	—1	0	**97**
67	68	71	75	78	75	6	1	12	12	9	8	4	1	**98**
64	67	70	74	74	..	2	6	9	9	8	—5	—5	..	**101**
69	71	73	76	79	77	5	8	12	12	10	5	6	4	**96**

CAUCASIAN MALE. RIGHT SIDE.—(Continued across.)

77	79	79	82	83	88	—2	4	8	10	11	11	10	9	**98**
76	76	76	80	85	81	15	20	21	18	8	3	11	8	**93**
72	75	76	79	84	78	1	9	12	12	12	2	7	7	**97**
77	79	80	82	86	85	8	14	16	17	16	1	5	4	**90**
79	81	84	87	91	82	2	12	15	15	13	1	10	6	**102**
71	74	75	75	79	84	2	9	12	14	15	17	15	16	**98**
76	76	80	81	85	80	8	14	15	14	12	4	4	—1	**93**
73	75	76	80	81	85	10	13	15	17	17	16	11	..	**97**
74	76	76	80	87	86	4	10	13	12	12	4	10	10	**105**
74	77	78	83	88	82	2	7	10	10	..	6	8	..	**96**
75	78	78	81	86	83	16	20	20	20	15	6	11	..	**96**
80	79	78	81	86	81	6	11	14	15	14	7	8	3	**93**
73	75	76	78	81	79	10	13	15	14	12	9	9	..	**94**
73	72	71	73	78	81	—2	3	6	6	0	3	5	4	**100**
75	76	75	78	85	85	0	5	8	10	9	4	3	3	**97**
72	75	75	78	82	75	11	14	16	16	15	2	—1	..	**94**
74	77	76	78	84	84	8	12	15	16	15	7	12	10	**96**
77	77	77	79	83	75	—3	0	2	4	5	1	4	2	**99**
75	76	77	78	84	81	9	12	16	14	14	5	8	5	**96**
70	71	75	76	80	86	1	4	6	9	6	—2	7	..	**101**
75	76	78	82	87	..	1	5	9	9	7	—5	—2	—3	**93**
74	77	78	81	84	83	5	12	14	15	12	8	9	..	**98**
75	77	79	82	84	75	3	11	12	14	12	9	..	..	**94**
75	75	74	76	82	73	7	11	14	14	12	1	9	..	**93**
75	76	78	85	88	83	13	16	17	18	15	4	10	..	**94**
74	75	74	74	76	80	3	8	13	13	8	8	8	..	**97**
70	74	75	78	80	82	—3	3	6	7	6	20	22	21	**101**
82	84	86	89	92	78	9	10	13	15	14	—4	4	2	**96**
76	76	76	77	78	73	5	10	12	12	8	2	5	..	**93**
65	65	67	70	75	80	7	10	12	14	13	8	9	..	**101**
65	68	71	77	61	70	11	13	17	16	14	2	2	..	**95**
69	72	75	76	80	80	7	12	17	18	15	4	10	..	**98**
67	66	67	69	75	74	5	10	12	13	9	10	7	..	**103**
71	73	74	78	80	74	7	11	13	14	10	2	..	..	**98**
73	75	76	76	81	80	5	9	13	13	11	5	7	6	**97**

CAUCASIAN FEMALE. RIGHT SIDE.—(Continued across.)

73	72	73	76	80	83	4	7	10	9	7	4	9	4	**96**
66	67	67	69	70	71	6	10	11	11	8	8	12	6	**100**
72	73	73	75	80	75	4	7	10	12	8	3	..	..	**94**
78	81	81	85	88	85	10	14	14	12	9	6	12	9	**90**
73	75	75	79	80	81	6	12	15	15	14	11	11	..	**93**
64	66	69	74	78	73	8	11	12	12	9	2	8	..	**98**
66	67	68	70	75	76	3	8	11	11	7	9	11	8	**100**
62	64	66	73	76	65	—1	6	9	10	9	0	..	..	**106**
69	71	72	75	78	76	5	9	12	12	9	5	12	7	**97**

TABLE III.—AVERAGE LENGTH OF THE RADII IN MM. OF THE ANTERIOR AND POSTERIOR HALVES OF THE BRAIN FOR THE PLANES—HORIZONTAL, VERTICAL, AND AT 45°.

	Negro Male.					
Number.	Left Side.			Right Side.		
	Brain axis.	Anterior.	Posterior.	Anterior.	Posterior.	Brain axis.
1189	176	69	70	68	69	176
1190	184	66	70	67	70	180
1451	178	69	71	70	72	176
1453	168	65	64	67	72	168
1456	164	64	71	61	71	164
1466	180	69	74	70	74	180
1470	164	60	65	62	67	160
1472	154	61	68	60	68	158
1473	160	60	70	61	71	160
1476	164	65	71	64	69	162
1478	166	62	69	63	69	170
1480	180	71	78	67	72	180
1486	174	66	69	64	71	176
1492	164	64	69	62	67	162
1495	170	64	69	64	69	166
1497	170	64	68	66	72	170
1502	164	66	69	64	70	166
1511	158	64	67	64	69	160
1519	160	63	71	62	68	160
1524	160	69	66	66	72	160
1528	176	68	73	70	76	176
1530	158	59	67	63	67	158
1533	160	64	69	64	68	160
1660	182	63	74	67	72	188
1661	156	60	65	60	66	156
1680	166	63	65	59	65	164
1691	172	63	68	60	67	170
1699	166	63	69	65	71	168
1701	167	66	72	67	75	169
1704	177	66	71	64	70	174
1706	173	66	70	66	71	169
1709	181	69	73	67	73	179
1711	159	62	71	63	70	160
1713	171	65	73	63	72	174
1718	156	62	70	63	70	157
1727	180	65	66	65	66	182
1728	182	65	75	64	68	180
1731	176	67	75	66	75	176
1736	164	63	73	65	72	167
1738	173	67	67	66	68	174
1739	157	66	77	65	70	164

TABLE III.—Continued.

Number.	Left Side.			Right Side.		
	Brain axis.	Anterior.	Posterior.	Anterior.	Posterior.	Brain axis.
			Negro Male.			
2469	176	62	68	66	72	176
2521	171	65	72	66	70	173
2522	171	67	72	65	72	169
2524	165	66	71	67	71	163
2535	160	62	69	62	69	159
87	165	65	70	64	69	162
172	168	58	63	60	63	170
173	171	61	67	64	71	173
193	166	58	63	55	61	166
105	148	68	75	61	72	148
107	142	60	60	60	59	143
109	133	53	57	53	58	136
110	...	..	..	47	53	131
111	114	42	47	42	45	115
112	113	45	51	47	53	113
113	126	45	55	47	51	124
			Negro Female.			
1449	164	64	68	65	65	162
1452	180	67	70	68	75	177
1459	162	59	65	66	66	164
1477	160	65	67	62	65	160
1479	162	62	67	63	68	162
1487	152	59	64	62	66	152
1493	166	60	65	61	67	166
1500	154	56	62	56	60	154
1501	144	64	68	65	67	142
1515	164	61	64	60	68	160
1521	170	64	68	65	68	166
1544	160	63	70	64	71	160
1659	168	61	65	59	66	168
1662	174	65	70	64	72	172
1678	158	62	65	63	65	160
1684	176	66	69	59	64	176
1685	160	63	69	63	65	158
1686	160	62	68	62	68	160
1687	165	56	61	53	57	165
1695	158	60	67	62	69	160
1700	161	64	70	64	70	161
1715	145	57	60	59	62	147
1722	149	63	68	62	68	151
1730	146	59	66	58	66	149
108	118	53	56	53	55	118
163	153	56	63	60	66	148

TABLE III.—Continued.

Number.	Caucasian Male.					
	Left Side.			Right Side.		
	Brain axis.	Anterior.	Posterior.	Anterior.	Posterior.	Brain axis.
1405	...	69	73	69	71	...
1455	172	67	69	69	73	172
1457	165	66	68	66	68	165
1458	174	68	74	68	74	172
1463	184	72	77	73	74	182
1469	172	68	71	66	69	172
1489	168	68	70	66	71	166
1490	173	71	73	68	71	170
1496	174	69	71	71	73	174
1512	166	71	72	68	68	170
1514	170	70	73	69	72	168
1520	178	69	77	70	76	176
1529	172	74	77	71	75	172
1538	162	66	72	67	69	162
1591	164	68	69	68	70	162
1682	170	67	66	72	75	170
1683	164	66	70	65	69	164
1693	166	78	75	80	79	166
1696	168	68	71	68	72	170
1702	164	67	66	66	65	160
1707	177	65	66	66	68	174
1708	169	69	70	70	71	170
1712	167	67	69	67	70	168
1716	165	65	70	66	71	162
1719	173	67	72	68	73	176
1723	161	67	70	69	71	163
1734	166	67	69	67	70	168
1748	185	74	75	73	77	183
1749	164	68	68	66	70	159
164	166	61	62	66	66	168
169	175	67	65	62	64	169
177	159	61	62	59	61	158
3G.	162	65	64	64	68	160
4G.	156	61	62	64	65	152
6G.	156	65	70	64	65	156

TABLE III.—CONTINUED.

CAUCASIAN FEMALE.

Number.	Left Side.			Right Side.		
	Brain axis.	Anterior.	Posterior.	Anterior.	Posterior.	Brain axis.
1485	158	61	64	62	68	158
1510	170	64	69	67	70	168
1522	150	62	63	62	62	148
1527	164	65	69	62	68	160
1583	180	65	73	65	74	176
1692	162	65	68	65	68	164
1697	156	60	63	59	61	157
1G.	160	64	65	59	59	158
2G.	156	62	62	59	62	152
5G.	150	62	62	62	69	150

TABLE IV.—SHOWING THE POSITION OF THE FISSURE OF ROLANDO IN DEGREES MEASURED FROM THE BRAIN CENTER.

CAUCASIAN MALE.

No.	Left Side.				Right Side.			
	Inferior end. °	Middle end. °	Superior end. °	Superior terminal. mm.	Inferior end. °	Middle end. °	Superior end. °	Superior terminal. mm.
1702	76	94	110	15	76	86	108	15
1707	76	83	108	20	78	82	107	20
1708	79	93	102	18	83	98	104	14
1712	68	74	97	9	75	77	99	14
1716	75	88	105	23	80	88	102	26
1719	65	70	100	30	75	80	100	24
1720	70	80	110	30	76	85	105	32
1723	75	88	113	20	70	88	105	18
1734	75	97	114	15	78	93	113	26
1748	70	92	...	28	70	84	107	32
1749	78	90	112	25	83	86	103	25
164	65	99	108	25	75	85	104	16
169	75	84	103	16	77	78	95	16
177	70	97	115	18	75	100	118	18
1490	..	83	110	..	..	93	105	..
1469	..	103	116	..	..	92	118	..
1455	..	104	105	..	..	98	115	..
1514	..	84	107	..	..	90	105	..
1G.	..	78	93	..	..	82	103	..
3G.	..	87	110	..	..	88	105	..
4G.	..	91	113	..	..	93	110	..
6G.	..	86	102	..	..	90	110	..

AVERAGES OF ABOVE COLUMNS—22, 73, 88, 107, 21, 77, 88, 106, 21.

TABLE IV.—CONTINUED.

No.	Left Side. Inferior end. °	Middle end. °	Superior end. °	Superior terminal. mm.	Right Side. Inferior end. °	Middle end. °	Superior end. °	Superior terminal. mm.
CAUCASIAN FEMALE.								
1697	68	86	103	15	74	76	105	19
2G.	..	85	113	..	..	88	113	..
5G.	..	78	104	..	..	82	104	..
AVERAGES OF ABOVE COLUMNS—3, 68, 83, 107, 15, 74, 82, 107, 19.								
NEGRO MALE.								
1699	75	90	112	30	80	85	114	31
1701	79	91	105	17	82	88	105	14
1704	77	85	106	24	77	80	105	23
1706	65	78	102	15	65	83	104	28
1709	83	97	113	32	88	90	113	29
1711	78	92	111	40	73	87	108	40
1713	78	87	108	28	86	93	112	22
1718	..	89	108	..	..	89	108	..
1727	77	86	111	24	75	86	114	24
1728	77	90	110	10	76	88	104	10
1731	80	84	104	32	78	90	107	34
1736	77	95	119	32	71	88	114	32
1738	70	85	103	8	72	86	107	10
1739	77	84	120	35	82	89	116	33
1741	80	88	105	26	80	88	109	26
2521	70	81	105	24	79	81	103	16
2522	76	85	106	20	76	85	110	18
2524	75	84	105	12	80	84	103	12
2535	70	91	110	18	80	85	111	18
173	78	106	105	20	82	92	107	15
193	77	78	105	18	77	76	105	15
107	77	95	107	10	74	95	107	8
109	78	96	..116	20	85	90	110	16
110	..	..	..	..	90	100	114	20
1470	..	88	117	..	..	90	117	..
1190	..	90	110	..	..	90	105	..
1189	..	83	103	..	..	83	103	..
AVERAGES OF ABOVE COLUMNS—27, 76, 88, 108, 22.5, 77, 87, 108, 21.								

TABLE IV.—CONTINUED.

NEGRO FEMALE.

No.	Left Side. Inferior end. °	Middle end. °	Superior end. °	Superior terminal. mm.	Right Side. Inferior end. °	Middle end. °	Superior end. °	Superior terminal. mm.
1678	78	87	102	15	80	88	116	16
1700	78	90	107	21	80	86	107	21
1715	82	90	112	21	85	93	112	23
1722	78	90	120	24	78	79	117	20
1730	85	92	116	20	85	89	116	14
163	79	95	120	25	79	85	115	23
108	77	92	118	14	84	92	113	14
1515	..	85	108	..	..	83	102	..
1479	..	94	108	..	..	87	108	..
1500	..	78	105	..	..	72	112	..
1684	..	95	106	..	..	93	115	..

AVERAGES OF ABOVE COLUMNS—11, 80, 90, 111, 20, 81, 86, 112, 19.

TABLE V.—GIVING THE AREA OF THE OUTLINE OF THE BRAIN IN FRONT AND BEHIND THE FISSURE OF ROLANDO IN THE DIFFERENT PLANES (45°, 90°, and 0°) WHICH INTERSECT THE BRAIN AXIS. THE MEASUREMENTS ARE IN SQUARE CENTIMETERS.

CAUCASIAN MALE.

No.	Total end.	Left Side. Plane at 45°. Anterior.	Plane at 45°. Posterior.	Horizontal Plane. Anterior.	Horizontal Plane. Posterior.	Vertical Plane. Anterior.	Vertical Plane. Posterior.	Right Side. Plane at 45°. Anterior.	Plane at 45°. Posterior.	Horizontal Plane. Anterior.	Horizontal Plane. Posterior.	Vertical Plane. Anterior.	Vertical Plane. Posterior.
1702	532.7	49.5	43.6	36.4	47.9	55.0	40.2	45.3	44.0	34.6	42.2	53.6	40.4
1707	572.6	47.7	49.4	40.0	50.0	57.2	45.0	47.2	48.1	41.4	48.4	55.4	42.8
1708	608.6	53.0	45.2	40.2	46.6	58.6	48.6	57.5	58.4	42.5	48.0	62.0	48.0
1712	579.8	46.8	50.2	35.6	53.2	60.2	41.8	47.0	55.8	39.0	48.7	59.5	42.0
1716	571.4	50.2	47.8	37.7	50.0	56.8	43.8	49.6	48.4	39.6	46.7	55.0	45.8
1719	630.0	47.8	58.5	35.2	55.5	64.4	51.0	52.8	56.2	41.0	53.0	64.0	50.6
1720	582.5	43.6	49.2	36.3	56.2	61.5	44.6	47.7	45.1	41.3	51.6	60.8	44.6
1723	559.5	46.6	48.0	34.7	49.2	57.8	40.8	49.3	47.8	34.7	49.6	56.0	45.0
1734	594.7	52.5	51.2	34.6	49.2	62.0	47.7	49.2	51.6	36.0	49.2	62.0	49.5
1748	689.9	62.7	52.8	43.5	59.1	125.5		60.3	59.1	44.6	62.1	71.5	48.7
1749	556.2	50.4	45.7	36.4	47.7	59.0	42.4	47.4	48.4	37.4	44.8	53.8	42.8
164	563.1	50.4	40.6	29.8	49.0	56.4	43.0	51.4	52.2	36.2	47.0	59.3	47.8
169	553.5	50.5	45.2	40.0	57.5	53.6	37.5	46.7	47.2	39.2	46.2	50.2	39.7
177	494.7	47.2	36.2	33.6	39.0	53.0	37.0	49.0	36.5	36.3	37.6	54.2	35.1
1490	631.2	55.0	53.0	100.0		69.0	45.0	57.5	51.3	88.4		66.4	45.4
1469	596.8	56.6	43.4	92.8		60.0	43.7	51.2	50.8	93.0		58.5	46.8
1455	614.2	62.0	44.0	90.0		62.5	44.2	60.6	48.8	91.7		71.0	39.4
1514	634.1	55.7	55.4	95.2		67.0	48.0	56.4	52.0	95.2		61.8	47.4
1G.	504.6	41.8	44.5	81.5		51.9	40.6	42.0	39.3	74.0		53.2	35.8
3G.	548.5	50.4	45.4	80.2		61.5	39.6	46.5	44.5	80.8		60.6	39.0
4G.	508.0	45.0	38.0	78.1		55.2	36.2	47.7	40.2	76.0		55.8	35.8
6G.	523.8	45.1	45.7	81.8		52.7	38.3	48.3		79.3	53.0	37.4	
Avs. 22	574.9	50.5	47.0	36.7	50.7	59.0	42.8	50.5	48.5	38.8	48.2	59.0	43.2

TABLE V.—Continued.

No.	Total end.	Left Side. Plane at 45°. Anterior.	Left Side. Plane at 45°. Posterior.	Left Side. Horizontal Plane. Anterior.	Left Side. Horizontal Plane. Posterior.	Left Side. Vertical Plane. Anterior.	Left Side. Vertical Plane. Posterior.	Right Side. Plane at 45°. Anterior.	Right Side. Plane at 45°. Posterior.	Right Side. Horizontal Plane. Anterior.	Right Side. Horizontal Plane. Posterior.	Right Side. Vertical Plane. Anterior.	Right Side. Vertical Plane. Posterior.
CAUCASIAN FEMALE.													
1697	475.3	41.4	36.8	30.3	45.3	47.5	35.0	36.8	42.2	32.0	43.5	48.7	35.8
2G.	484.2	42.5	42.1	73.2		51.7	34.4	41.5	39.4	73.0		50.8	35.6
5G.	434.6	35.0	37.1	71.4		45.3	28.4	37.5	35.6	70.3		46.0	28.0
Avs. 3	464.4	39.6	38.7	30.3	45.3	48.2	32.6	38.6	39.1	32.0	43.5	48.5	33.1
NEGRO MALE.													
1699	562.1	46.5	46.5	34.6	48.6	55.2	44.5	48.0	50.5	37.8	48.0	57.3	44.6
1701	609.8	52.0	48.5	41.2	50.4	60.4	47.0	52.0	55.4	40.6	51.6	61.5	49.2
1704	598.5	52.0	52.4	40.6	51.6	60.3	44.2	47.8	53.4	41.0	54.0	58.3	42.9
1706	594.4	48.6	54.5	34.5	50.8	60.0	48.0	50.4	51.6	34.5	54.5	60.8	46.2
1709	644.4	60.8	53.8	42.4	47.8	70.6	49.4	56.8	54.8	44.0	48.5	68.7	46.8
1711	530.4	45.0	45.0	33.5	45.0	56.8	38.6	47.0	46.0	33.2	45.5	55.0	39.8
1713	583.9	47.5	54.3	38.0	46.2	55.5	46.8	50.4	53.4	39.4	46.4	57.8	48.2
1718	531.8	44.6	45.2	72.2		56.3	43.1	45.6	46.6	77.2		57.9	43.1
1727	607.2	51.0	51.9	38.2	48.5	63.0	47.8	50.0	52.6	38.4	49.0	65.7	51.2
1728	615.3	52.0	53.0	40.4	53.0	61.0	48.8	49.5	55.8	41.5	49.2	60.6	50.5
1731	634.3	52.0	57.2	40.7	50.5	63.7	50.0	53.2	57.1	42.4	52.5	61.8	53.2
1736	569.7	48.6	47.6	36.0	50.8	59.2	41.0	49.0	51.1	36.3	50.8	56.5	42.8
1738	576.2	48.4	49.8	34.4	49.2	61.2	47.9	48.4	45.4	35.2	49.5	62.8	44.0
2521	598.6	48.6	53.6	35.5	52.2	60.1	47.5	49.4	54.2	41.0	49.1	58.7	48.7
2522	597.3	51.4	51.0	39.2	50.0	64.4	46.2	48.6	52.0	36.8	48.7	61.8	47.2
2524	583.4	49.5	50.5	36.6	50.5	60.5	46.0	48.3	51.0	36.9	47.2	59.8	46.6
2535	539.9	44.7	45.2	29.5	46.2	57.5	45.8	42.8	50.4	34.3	43.6	56.3	43.6
173	582.1	54.7	41.0	37.7	47.4	60.3	43.5	52.8	50.0	39.1	47.0	62.0	46.6
193	486.8	40.0	44.0	33.2	45.0	49.6	34.4	38.6	39.4	34.0	46.0	47.1	35.5
1470	544.9	45.6	48.6	81.2		57.3	43.7	44.8	44.2	79.6		58.2	41.0
1190	635.3	54.1	52.8	105.6		63.8	50.6	50.4	54.2	88.6		63.4	51.8
1189	624.4	53.6	56.0	94.4		63.0	47.3	53.2	55.7	92.6		61.3	47.3
Avs. 22	583.7	49.6	50.1	37.0	49.1	60.0	45.5	48.9	51.1	38.1	49.1	59.7	45.5
NEGRO FEMALE.													
1678	512.1	42.5	47.0	31.5	42.5	50.0	44.0	42.0	45.2	32.0	40.6	55.8	39.0
1700	549.5	47.4	44.5	35.7	47.0	55.0	40.4	46.6	49.2	39.7	47.2	55.0	41.8
1715	449.9	36.7	36.2	29.2	32.8	45.8	36.4	37.8	39.5	32.7	37.8	47.3	37.7
1722	490.4	41.0	41.6	33.3	43.3	51.2	35.2	37.6	47.3	32.0	41.4	47.9	38.6
1730	456.4	37.0	35.4	31.6	38.7	48.8	32.8	37.8	41.0	30.4	39.8	49.3	33.8
163	485.8	40.3	41.7	30.0	38.4	52.9	39.0	39.7	45.0	30.3	37.5	51.0	40.0
1515	498.0	43.6	45.5	81.8		50.3	34.9	40.0	42.7	76.4		46.6	36.2
1479	547.5	46.0	45.6	80.4		55.8	48.0	45.0	47.6	80.4		54.4	44.3
1500	464.5	37.2	41.9	74.6		47.0	33.5	33.5	42.3	72.5		48.6	33.4
1684	566.2	53.0	51.9	91.8		53.0	44.0	50.8	47.0	80.3		54.2	40.2
Avs. 10	492.0	42.5	43.1	31.9	40.4	51.0	38.8	41.1	44.7	33.0	40.7	51.0	38.5

TABLE VI.—AREA OF THE ANTERIOR AND POSTERIOR LINEAL HALVES OF THE CORPUS CALLOSUM IN SQUARE CENTIMETERS.

Negro Male.			Caucasian Male.			Negro Female.			Caucasian Female.		
No.	Anterior.	Posterior.	No.	Anterior.	Posterior.	No.	Anterior.	Posterior.	No.	Anterior.	Posterior.
1189	4.20	3.55	1216	4.45	3.40	1449	3.20	3.00	1485	1.80	2.50
1190	3.50	3.25	1405	3.85	3.05	1452d	3.50	3.00	1510	3.00	2.55
1246	2.95	3.40	1455	3.60	2.60	1459	3.20	2.85	1522	3.55	2.70
1451d	4.25	3.60	1457	3.05	2.75	1477	2.40	2.50	1527	3.60	3.55
1453	2.35	2.45	1458	3.90	3.25	1479	2.85	3.25	1583	3.60	3.70
1454	2.95	3.15	1463	4.10	3.10	1487	1.70	1.90	1692	3.70	3.00
1456	3.30	3.55	1469	2.35	2.20	1493	2.65	2.50	1697	3.20	2.15
1466m	3.50	3.50	1489	4.10	3.50	1500	2.25	2.05	Ger. 2	2.65	3.00
1467	3.05	3.35	1490	4.90	3.95	1501	2.70	3.35	" 5	3.40	2.70
1470	2.50	2.75	1496	4.45	3.05	1515	2.40	2.55			
1472	2.05	2.25	1512	3.70	2.80	1521	3.30	3.40			
1473	2.60	2.75	1514	4.00	3.00	1544	3.35	3.00			
1476	2.80	2.70	1520	4.65	3.45	1653	3.70	4.10			
1478	3.20	3.50	1529	3.50	2.70	1659	2.10	2.90			
1480d	4.00	3.10	1538	3.80	3.00	1662	3.75	3.45			
1486	3.45	3.20	1591	3.60	3.10	1678	2.95	3.00			
1492	2.50	2.10	1682	3.90	3.10	1685	3.10	3.20			
1495	2.80	2.65	1683	3.05	3.05	1686	3.75	3.45			
1497	3.10	3.35	1690	4.10	3.55	1687m	2.60	2.20			
1502	2.80	2.75	1693	3.75	2.10	1695m	2.55	2.35			
1511	3.00	3.05	1696	4.10	3.35	1700	3.65	3.40			
1519	2.65	2.40	1702	3.60	2.85	1715	2.00	2.05			
1524m	3.35	2.55	1707	2.40	2.10	1722	2.70	2.70			
1528	4.50	4.70	1708	3.25	2.70	1730	2.60	2.70			
1530	3.45	3.55	1712	3.80	3.30	163	2.50	2.60			
1533	2.75	2.80	1716	2.65	2.40						
1582	2.80	2.80	1719	4.80	4.30						
1650m	4.00	3.80	1720d	3.80	3.75						
1660	3.50	3.40	1723	2.85	2.65						
1661	2.60	2.70	1734	3.80	3.40						
1667m	3.30	3.20	1748	4.30	3.80						
1680	2.15	2.15	1749	4.10	3.40						
1691	2.15	2.40	B.V. 164	3.20	2.60						
1699	3.50	2.85	" 169	2.60	1.75						
1701	3.70	3.45	" 177	2.35	2.15						
1704	3.45	3.70	Ger. 1	3.25	2.65						
1706	3.75	3.50	" 3	4.30	3.50						
1709m	4.55	3.70	" 4	3.20	2.60						
1711m	3.50	2.65	" 6	2.90	2.40						
1713	3.55	3.50	Leidy.	6.00	5.10						
1718	3.75	3.85	Seguin.	4.20	3.90						
1727	3.70	3.65	Laborer, S.	2.90	2.30						
1728	3.20	3.60									
1731	4.10	4.40									
1736	2.90	2.90									
1738	2.80	2.60									
1739d	2.60	3.20									
2469d	3.15	2.75									
2521	2.40	2.50									
2522	3.05	3.40									
2524	3.50	3.30									
2535	3.10	3.10									
B.V. 87	3.50	3.10									
" 172	3.10	3.30									
" 173	3.25	3.65									
" 193	2.20	2.10									
105	2.25	2.15									
107m	2.85	2.50									
109	1.30	1.50									
111	1.15	1.60									
112	1.05	1.35									
Laborer, S.	3.00	2.50									

d = distorted. m = mulatto.

TABLE VII.—RELATION OF THE PARTS OF THE CORPUS CALLOSUM TO ONE ANOTHER.

Negro Male.					Caucasian Male.					Caucasian Female.				
No.	Splenium.	Isthmus.	Body	Genu.	No.	Splenium.	Isthmus.	Body.	Genu.	No.	Splenium	Isthmus.	Body.	Genu.
1189	200	105	145	310	1216	180	110	175	335	1485	130	90	90	160
1190	190	90	145	240	1405	155	100	175	300	1510	135	85	125	230
1246	225	75	130	210	1455	140	90	150	280	1522	150	95	135	260
1453	160	55	115	160	1457	175	85	140	220	1527	205	95	130	280
1454	165	95	140	220	1458	180	105	150	300	1583	200	100	180	270
1456	200	100	165	240	1463	165	115	115	330	1692	170	80	155	295
1466	205	115	160	235	1469	125	65	90	185	1697	100	75	135	245
1467	200	90	140	205	1489	160	120	170	310	2 G.	150	85	130	205
1470	160	75	105	195	1490	220	110	200	370	5 G.	155	65	125	255
1472	150	50	100	140	1496	155	85	175	335	R. 1	210	90	170	330
1473	170	70	110	170	1512	165	85	150	280	R. ..	180	100	160	240
1476	155	70	125	195	1514	180	80	140	300	R. 4	140	80	110	220
1478	200	80	155	220	1520	195	85	160	370	R. 6	160	90	150	240
1480	210	80	145	300	1529	140	85	140	260	R. 7	180	120	140	210
1486	195	80	155	245	1538	170	90	160	270	R. ..	110	40	80	170
1492	110	55	100	200	1591	185	95	150	260	R. ..	170	100	140	250
1495	160	65	135	210	1682	165	110	170	285	§	190	110	150	240
1497	210	85	150	220	1683	155	100	140	235					
1502	180	60	130	190	1690	195	100	170	315	§ Kovalewski.				
1511	190	80	130	210	1693	120	55	115	290					
1519	140	60	120	170	1696	190	90	165	305					
1524	135	90	155	220	1702	145	90	140	260					
1528	290	125	170	320	1707	110	70	100	190					
1530	225	70	150	235	1708	165	65	140	240					
1533	170	60	120	195	1712	175	125	145	275	Negro Female.				
1582	180	70	120	190	1716	130	85	105	195	1449	170	90	140	220
1650	255	110	140	275	1719	225	135	205	340	1452	190	85	130	255
1660	200	85	170	255	1720	215	115	175	260	1459	175	70	140	225
1661	155	75	115	190	1723	135	100	120	205	1477	155	70	125	165
1667	210	60	120	220	1734	185	95	165	285	1479	210	80	125	200
1680	130	60	110	160	1748	205	125	170	320	1487	115	45	85	110
1691	155	75	95	150	1749	205	90	155	300	1493	160	65	95	200
1699	160	80	160	245	164	160	70	120	245	1500	120	55	100	160
1701	190	105	170	260	169	100	50	100	200	1501	220	85	135	185
1704	205	120	160	240	177	110	75	110	160	1515	160	65	110	165
1706	210	110	150	260	1 G.	135	90	125	255	1521	225	90	145	220
1709	200	100	210	315	3 G.	185	100	180	325	1544	190	95	150	230
1711	145	85	155	240	4 G.	140	85	140	235	1653	260	85	130	230
1713	200	100	160	255	6 G.	135	75	130	205	1659	180	80	125	140
1718	225	110	170	260	R. 3	170	110	170	260	1662	200	110	155	280
1727	210	85	150	260	R. 5	160	70	150	220	1678	185	60	145	205
1728	210	100	140	225	R. ..	190	90	140	280	1685	195	100	135	210
1731	215	145	185	285	R. ..	180	90	170	240	1686	210	110	140	260
1736	160	80	145	200	R. 16	200	130	180	330	1687	135	60	115	175
1738	145	80	105	195	R. 18	190	130	180	280	1695	135	80	130	155
2469	185	80	110	210	R. 20	210	100	160	310	1700	190	100	165	260
2521	145	70	110	175	R. 22	270	140	190	370	1715	100	65	90	155
2522	195	85	145	215	R. ..	140	90	140	250	1722	140	90	125	180
2524	195	90	155	235	R. 25	180	90	140	280	1730	155	70	120	190
2535	165	85	140	220	R. 28	200	80	160	280	163	145	65	120	170
87	180	90	120	210	R. 30	140	60	110	220					
172	200	100	120	230	R. ..	180	80	110	270					
173	185	110	160	230	R. 33	180	90	140	310					
193	120	55	95	165	R. 38	170	110	180	240					
105	145	65	100	155	*	260	120	170	290					
107	145	65	130	215	†	170	90	130	220					
109	80	40	60	90	‡	220	100	150	250					
111	95	40	55	70										
112	80	40	50	65	* Gylden. † Siljestrom. ‡ Statesmen.									
114	110	60	85	130										
Avs. 60	176	81	133	212	57	172	76	149	272					

R = Retzius.

ON OSSIFICATION CENTERS IN HUMAN EMBRYOS LESS THAN ONE HUNDRED DAYS OLD.

BY

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WITH 6 TEXT FIGURES AND 7 TABLES.

As the study of the bones preceded that of the other structure of the human body, so their ossification was the first subject investigated in embryology. The early anatomists became interested in the development of bones on account of the difference between them in adults and children, and it was but a step to their study, first in the fœtus and then in the embryo.

More than one hundred years ago the early ossifications were studied with vigor and in a short time the subject was closed, and we may say that our present knowledge dates mostly from before 1820. With the improvement in embryological methods so many new fields were opened that it did not seem worth while to destroy good specimens nor to make laborious reconstructions to study a subject which seemed so unpromising in results. However, it is apparent that there is considerable difference of opinion regarding the time of ossification as well as the number of centers in certain bones, which frequently diminish as they are studied more carefully.

We notice in looking over the older literature that the ossification was studied by means of ordinary dissection after which the very small specimens were dried upon a glass slide. Such specimens show sharply marked bone centers, and are very useful, but unfortunately the embryos are pretty well destroyed in their preparation. Furthermore, very small centers and delicate attachments are difficult to see, and this defect in the specimens has led to numerous erroneous conclusions. The time of ossification and the order of the appearance of the centers has never been definitely settled, mainly because the specimens were not numerous and were much injured in their study, and because the various investigators did not determine correctly the age of the embryos. Thus, for instance, we read in Béclard's article that the centers for the mandible, maxilla, clavicle, humerus, ulna, radius, femur and tibia are present in an embryo 35 days old (16 lines long) which, according to my table, must be 54 days old. Numerous other embryos are studied in this article, each

time giving their age in days or weeks, and usually omitting their length. A similar criticism may be made regarding Meckel's great paper from which is derived our main information regarding the development of the spinal column and skull.[1]

The older illustrations of primary bone centers are not especially good, for in them the finer details are obscure and the enlarging glass did not aid to make them sharper. However, some of Meckel's pictures are still used in the anatomies,[2] but the small dried arms pictured in Bell's Anatomy [3] and in Rambaud & Renault's Atlas [4] have not been copied extensively. Semidiagrammatic illustrations, *i. e.*, older bones with the earlier centers marked in them have gradually taken their place. And it is only in very recent years that suitable illustrations from X-ray pictures,[5] from transparent embryos [6] or from reconstructions [7] are taking their place.

The newer methods enable us to recognize and to follow the early ossification centers with much greater precision than was possible in Meckel's time. Instead of a dissected and dried specimen we now have sections and reconstructions, and what is still better transparent specimens made according to the method of Schultze, which enable us to detect the minutest bone (0.1 mm. in diameter) and to study it in relation to the rest of the skeleton without destroying the embryo. And last, but not least, we have a standard of measurement (the crown-rump line of His) from which we can determine the age of the embryo with an error of but a few days. It naturally follows that all that is now required is a good collection of transparent embryos to determine the time of appearance and order of development of the bone centers.

During the past half dozen years I have cleared from time to time human embryos which were shrivelled or otherwise made unfit to cut into serial sections. Gradually the number increased so that now I have some 60 transparent specimens in my collection with crown-rump measurements ranging from 10 to 110 mm. in length. Most of these specimens are used in this study.

[1] Béclard, Meckel's Archiv, 1820.

[2] Meckel, Meckel's Archiv, 1815.

[3] Bell's Anatomy, New York, 1834, p. 166.

[4] Rambaud & Renault, Origin et Dével. d. Os., Paris, 1864.

[5] Lambertz, Entwickl. d. Mensch. Knochengerüstes während d. foetelen Lebens, Hamburg; Bade, Arch. f. Mik. Anat., 55; Cunningham's Anatomy, 2d Edition, 1905.

[6] Schultze, Grundriss d. Entwickl. d. Menschens, 1897.

[7] Gaupp, Hertwig's Handbuch d. Entwickelungslehre, Jena, 1905; Bardeen, Amer. Journal of Anatomy, IV, 1905.

We have gradually learned that it is best to clear specimens which have been well shrivelled in alcohol in a 1% solution of KOH for a few hours and not in the strong solution recommended by Schultze. With the weaker solution the tissues, of the smaller embryos especially, remain firm, and, in the end, the specimen is perfectly transparent with all the bones held in place. After the treatment with potash the embryo is placed in the following solution for days, or even for months:

Water	79
Glycerine	20
Potash	1

From time to time the embryo may be returned to a 3% solution of potash for a number of hours in order to hasten the process. The action of the glycerine has been to make the tissue more resistant, and for this reason the strength of the potash is increased. In case there is much blood pigment in the embryo or it is otherwise colored through age, this may be removed by placing the specimen for a number of days in the strongest ammonia to which a little potash has been added, as recommended by Hill.[8] In case the embryo is stained with alum cochineal before it is cleared the bones alone are colored red, and for the study of their finer connections this is often an advantage.

Many of the embryos received recently have been preserved in formalin and for a long time it was practically impossible to clear them in the ordinary way. Finally, in desperation, I placed such an embryo in a 10% solution of potash and to my astonishment I found that it began to clear at the end of a month. By further treatment it was found that such embryos could be cleared perfectly well, and in case the bones are not decalcified by the formalin the very best specimens are obtained. In them the tendons and other white fibrous tissue are rendered tougher than in the specimens treated with alcohol alone. Finally when the embryos are well cleared they are gradually transferred to stronger and stronger glycerine until all the water is removed from them.

Before clearing the embryo it is well to cut it through the sagittal plane into two equal parts, for by this treatment the bones are all brought into view in the finished specimen. Later these halves may be fixed to glass slides with gelatin, as recommended by Bardeen. The specimen is to be taken from pure glycerine and wiped gently and then placed upon a glass plate which is covered with melted gelatin. As soon as the gelatin is cool this slide with the embryo attached is returned to pure glycerine

[8] Hill, Johns Hopkins Hospital Bulletin, 1906.

which extracts the water from the gelatin and makes it very firm. The ossification centers show best when viewed with a large lense over a dark background in direct sunlight.

I have made numerous attempts to study the ossification centers in serial sections stained in carmin and in hæmatoxylin, but find that they are by no means as satisfactory for this purpose as are the Schultze specimens. An embryo 20 mm. long (No. 22), which we have studied with great care shows no ossification centers in the sections, while embryos 15 mm. long show them when cleared.[9] The model made by Ziegler, which is from Hertwig's reconstruction of an embryo 80 mm. long does not show the bones of the skull developed to as great an extent as they are in cleared specimens. So for the present the Schultze method enables us to locate the first bones with much greater certainty than do sections, with the possible exception of those colored with Mallory's connective-tissue stain.

Ossification Centers in the Second Month.

All anatomists agree that the clavicle is the first bone to ossify and that it appears about the middle of the second month or during the sixth week. Béclard states that he found it in an embryo 30 days old, but as E. H. Weber remarks in Hildebrandt's Anatomy, he has probably underestimated the age of the embryo. Judging by the number of ossification centers present Béclard's embryo must have been 44 days old, for it corresponds with my specimens 263, C and A. Meckel must also have underestimated the age of an embryo in which the clavicle was found to be three lines long, for he places it in the middle of the second month. According to my reckoning, his specimen is fully 56 days old, for it corresponds with my specimen 263, b. 2, Table II. Similar differences of opinion will be found regarding the time of ossification of all sharply defined bones due, no doubt, to the difficulty in determining the age of embryos 100 years ago. Therefore, little is to be gained in reviewing the literature upon this subject for such specimens, since the results are not satisfactory when compared with a good series of Schultze specimens. Especially is this true regarding the literature of obscure bones which are said to arise from more than one center. In my description I shall, therefore, confine myself closely to my own specimens.

[9] The same is shown in Bardeen's studies on the development of bones (Anat. Anz., XXV & Amer. Jour. Anat., IV). The earliest stage of different centers he describes are always a little later than those in which they may be seen in embryos cleared in potash.

Table I shows at a glance the ossification centers which appear during the second month. Five embryos of the 39th day were cleared, and in two of them the clavicle and mandible are found, while in one it is uncertain whether or not the clavicle is ossified. When the question mark (?) is inserted in the table, for instance for specimen E, it indicates that a hyaline body is seen, but that it is not as white as an ossification

TABLE I.

Giving the ossification centers present in embryo of the second month. The first horizontal column gives the number of the embryo, the second their crown-rump length in millimeters, and the third their probable age in days. * indicates that the bone given in the first column is ossified; ? that ossification is uncertain; and 0, that the specimen is injured.

Number	59	156	214	167	G	E	168	53	D	263,b	42	271	C	263,c	A	56	333	B	202	274
Length	10	12	14	14.5	15	15	15	15	16	16	18	18	18	19	..	24	..	29	30	31
Age	32	35	38	39	39	39	39	39	40	40	42	42	42	44	..	49	..	54	55	56
Clavicle	..	..	..	..	..	?	*	*	*	*	*	*	*	*	*	*	*	*	*	*
Mandibula	..	..	..	..	..	..	*	*	*	*	*	*	*	*	0	*	*	*	*	*
Maxilla	..	..	..	..	..	..	..	..	..	*	*	*	*	*	0	*	*	*	*	*
Humerus	..	..	..	..	..	..	..	..	..	..	..	..	*	*	*	*	*	*	*	*
Femur	..	..	..	..	..	..	..	..	..	..	..	..	*	*	*	*	*	*	*	*
Radius	..	..	..	..	..	..	..	..	..	..	..	..	..	*	*	*	*	*	*	*
Tibia	..	..	..	..	..	..	..	..	..	..	..	..	..	*	*	*	*	*	*	*
Ulna	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	*	*	*	*	*
Fibula	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	?	*
Scapula	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	*
Supraoccipital	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	?	*
Frontal	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	*
Zygomatic	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	*
Temporal	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	*
Ilium	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	*
Parietal	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	*
Terminal phalanx of thumb	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	*
Ribs 1	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..
Ribs 2	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	*
Ribs 3	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	*
Ribs 4	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	*
Ribs 5	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	*	*
Ribs 6	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	*	*
Ribs 7	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	*	*
Ribs 8	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	*	*
Ribs 9	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	*	*
Ribs 10	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	*
Ribs 11	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	*
Ribs 12	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..

center should be. During the 40th day the maxilla also appears, to which are added the humerus and femur on the 42d day. Next come the radius and tibia on the 44th day and then the ulna on the 49th day. Several days elapse before the fibula appears, as was known to the older anatomists. In less than a day later the scapula follows and on the 56th day the supraoccipital begins to ossify. During the 55th day the ribs make their appearance, and judging by their size, it is the 6th and 7th which ossify first. The ossification spreads rapidly in both directions and on the next day (56th) it is present in all of the ribs, with the exception of the 1st and the 12th. On the 56th day we also find the beginning of ossifica-

tion in the frontal, zygomatic, squamo-zygomatic and parietal bones in the skull, the ilium in the lower extremity and the terminal phalanx of the thumb in the hand.

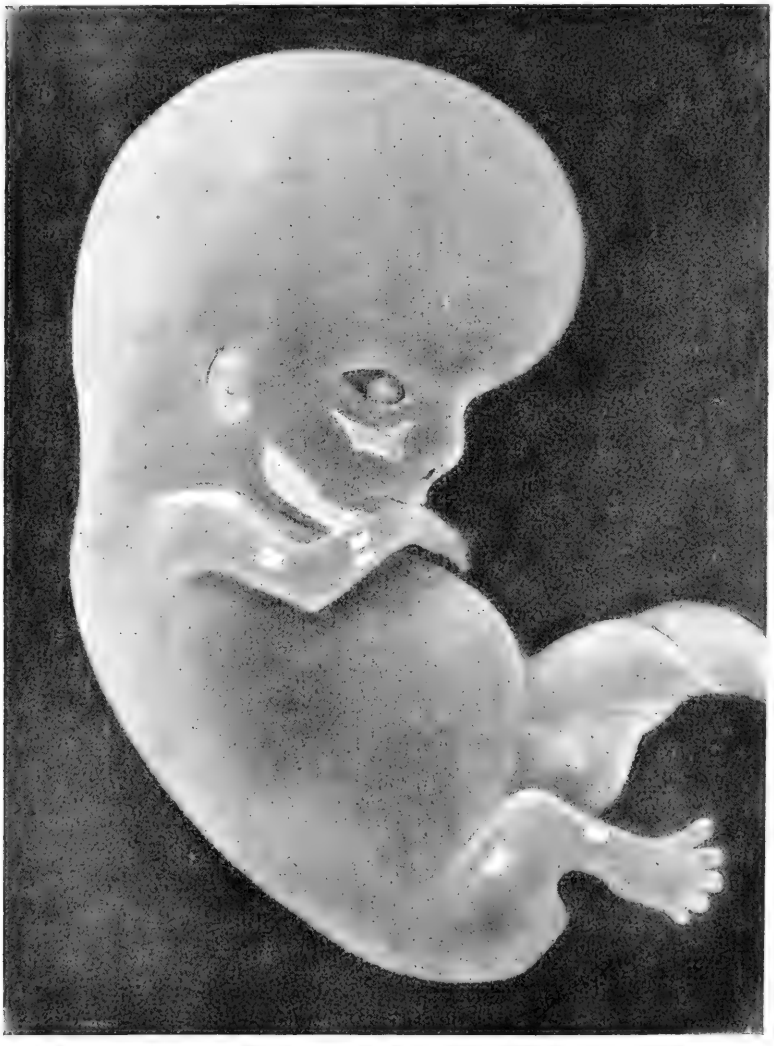


FIG. 1. Embryo No. 333, enlarged 5 times. The outlines of the embryo were taken from a fresh specimen.

The remarkable regularity of the appearance of the bones make of them the best index of the size and age of an embryo we now possess. The measurement of the crown-rump length of an embryo is modified by the

method of preservation and the amount of distortion, but neither of these factors influences the order of ossification. I may add that the length of the embryo was determined by direct measurement from crown to rump, but in case the embryo was distorted this measurement was deduced from the neck-rump length which is the least variable of external measurements. The age of the embryos in days was obtained by multiplying the square root of the crown-rump length in millimeters by ten.[10] These ages correspond with the estimations given by embryologists. If in the course of time our data enable us to determine the ages more accurately, the ages I have ascribed to the various embryos can easily be changed, for in all the specimens the length from crown to rump is also given.

Ossification Centers in the Skull.

Mandibula.—The mandible appears a very little later than the clavicle. In two embryos, 15 mm. long, 39 days old, it is present as a finely granular or reticular mass, half a millimeter long, immediately below the epidermis towards the free end of the first arch, representing, of course, the body of the jaw. In the next specimen, 263, b, the mandibula is found to be a slender but compact bone about one millimeter long reaching nearly to the midventral line; in D it is a little larger and more sharply defined. On the 42d day (No. 42) the bone measures 2 mm. and shows the beginning of the ramus and of the alveolar process. It now gradually enlarges, measuring 3 mm. in No. 56 and No. 333. The lower jaw in these embryos is thin and transparent, showing a delicate structure. The alveolar process and the ramus are very transparent, while the base and the part of the body near the midventral line is thick and opaque. Much the same appearance is seen in No. 202 (55 days), while in 274 the jaw is fully 5 mm. long and shows the beginning of the coronoid process and the condyle. A day later, No. 263, b, 2, the jaw is 6 mm. long, and within it can be seen three lines of thickened bone radiating from the symphysis, one towards the angle, one into the condyle, and one into the coronoid process. The next day a few sockets for the teeth may be seen in the alveolar process. This condition remains, the jaw only growing in size, until the 58th day (272) when the jaw appears hollow and the three radiating lines no longer reach to its anterior end. By the 75th day (288, b) the jaw has grown to be 10 mm. long and meets its fellow to form the symphysis on the midventral line. The

[10] See Mall, Amer. Jour. of Anat., II, 335; Johns Hopkins Hospital Bulletin, 1903; and Johns Hopkins Hospital Reports, IX, 1900.

condyle has become much more sharply defined and its bone fibers again reach to the symphysis. About this time the mylohyoid line and the lingula appear. On the 83d day the ramus is becoming relatively thinner and broader, the coronoid process has moved farther away from the condyle, the angle has become more marked, and the alveolar process has increased in length. The mandibula has now its characteristic shape, and measures 14 mm. in length. In older embryos its form is characteristic; it gradually increases in size, measuring 19 mm. in embryo P.

Maxilla.—The maxilla, according to my specimens, arises from two centers only, one to form the premaxillary bone and one to form the main body. In one of the youngest specimens (263, b) the maxilla is marked by a mass of granules, together one-half millimeter in diameter, lying spread out just beneath the eye and one millimeter from the middle line. In another specimen (C) it is impossible to find the maxilla, but a very small premaxillary is found measuring one-fourth of a millimeter in diameter. A second specimen of the 42d day (42) shows both centers present as granular masses. The maxilla is just beneath the eye, over a millimeter in diameter and the premaxillary is as small as in C and separated by a millimeter from the maxilla. A little later (56 and 202) the two bones are denser in structure and both have parallel processes which no doubt are to form the frontal process. These two bones are found united along the alveolar border on the 56th day (274), but the frontal processes remain separated for a long time. In another embryo of the 56th day (263, b, 2) the frontal process is found to be distinctly double, one-half coming from each center, and the alveolar process is large and includes both of them. The orbital plate and the palatine process are just beginning and the zygomatic process is well developed. By grasping the different sides of the bone between two needles it is easily demonstrated that there is but one bone from this time onward. If much pressure is exerted it is observed that the bone bends, and if too much, it breaks. In later stages when the palate, temporal and zygomatic bones are present it is easily seen that these are separate, although they come in contact with the maxilla. At no time are more than two centers present, and these unite in the very beginning of the third month. In Table II the parallel lines are inserted between the columns for the maxilla and the premaxillary for specimens, in which these two bones can still be recognized, but they are firmly united. However, these processes, especially the zyogmatic, are easily broken off, and, judging by the illustrations of this bone in Rambaud & Renault, their numerous centers are due to such breaks. In the next specimen (266) the premaxillary is separate, and not united with the body of the maxilla; in all of the older embryos they are united.

At about this time the palate bone is well developed and overlaps the palatine process of the maxilla. A little later (263, b, 1) a small palatine process arises from the premaxillary process and in older stages (300)

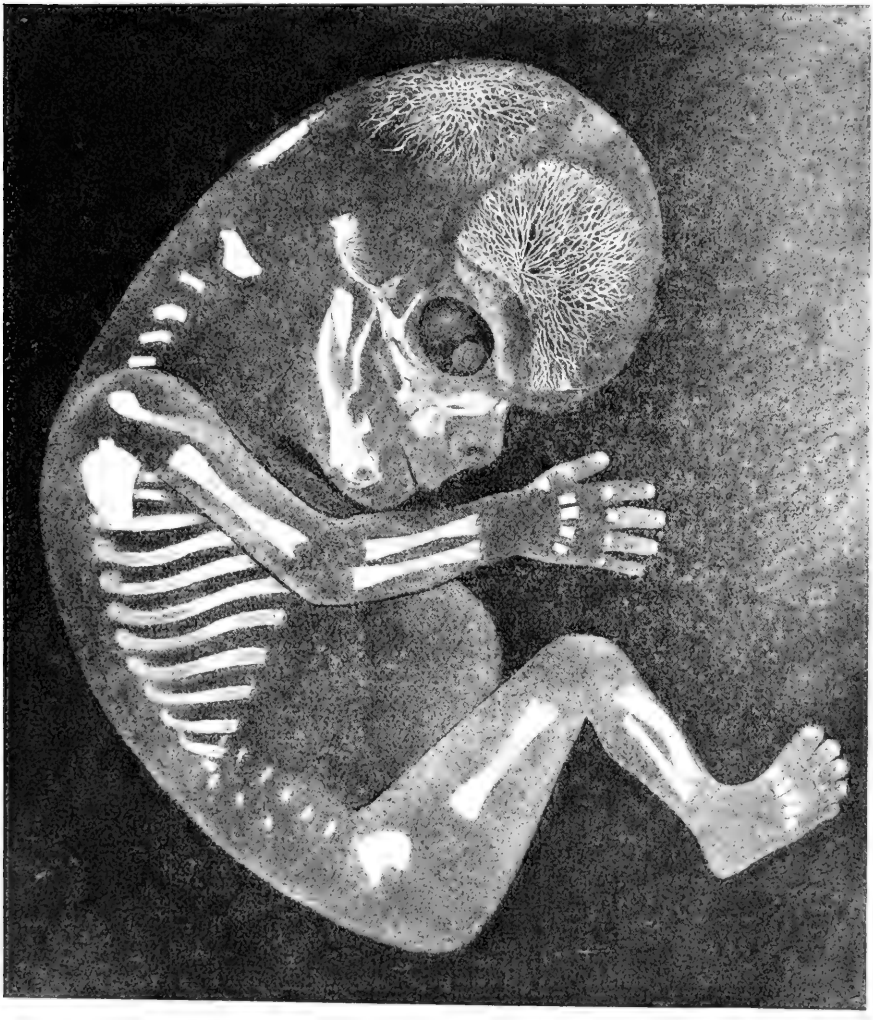


Fig. 2. Embryo No. 284 (54 mm. long), enlarged nearly 2½ diameters. Immediately beneath the squamo-zygomatic the alisphenoid may be seen.

the bony palate reaches nearly to the vomer. In this embryo the infra-temporal and orbital plates are well formed, and the bone is well hollowed out, forming the well-marked hiatus which communicates freely with the cavity of the nose.

TABLE II

Giving the time of ossification of the bones of the skull.

No.	168	53	D	263,b	42	C	263,c	333	56	202	274	263,b,2	266	263,b,1	272	282	K	284	288,b	M	N	300	O	S	Q	306,b	P	R
Length	15	15	16	16	18	18	19	..	24	30	31	32	33	34	34	42	53	54	57	69	70	73	73	75	81	105	105	110
Age	39	39	40	40	42	42	44	..	49	55	56	56	57	58	58	65	72	73	75	83	83	85	85	87	90	105	105	110
Mandibula	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*
Maxilla	*	*	*	*	*	..	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*
Premaxillary	..	..	..	..	*	*	*	..	*	*	=	=	..	=	=	=	=	=	..	..	..	..	*	*	*	*	*	*
Supraoccipital	..	..	..	..	..	..	..	..	..	?	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*
Exoccipital	..	..	..	..	..	..	..	..	..	..	..	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*
Interparietal	..	..	..	..	..	..	..	..	..	..	..	..	..	..	*	*	*	*	*	*	*	*	*	*	*	*	*	*
Basioccipital	..	..	..	..	..	..	..	..	..	..	..	..	..	0	*	*	*	*	*	*	*	*	*	*	*	*	*	*
Zygomatic	..	..	..	..	..	..	..	..	..	..	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*
Squamo-zygomatic	..	..	..	..	..	..	..	..	..	..	..	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*
Tympanic ring	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..
Frontal	..	..	..	..	..	..	..	..	..	..	..	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*
Parietal	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..
Pterygoid	..	..	..	..	..	..	..	..	..	..	..	..	..	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*
Alisphenoid	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..
Orbitasphenoid	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..
Basisphenoid	..	..	..	..	..	..	..	..	..	..	..	..	..	*	*	*	*	*	..	*	*	*	*	*	*	*	*	*
Palate	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..
Vomer	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..
Nasal	..	..	..	..	..	..	..	..	..	..	..	..	..	0	0	..	..	..	..	..	..	..	..	..	..	..	..	..
Lachrymal	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..

After the premaxillary bone joins the maxilla it is in general cubical in shape, measuring on a side 3 mm. on the 65th day; 4½ mm. on the 75th day (288, b); and 6 mm. on the 85th day (300).

It is said by Rambaud & Renault that the primary ossification centers unite during the third month. Toldt states that they appear at the end of the second month and unite at the end of the fourth month. If Toldt's statement is correct the primary centers should be present in practically all of the embryos given in Table II. Toldt is a very reliable observer, and in order to verify his statement I tested the maxillæ in all of my embryos by squeezing them between two needles, but at no time could I demonstrate more than one center, exclusive of the premaxillary. The

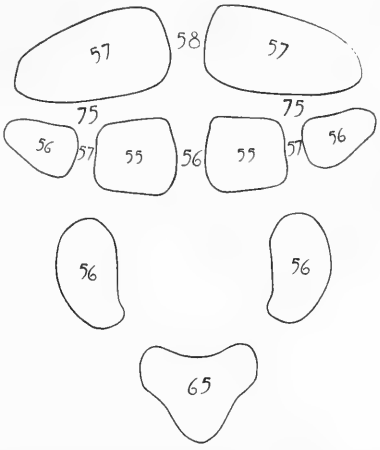


Fig. 3. Diagram of the ossification of the occipital bone. The figures upon or between the centers indicate the time in days in which they appear or unite.

bone was dissected out in embryo 288, b, and was found to be a single bone, and was broken with difficulty when handled with two needles in glycerine. The opinion I have reached regarding the ossification of the maxilla is fully confirmed by Ziegler's copy of Hertwig's model of the skull of an embryo 8 cm. long, and by Schultze's illustration of the bones of the skull of an embryo of the third month.

Occipital bone.—According to the embryos I have studied, the occipital bone arises from nine primary centers, two less than described by Meckel. On the 55th day (202) two cartilages which are thin and transparent, and may be ossified, are seen just above the foramen magnum. A little later these cartilages are fully ossified, are beginning to unite across the

middle line, and lateral to them two small new centers are seen. On the next day all four centers begin to unite to form a single bone, the *supra-occipital,* measuring 2 mm. in height and 6 mm. in width. In the same embryo (263, b, 2) two very small *exoccipitals* one-quarter of a millimeter in diameter are seen. On the 57th day (266) the *supraoccipital* centers are well blended and the *interparietals* make their appearance as two reticular membrane bones, which unite during the next day (263, b, 1 and 272). On the 65th day the *basioccipital* makes its appearance and on the 75th day all the bones above the foramen magnum are united into the single tabular part of the occipital bone. The accompanying diagram shows the general arrangement of the centers with the date of their appearance marked upon them and their time of union marked between them.

Although there are over 20 perfect specimens in the cleared embryos of the proper ages, in none of them is there any sign of more than two centers for each interparietal as is asserted by Meckel, Ranke[11] and Bolk.[12] The supraoccipital is 6 mm. long, crossing the middle line in an embryo 56 days old, and above it lie the interparietals which together are a little narrower and nearly as long. At this time the exoccipital is about a millimeter long. They all grow quite rapidly and on the 73d day the supraoccipital and the interparietal begin to unite. Now the former measures 4 x 10 mm. and the latter 2 x 7 mm. The common squamous portion of the occipital bone measures 6 x 10 mm. on the 75th day, 12 x 16 on the 85th day, and 18 x 30 on the 105th day. The growth of the exoccipital and basioccipital is not so rapid, the former is 3 mm. long on the 73d day and 7 mm. on the 105th day; during the same time the basioccipital grows to measure 2 x 6 millimeters.

The zygomatic bone.—The malar bone appears as a small three-cornered center just beneath and to the lateral side of the eye on the 56th day. On the 58th day it is four-cornered and nearly two millimeters long. Two of the horns, that is two of the corners, encircle the orbit and one of the remaining two corners points towards the maxilla and the other towards the temporal bone. The zygomatic grows larger and gradually becomes hour-glass shaped, the constriction or narrow stem connecting the orbital side with the temporal and maxillary processes. By the 75th day the bone has an area of 10 square mm., and the orbital end has given rise to the orbital surface which now grows more rapidly than the rest of the bone. On the 105th day the orbital surface is fully twice

[11] Ranke, Abhandl. d. K. Bayer. Akademie u. Wiss., XX.

[12] Bolk, Petrus Camper, II.

as long as the distance between the maxillary and temporal processes and gives the appearance of the horns upon an ox's head. At no time is a second center visible.

Temporal bone.—This bone also appears on the 56th day as a small hook-like nucleus about a millimeter long representing mostly the zygomatic process. The slightly enlarged dorsal end marks the beginning of the squamous portion. The primary and only nucleus of the *squamo-zygomatic* gradually enlarges, the zygomatic process growing longer and the squamous portion spreading out over the temporal region of the head. By the 58th day the squamous portion measures 2.5 mm. in diameter and the zygomatic portion is also as long. At no time are these two parts of the bone separated, but they are firmly attached to each other as may be demonstrated by pressing them between two needles. They gradually enlarge and on the 65th day a small nucleus appears below the junction of the zygomatic process with the squamous portion to which is attached the delicate tail-like ring. The *tympanic ring* is present only on the right side of this embryo (282). Gradually the ring enlarges and finally makes the circle complete on the 85th day. The squamous portion at first begins to radiate from its point of junction with the zygomatic, but as the bone grows larger an axis is extended partly through the middle of the squama from which this net-work of bone now radiates. During this time the squamous portion grows below the zygomatic; on the 73d day it measures 3 mm. in length, on the 85th day 7 mm., and on the 105th day 11 mm.

Frontal bone.—The frontal also appears on the 56th day, a little later than the time given by Toldt. In embryo No. 274 it forms a reticular nucleus about 4 mm. in diameter with the orbital plate a little more developed than the rest of the bone. On the 58th day it measures 8 mm. in diameter; on the 73d day 10 mm. and on the 85th day 15 mm.

Parietal bone.—This bone is a little behind the frontal in its appearance, judging by its transparency and extent. On the 56th day it appears as a very delicate reticular nucleus, about 3 mm. in diameter, which can be seen only with difficulty. A few days later (272) it is found spreading towards the occipital bone and the middle line. It is now hour-glass shaped, each end of which is about 4 mm. in diameter and may represent the two centers described by Toldt. At this time the nucleus near the sphenoidal angle is more extensively ossified, and its reticular structure is coarser than in the nucleus near the occipital angle of the parietal bone. The bone now grows rapidly, keeping pace pretty well with the frontal. On the 105th day it measures 22 x 26 mm.

Sphenoid bone.—The first center of the sphenoid to appear is that of the *pterygoid* which may be seen just behind the palate in an embryo 57 days old. It is a very small bone about one millimeter long with a small T-shaped handle above, which possibly represents the vaginal process. A day later (263, b, 1) the *alisphenoid* is present as a small rectangular bone measuring about a millimeter on each side. Both centers grow slowly until the 75th day when the alisphenoid measures 4 mm. in length. At this time the main body of the pterygoid is not much larger than it

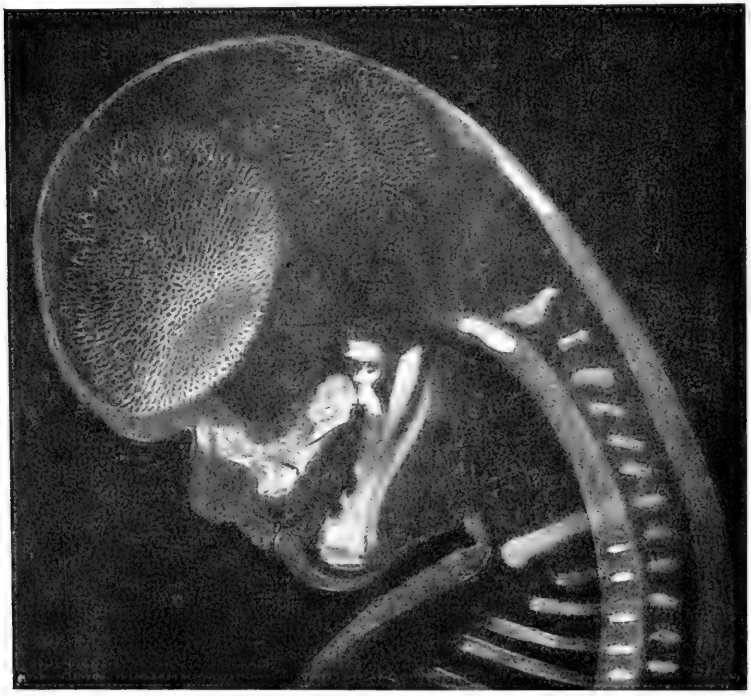


Fig. 4. Mesial view of the head of embryo No. 284 (54 mm. long), enlarged 2½ diameters. The centers of the occipital bone surround the foramen magnum. Behind the maxilla may be seen the palate, the pterygoid, and the alisphenoid in order.

was at first, but the cross piece of the T is nearly a millimeter long. On the 83d day (M) the *orbitosphenoid* is present as a rectangular bone about a millimeter long. In the same embryo (M) the alisphenoid measures 3 x 6 mm. and the pterygoid is fully two millimeters long. A little later (N) the *basisphenoid* appears as two fairly large granules of bone, one on either side. In an older embryo (300) the basisphenoid granules are

smaller than before, but after this they grow in size, and on the 105th day they are united. The alisphenoid grows rapidly, is 8 mm. long on the 73d day, and reaches out towards the frontal and temporal on the 105th day. By the same time the orbitosphenoid is hook-shaped, encircles the optic foramen and measures 3 x 4 millimeters.

Palate bone.—On the 57th day both horizontal and vertical parts of the palate bone may be seen. They are very thin, are united, and each part measures a square millimeter in area. Next day the horizontal part is larger than the vertical and from now on they grow gradually, the parts remaining of equal size. On the 58th day the area of each part is 4 square millimeters; on the 90th day 6 square millimeters, and on the 105th day 9 square millimeters

Vomer.—The vomer is present in embryo No. 266 as a delicate double bone measuring about 2 mm. in length; in 263, b, 1, it is a little shorter. On the 58th day the two centers are 3 mm. long, and on the 65th day they are no longer, but are united at a single point near their anterior end. The union spreads rapidly throughout the length of the bone, and on the 73d day it appears as a single groove-like bone 4 mm. long. On the 83d day it is 6 mm. long; on the 90th day 2 mm. high and 7 mm. long; and on the 105th day it is 10 mm. long.

Nasal bone.—The nasal bone also begins on the first day of the third month, although Toldt states that it ossifies during the 12th week. It can barely be seen on the 57th day, and is well marked on the 65th day, measuring at this time one square millimeter in area. It grows slowly, being but 1.5 mm. square on the 83d day and but 2 mm. on the 105th day.

Lachrymal bone.—The lachrymal bone is the last of the bones of the head to appear during the first 100 days of embryonic life. Gaupp states that it appears at the end of the second month and Quain states that it appears during the 8th week. Cunningham and Gray make similar statements. Béclard, who always places the time of ossification too early, states that the lachrymal appears on the 55th day, and Meckel, who is a much more competent observer, states that it does not ossify until the 5th or 6th month. I find it present in an embryo of the 83d day, and not before, as a narrow and very thin bone nearly 2 mm. long. In all the specimens studied the eyes were removed in order to bring the region of the lachrymal well into view and these specimens were studied with the greatest care under the enlarging glass in direct sunlight. In an embryo 85 days old the bone is much smaller, and in an embryo of the 90th and one of the 105th day, it is again about 2 mm. long and a little more opaque than before. In the model from Hertwig's laboratory, which is

from an embryo 90 days old, the lachrymal is not over half as large as the nasal, and Schultze's picture of an embryo of the 3d month does not show it at all.

It appears then that the blunder of Béclard regarding the time the lachrymal begins to ossify, made nearly a century ago, has crept into the anatomies and has remained there unchallenged.

Ossification of the Ribs and Vertebræ.

The ribs.—The ribs appear on the 55th day (202), no ossification centers being present in an embryo of 54 days. In the embryo in which they first appear they are not quite symmetrical on the two sides, the left side having two centers more than the right. On the right side the 6th rib is the largest, while on the left side it is the 7th, these two probably being the first to ossify in this embryo. They evidently make rapid progress in their growth, for on the 56th day 10 ribs are present, giving a well proportioned thorax. On the 57th day the first rib makes its appearance, and it is not missing in any of the succeeding embryos. The first rib is then the 11th to appear and the 12th rib the last. Table III shows at a glance that the 12th rib is variable, not being present in all cases. According to Bardeen,[13] this variation is very rare, being present but once in 46 embryos studied by Paterson, Rosenberg and himself. The 13th rib, which is not present in the specimens I have studied, seems to be quite common in those reported by Bardeen. In adult skeletons, according to Bardeen, the absence of the 12th rib is about as common as the presence of a 13th rib, being nearly 1% for each variation in 908 skeletons studied.

A cervical rib is present in two of my specimens, and were it not for a very accurate count, it would be easy to call the number of ribs in one of them (288, b) normal, and those in the other (300) as an embryo with a 13th rib. Of the 908 skeletons referred to above a cervical rib was found but twice (having been found by Topinard in 350 skeletons) being, therefore, much rarer in the adult than in the embryos I have studied. However, we have found it three times in about 250 subjects[14] dissected in our dissecting room, without looking for it especially, and for this reason we do not know with certainty whether we found every cervical rib. The nucleus representing a cervical rib was noticed by Albinus nearly two centuries ago, and is spoken of by Meckel, Oken and Béclard as a rudiment of a cervical rib[15] I think it probable, therefore, that careful search will

[13] Bardeen, Anat. Anz., 25, 1904.

[14] Brush, Johns Hopkins Hospital Bulletin, 1901.

[15] Hildebrandt, Anatomie, II, 1830, p. 164.

TABLE III.

Giving the time of ossification of the ribs.

No.	202 right	202 left	274	263 b,2	266	263 b,1	272	J	329	I	282	K	L	284	326	288 b	M	N	300	O left	O right	306 c	S	Q	306 a	P	306,b right	306,b left	R
Length	30	30	31	32	33	34	34	36	39	41	42	53	54	54	56	57	69	70	73	73	73	75	75	81	100	105	105	105	110
Age	55	55	56	56	57	58	58	60	62	64	65	72	73	73	74	75	83	83	85	85	85	87	87	90	100	105	105	105	110
Ribs 7	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	*	..	..	*	..	..	..	..	..	..	..	..	..	..
Ribs 1	..	..	..	..	*	*	*	*	*	*	*	*	*	*	*	*	..	*	*	*	*	*	*	*	*	*	*	*	*
Ribs 2	..	..	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*
Ribs 3	..	..	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*
Ribs 4	..	..	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*
Ribs 5	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*
Ribs 6	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*
Ribs 7	..	..	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*
Ribs 8	..	..	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*
Ribs 9	..	..	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*
Ribs 10	..	..	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*
Ribs 11	..	..	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*
Ribs 12	..	..	..	..	..	*	*	..	*	..	..	*	*	*	..	..	..	..	*	..	*	*	*	*	*	*	..	*	*

TABLE IV.

Giving the time of ossification of the arches of the vertebræ.

	No.	202	274	263 b,2	266	263,b1 right	263,b1 left	272	J	I	282	K	284	288 b	M	N	300	O	S	306 c	Q	306 a	306 b	P	R
	Length	30	31	32	33	34	34	34	36	41	42	53	54	57	69	70	73	73	75	75	81	100	105	105	110
	Age	55	56	56	57	58	58	58	60	64	65	72	73	75	83	83	85	85	87	87	90	100	105	105	110
Arches of the Vertebrae	1	..	..	..	..	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*
	2	..	..	..	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*
	3	..	..	..	..	..	..	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*
	4	..	..	..	..	..	..	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*
	5	..	..	..	..	..	..	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*
	6	..	..	..	..	..	..	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*
	7	..	..	..	..	*	..	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*
	8	..	..	..	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*
	9	..	..	..	..	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*
	10	..	..	..	..	..	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*
	11	..	..	..	..	..	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*
	12	..	..	..	..	..	..	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*
	13	..	..	..	..	..	..	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*
	14	..	..	..	..	..	..	*	*	*	..	*	*	*	*	*	*	*	*	*	*	*	*	*	*
	15	..	..	..	..	..	..	*	*	*	..	*	*	*	*	*	*	*	*	*	*	*	*	*	*
	16	..	..	..	..	..	..	*	*	*	..	*	*	*	*	*	*	*	*	*	*	*	*	*	*
	17	..	..	..	..	..	..	*	*	*	..	*	*	*	*	*	*	*	*	*	*	*	*	*	*
	18	..	..	..	..	..	..	*	*	*	..	*	*	*	*	*	*	*	*	*	*	*	*	*	*
	19	..	..	..	..	..	..	*	*	*	..	*	*	*	*	*	*	*	*	*	*	*	*	*	*
	20	..	..	..	..	..	..	..	..	*	..	*	*	*	*	*	*	*	*	*	*	*	*	*	*
	21	..	..	..	..	..	..	..	..	*	..	*	*	*	*	*	*	*	*	*	*	*	*	*	*
	22	..	..	..	..	..	..	..	..	..	..	*	*	*	*	*	*	*	*	*	*	*	*	*	*
	23	..	..	..	..	..	..	..	..	..	..	*	*	*	*	*	*	*	*	*	*	*	*	*	*
	24	..	..	..	..	..	..	..	..	..	..	*	..	..	*	*	*	*	*	*	..	*	*	*	*
	25	..	..	..	..	..	..	..	..	..	..	*	..	..	*	*	*	*	*	*	..	*	*	*	*
	26	..	..	..	..	..	..	..	..	..	..	*	..	..	..	..	..	..	..	..	..	..	..	..	*
	27	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	*
	28	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	?

find this rib much more common in the embryo than we think it is,—possibly in 5% of the specimens.

The arches.—The arches of the vertebræ appear on the 57th day as two small granules of bone, one for the second vertebra and one for the eighth. On the next day these two points of ossification have increased on both sides of the embryo. In the cervical region the first and second arches are present on the right side and the first three arches are on the

TABLE V.

Giving the times of ossification of the bodies of the vertebræ.

No.	202	274	263, b, 2	266	263, b, 1	272	282	284	288, b	M	N	300	O	S	306, c	Q	306, a	306, b	P	R
Length	30	31	32	33	34	34	42	54	57	69	70	73	73	75	75	81	100	105	105	110
Age	55	56	56	57	58	58	65	73	75	83	83	85	85	87	87	90	100	105	105	110
Bodies of the Vertebræ 1	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..
2	..	..	..	..	..	..	..	..	..	..	*	..	0	..	..	*	..	..	*	*
3	..	..	..	..	..	..	..	..	..	..	*	..	0	*	..	*	..	*	*	*
4	..	..	..	..	..	..	..	..	..	*	*	..	0	*	*	*	*	*	*	*
5	..	..	..	..	..	..	..	..	..	*	*	*	0	*	*	*	*	*	*	*
6	..	..	..	..	..	..	..	..	*	*	*	*	0	*	*	*	*	*	*	*
7	..	..	..	..	..	..	..	..	*	*	*	*	0	*	*	*	*	*	*	*
8	..	..	..	..	..	..	..	*	*	*	*	*	0	*	*	*	*	*	*	*
9	..	..	..	..	..	..	..	*	*	*	*	*	0	*	*	*	*	*	*	*
10	..	..	..	..	..	*	*	*	*	*	*	*	0	*	*	*	*	*	*	*
11	..	..	..	..	..	*	*	*	*	*	*	*	0	*	*	*	*	*	*	*
12	..	..	..	..	..	*	*	*	*	*	*	*	0	*	*	*	*	*	*	*
13	..	..	..	..	..	*	*	*	*	*	*	*	0	*	*	*	*	*	*	*
14	..	..	..	..	..	*	*	*	*	*	*	*	0	*	*	*	*	*	*	*
15	.	..	..	..	..	*	*	*	*	*	*	*	0	*	*	*	*	*	*	*
16	..	..	..	..	..	*	*	*	*	*	*	*	0	*	*	*	*	*	*	*
17	..	..	..	..	..	*	*	*	*	*	*	*	0	*	*	*	*	*	*	*
18	..	..	..	..	..	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*
19	..	..	..	..	..	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*
20	..	..	..	..	..	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*
21	..	..	..	..	..	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*
22	..	..	..	..	..	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*
23	..	..	..	..	..	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*
24	..	..	..	..	..	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*
25	..	..	..	..	..	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*
26	..	..	..	..	..	..	..	*	*	*	*	*	*	*	*	*	*	*	?	*
27	..	..	..	..	..	..	..	*	*	*	*	*	*	*	*	*	*	*	..	*
28	..	..	..	..	..	..	..	..	*	..	*	..	*	..	..	..	..	*	..	*
29	..	..	..	..	..	..	..	..	..	..	*	..	..	..	..	..	..	*	..	..

left side. Lower down the 7th to the 10th arches inclusive are on the right side and the 8th to the 11th on the left side. In embryo 272 and J, all the arches are present to the 19th, *i. e.*, through the cervical and thoracic region on both sides; on the 64th day they extend to the third lumbar vertebra. The next day they extend to the 13th vertebra only, and on the 72d day they reach to the 27th vertebra. From now on the ossification centers are irregular in number, fluctuating around the first sacral vertebra. A glance at Table IV shows that the lower vertebræ vary in their appearance, corresponding somewhat with the condition found in the appearance of the ribs.

Up to the 65th day the second arch is the largest in the cervical region

and the eighth or ninth, or both, in the upper thoracic region. This appearance indicates that the arch first to appear is the largest for a considerable time after other arches appear, a conclusion which the earlier anatomists deduced in studying the ossification centers of the ribs, arches and bodies of the vertebræ.

The bodies.—The bodies are present in large number in an embryo 58 days old, although none are present in another embryo of the same age as well as in a few excellent specimens a little younger. In this specimen (272) the bodies extend from the 10th to the 25th vertebræ being very small above and below, the 19th, 20th and 21st being the largest. A specimen of the 65th day shows much the same appearance. They now extend more rapidly towards the head than into the sacrum, fluctuating in number on both ends of the spinal column. Until the 90th day the bodies on the 20th and 21st vertebræ are the largest, indicating that these two bones were the first to ossify. At no time were accessory ossification centers seen nor were the bodies found to arise from two centers. Meckel made the same observation in 1815, but several writers since his time have spoken in favor of double ossification centers in the bodies of the vertebræ, an idea much in vogue about the time of Haller.

Ossification of the Bones of the Arm.

The clavicle.—The ossification center for the clavicle is present in two, and uncertain in one, out of five embryos of the 39th day. In these specimens, as well as in older ones, the clavicle is clearly made up of two centers, a large one about .5 mm. in diameter near the median line and a smaller one (.2 mm. in diameter, and .5 mm. long) reaching something like a handle from the first, towards the shoulder joint. Together they are about a millimeter long. A few days later (No. 42) these two bones measure nearly 2 mm. together, the inner one, however, is much the larger, and fully separate from the outer one. Towards the 45th day the two centers blend, and a recent series of sections of an embryo 20 mm. long (No 240), which had been stained in iron hæmotoxylin, shows them fairly well united. This specimen also shows the anlage of the clavicle as composed of a peculiar cartilage with a deposit of granules between the cells. The appearance is unlike that seen in the mandible or in the humerus. By the 49th day the two centers are fully united, and appear in a single bone 2 mm. long as is shown in an excellent specimen (No. 333). By the 55th day it is 3 mm. long; the 58th day, 5 mm.; the 75th day, 9 mm., and the 85th day, 12 mm.

The humerus.—This bone appears on the 42d day as a very small cylin-

TABLE VII.

Giving the time of the ossification of the bones of the arm.

		E	168	53	D	263,b	42	271	C	263,c	333	56	B	202	274	263,b,2	266	263, b,1	272	J	I	282	K	L	284	288,b	M	N	300	O	S	306,c	Q	306,b	P	R
No																																			
Length		15	15	15	16	16	18	18	18	19	..	24	29	30	31	32	33	34	34	36	41	42	53	54	54	57	69	70	73	73	75	75	81		105	110
Age		39	39	39	40	40	42	42	42	44	..	49	54	55	56	56	57	58	58	60	64	65	72	73	73	75	83	83	85	85	87	87	90	105	105	110
Clavicle		?	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*		*	*	*	*
Humerus									*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*
Radius																																				
Ulna																																				
Scapula																																				
Metacarpal	1																																			
	2																																			
	3																																			
	4																			0																
	5																																			
Phalanges I	1																																			
	2																																			
	3																																			
	4																																			
	5																																			
Phalanges II	2																																			
	3																																			
	4																																			
	5																																			
Phalanges III	1																																			
	2																																			
	3																			0				0	0											
	4																			0				0	0											
	5																																			

drical center, but half a millimeter in length. It grows quite rapidly, being 5 mm. long on the 58th day; 9 mm. on the 75th day; and 14 mm. on the 85th day.

The radius and ulna.—The radius and tibia both arise on the 42d day, and the ulna appears a few days later. They are hollow cylinders, a millimeter long on the 56th day, and 3 mm. on the 58th day. From now on the ulna is always a little longer than the radius; on the 85th day they are 12 mm. long.

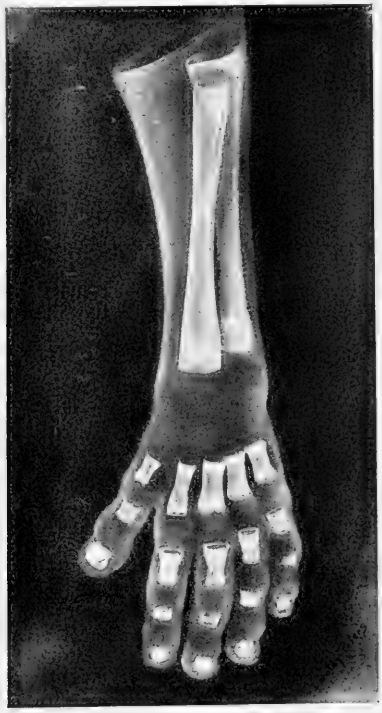


FIG. 5. Hand of embryo No. 300 (73 mm. long), enlarged 3 diameters.

Scapula.—The first center appears about the middle of the region of the spine as a small granule on the 55th day. By the 58th day it is 2½ mm. in diameter, and on the 85th day it is 9 mm. long.

Metacarpal.—The second and third metacarpal bones begin at the same time, for they are equally large in an embryo of the 57th day. On the next day (263, b, 1) small fourth and fifth metacarpal bones are also present, and in an embryo of the same age (272) the first one is added as a small crescent-shaped bone with the opening of the ring turned

toward the volar side of the hand. On the 75th day these bones are 1 mm. long and on the 85th day they are 2 mm. long.

Phalanges, I.—The second and third bones of the first row of phalanges are present in an embryo 58 days old as two small crescent-shaped bones open toward the volar side of the hand. In an embryo two days older four of the bones of this row are present. On the 64th day and thereafter all five are present. On the 90th day they are 1½ mm. long.

Phalanges, II.—The second row are the last of the phalanges to appear. On the 75th day the center in the second phalanx is well formed and those of the third and fourth phalanges are each represented as two very small nuclei, the one on the radial side being a little longer than the one on the ulnar side. On the 83d day (N) a single center appears in the fifth phalanx. It is crescent-shaped with its closed side outwards, and its open side directed towards the radial side of the hand. It retains this form, growing only in size in embryos up to 105 days old. At this time each of the bones of this row is about half a millimeter long.

Phalanges, III.—The first terminal phalanx is the first bone of the hand to appear, being present in an embryo of the 56th day. It is club-shaped being developed, unlike the rest of the phalanges, in connective tissue. Immediately following the appearance of the first terminal phalanx the rest of the terminal phalanges appear, the fifth being very minute. Lambertz first demonstrated that these bones appear before any other bones of the hand, while Rambaud and Renault thought that they were the last of the phalanges to develop, and actually picture a hand of an embryo with the ossification centers present in the second, but not in the terminal row. Bade's X-ray pictures are too hazy to give any clear idea regarding this point.

Ossification of the Bones of the Leg.

The femur.—The ossification center of the femur appears on the 42d day and grows gradually, being 1.5 mm. long on the 55th day. On the 58th day it is 4 mm. long, on the 75th, 8 and on the 85th 15 mm. long.

Tibia and Fibula.—The tibia appears on the 44th day and the fibula on the 55th day, at which time the former is about one millimeter long. Throughout the early development the tibia remains a little longer than the fibula, and about 25% shorter than the femur.

Ilium.—The center of the ilium appears a little anterior to its center on the 56th day, and by the 58th day it measures 2 mm. in diameter. It soon has a knob-like process on its posterior border which often appears as a small adjacent nucleus above the great ischiatic notch. By the 85th day the antero-posterior length of the center is 6 mm.

TABLE VI.

Giving the time of ossification of the bones of the leg.

No	C	263,c	A	56	333	B	202	274	263,b,2	266	263,b,1	272	J	I	282	K	L	284	288, b	M	N	300	O	S	Q	306,a	306,b	P	R
Length	18	19	..	24	..	29	30	31	32	33	34	34	36	41	42	53	54	54	57	69	70	73	73	75	81	100	105	105	110
Age	42	44	..	49	..	54	55	56	56	57	58	58	60	64	65	72	73	73	75	83	83	85	85	88	90	100	105	105	110
Femur	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*
Tibia	..	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*
Fibula	..	..	..	..	..	?	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*
Ilium	..	..	..	..	..	..	..	..	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*
Metatarsal 1	..	..	..	..	..	..	..	..	..	..	..	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*
Metatarsal 2	..	..	..	..	..	..	..	..	..	..	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*
Metatarsal 3	..	..	..	..	..	..	..	..	..	..	..	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*
Metatarsal 4	..	..	..	..	..	..	..	..	..	..	..	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*
Metatarsal 5	..	..	..	..	..	..	..	..	..	..	..	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*
Phalanges I 1	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	*	*	*	*	*	*	*	*	*	*
Phalanges I 2	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	*	*	*	*	*	*	*	*	*
Phalanges I 3	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	*	*	*	*	*	*	*	*
Phalanges I 4	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	*	*	*	*	*	*	*	*
Phalanges I 5	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	*	*	*	*	*	*	*	*
Phalanges II 2	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..
Phalanges II 3	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..
Phalanges II 4	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..
Phalanges II 5	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..
Phalanges III 1	..	..	..	..	..	..	..	..	..	..	*	..	..	0	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*
Phalanges III 2	..	..	..	..	..	..	..	..	..	..	..	*	*	0	*	0	0	*	*	*	*	*	*	*	*	*	*	*	*
Phalanges III 3	..	..	..	..	..	..	..	..	..	..	..	*	*	0	*	0	0	*	*	*	*	*	*	*	*	*	*	*	*
Phalanges III 4	..	..	..	..	..	..	..	..	..	..	..	*	*	0	*	0	0	*	*	*	*	*	*	*	*	*	*	*	*
Phalanges III 5	..	..	..	..	..	..	..	..	..	..	..	..	..	..	*	..	..	..	..	*	*	..	..	..	..	..	..	..	..
Os calcis	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..

Ischium.—This nucleus appears first on the 105th day in the body of the ischium. It is one millimeter in diameter in embryo R.

Calcaneum (variation).—A nucleus one millimeter in diameter is in the middle of of this bone on both sides on the 65th day. It is not present in any of the older embryos.

Metatarsal bone.—The second metatarsal bone is the first of this row to appear and is present as a small nucleus in an embryo 58 days old. In an embryo 60 days old the second, third and fourth metatarsal bones have begun to ossify and in a second embryo 58 days old all five bones

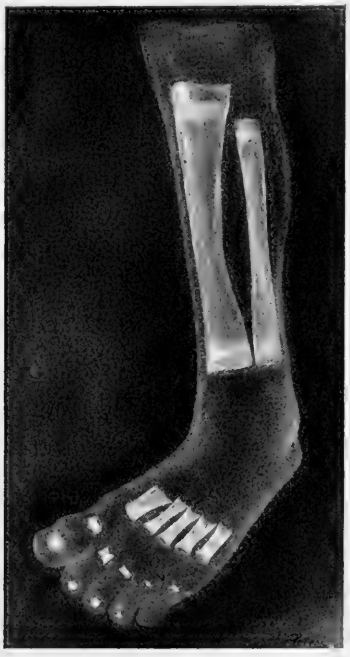


FIG. 6. Foot of embryo No. 300 (73 mm. long), enlarged 3 diameters.

are present, the second being the largest. On the 75th day they are about one, and on the 90th day about two millimeters long.

My data correspond in time with those given by Quain, who states that the metatarsal ossify in the 8th or 9th week. Other anatomists find the time of their appearance all the way from 6 weeks (Gegenbaur) to 5 months (Schwegel). Hasselwander,[16] who has made an exhaustive study of the ossification of the bones of the foot, fixes the time of the appear-

[16] Hasselwander, Zeit. f. Morph. u. Anthropol., 5, 1903.

ance of the metatarsal between the 9th and 10th weeks. Unfortunately Hasselwander does not give the crown-rump measurements of his specimens, making it difficult for me to estimate their age. However, I am inclined to think that he has overestimated the ages of all of his embryos.

Phalanges, I.—The center for the first bone of this row is present in an embryo 83 days old and all of them are present in another embryo of the same age. On the 85th day a specimen shows but the first and second bones. In the earliest stages the bones often appear as double centers, one on the dorsal side and one on the volar side of the bone. Soon two delicate half rings unite the primary centers to form the shaft which in the older embryos of my list is but half a millimeter long. Hasselwander places the time for the appearance of this row all the way from 11 weeks to 4 months, the centers not being constant until the latter part of the 4th month.—The second row of phalanges is not ossified in any of my preparations. They appear shortly after the 110th day, according to Hasselwander, although the older French anatomists place the time much too early, Rambaud and Renault on the 45th day!

Phalanges, III.—The first terminal phalanx appears on the 58th day and is therefore one of the first bones of the foot to ossify, as was correctly stated by Meckel nearly a century ago. After this time, in all the embryos studied, the first four of the terminal phalanges are present, while the fifth is not constant until the 90th day, although it is present in three younger embryos. Rambaud and Renault fix the time of ossification of the first four terminal phalanges in the 4th month, and the fifth after birth.

It may be noted again that early ossification centers are best seen in Schultze specimens viewed in direct sunlight with a purple background, with a large lense which magnifies two or three diameters, in order that both eyes may be used. The very earliest deposit of bone cannot be seen with certainty in ordinary serial sections stained with hæmatoxylin and eosin or with carmine.

Much of the trouble in determining the time of ossification is due to the uncertainty regarding the age of the embryos studied. In my specimens the age is estimated in days by multiplying the square root of the crown-rump length in millimeters by 10. This measurement was made with great care, and is given each time with the age. If the calculations of embryologists are correct, my estimations of the age of the embryos cannot be out of the way more than a few days. Estimations of the age from the last menstrual period alone may be fully a month in error and are nearly always in need of correction.

DESCRIPTION OF A 4-MM. HUMAN EMBRYO.

J. L. BREMER.

With 16 Text Figures.

This embryo, series No. 714 of the Harvard Embryological Collection, noted as about three weeks old, is an excellent subject for study because of its good preservation and successful sectioning. Unfortunately the drawings of the whole embryo are inadequate, so that the sketch given here has been altered slightly to conform more accurately with the shape as we find it in the serial sections. The necessity for a part of this alteration may, of course, be due to shrinkage, but the form, as given in Fig. 7, is certainly at least approximately correct. This shows a large head, flexed sharply on the body, a curving back ending in a curled tail, twisted spirally to the right; a marked protuberance below the head for the heart, and an outgrowth for the fore limb. There is no trace of posterior limb. Appended to the ventral surface below the heart is the yolk-sac, represented here as cut irregularly, and at the right side of this, posteriorly, the body-stalk, cut near the chorion. Four pocket-like depressions, the gill clefts, lie behind a larger depression, the mouth. There is no surface marking to indicate the eye. Protuberances corresponding to the primitive segments are not shown in the original drawings, though plainly visible in a model of the rump region (Figs. 15, 16)—perhaps the irregularities of surface have increased with shrinkage. There is no sign of distortion or injury.

The embryo was preserved in 10 per cent formalin, imbedded in paraffin, and cut in a transverse plane. On microscopic examination the tissues are found to be excellently preserved; even the frequent mitotic figures in multiplying cells show clearly. In fact, by the histological condition, as well as by the external appearances, we are led to believe that the specimen is normal, and yet the state of growth of some parts and the form of others do not agree with the descriptions of human embryos of the same size given by His and other investigators.

The most notable difference is in the stage of growth of the nervous system. His describes the thickened medullary plates as having already, in an embryo of this size and general stage of development, been changed throughout their entire extent from a groove to a closed tube; but in

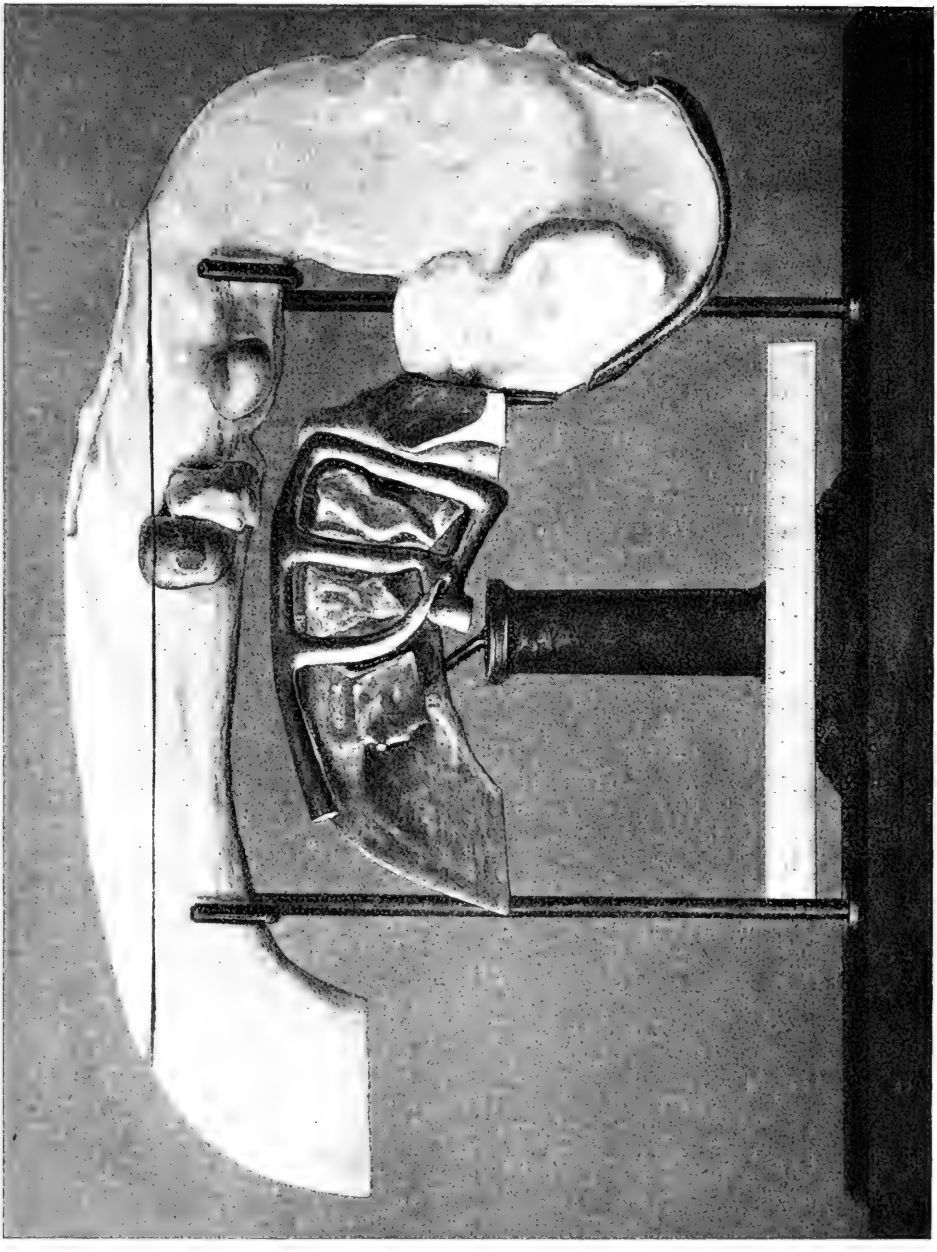


Fig. 1. Models of Brain and Pharynx. × 60.

this embryo the closure is incomplete in the anterior part of the head, and also for a considerable distance from the end of the tail, giving both an anterior and a posterior neuropore. This is the more interesting when we consider that in both the pig embryo and the rabbit embryo,

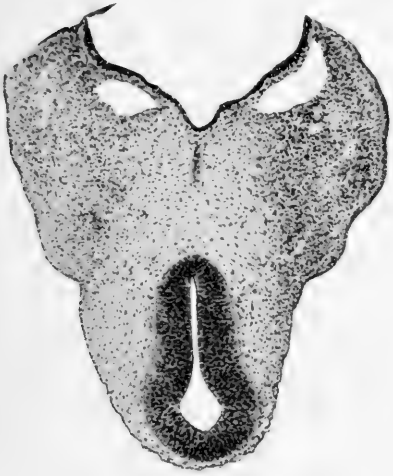


FIG. 2.

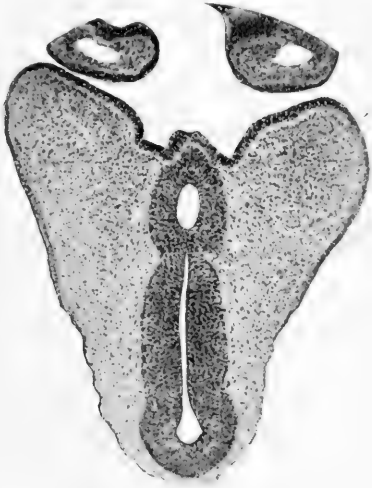


FIG. 3.

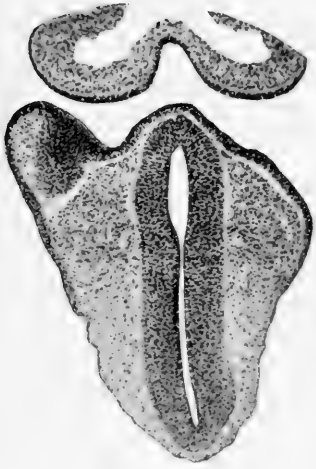


FIG. 4.

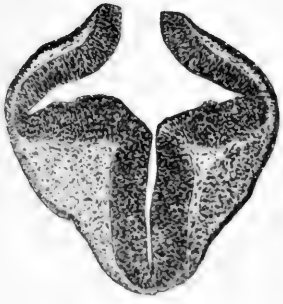


FIG. 5.

FIG. 2, 3, 4 and 5. Sections through head. × 60.

as described in Keibel's Normentafeln, the closure of the medullary tube is completed only after the formation of the head and neck bends, as in this human embryo.

Description of Models.

Model of brain.

This model is shown in side view in Fig. 1, and again in Fig. 6, there turned so as to be seen nearly from below. The fore brain is divided into two parts, one of which includes the anterior neuropore (marked by the rolled ectoderm, shown only where that of the brain joins that of the skin), the lateral pocket of the optic vesicle, and further from the anterior end on the ventral side the enlargement for the hypophysis, which fits between the two lobes of this gland arising from the pharynx.

Fig. 6. Model of brain, seen from below. × 60.

The relations of the pharyngeal and medullary portions of the hypophysis are shown in Fig. 3, the photograph of a section of this part of the embryo at this level. Of the other photographs of the brain, Fig. 4 represents a section of the anterior part of the hypophysis, showing its continuity with the cavity of the brain, and Fig. 5, a section passing through the optic vesicle and the anterior neuropore. The plane of section is shown by the line between the two pieces of the model.

In this first portion of the fore brain there are, beside the anterior

neuropore, two other areas, shown in the model, where the ectoderm of brain and skin are continuous; one in the mouth region, and one behind (following the curve of the brain) the neuropore. Moreover just behind this last area there is a distinct prominence of the brain tube, not

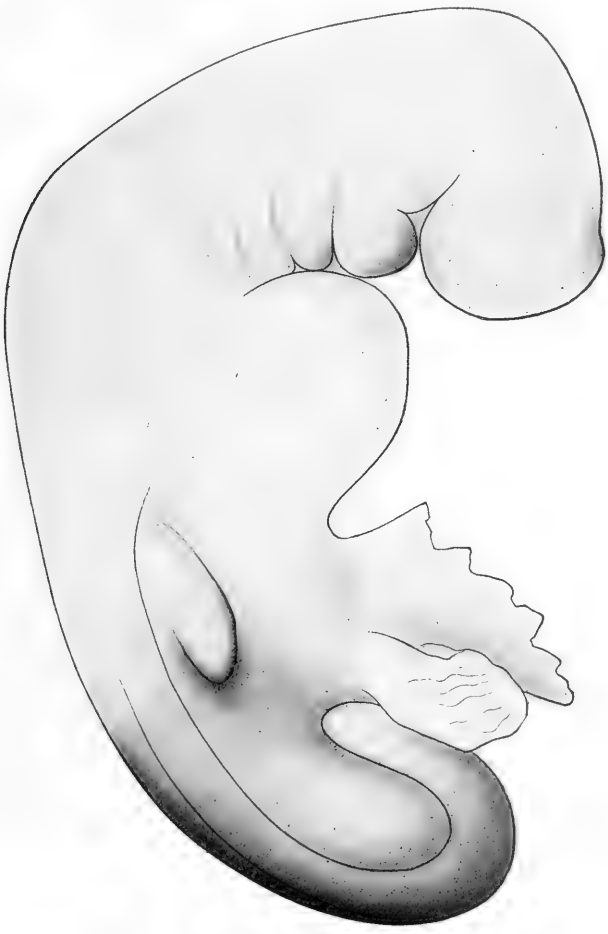


FIG. 7. Drawing of whole embryo. × 30.

attached to the skin, which simulates a neural crest, as though for a pair of cranial ganglia. This of course must soon disappear, as no such pair of ganglia exists at a later period, but its presence is interesting as showing a tendency of this anterior portion of the brain to develop as do the more posterior portions.

Between this anterior part of the fore brain and the mid brain, not marked distinctly from either, is found a narrow strip of less caliber than the anterior portion, with no special prominence and no connection with the ectoderm of the skin. A cross section of this is shown in Fig. 2.

The mid brain, occupying the head bend, is also without special interest, except that it is of smaller caliber than might be expected. It merges without line of demarcation into the hind brain. The hind brain is again divisible into two parts; one posterior, smooth, of nearly even caliber, merging gradually at the neck bend into the spinal cord; and one anterior, showing a further development. The roof of this anterior part is thin, has become shrunken and crumpled. As yet, however, there is no sign of a Varolian bend. Attached to the side, ventrally, are two

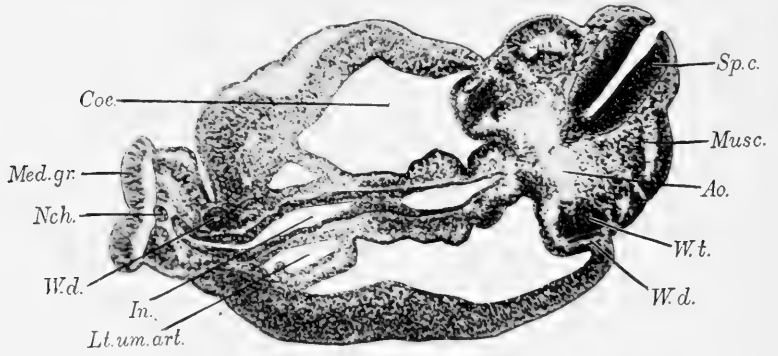


Fig. 8. Section through body of embryo, near the cloaca. × 60.

ganglia, one for the Trigeminal nerve, and one for the Acoustico-facial complex, in which there is only a faint indication of separation into distinct nerves. Behind this second ganglion, applied closely to it, lies the otocyst, a rounded hollow vesicle, close to the brain, but not touching it, and still attached externally to the ectoderm, a patch of which can be seen on its outer surface. Of the other cranial ganglia nothing is shown in the model, as they are represented only by diffuse groups of cells on each side of the hind brain, not yet divided into separate ganglia, and not attached to the brain wall. There is a peculiar notch in the floor of this part of the hind brain, shown in Fig. 1, between the two ganglia, bounded by two rounded prominences seen better in Fig. 6, the significance of which I do not know.

On taking off the top part of the model, we find that the cavity of the hind brain is marked by a median ventral furrow. The sides are

smooth, except in the anterior portion, where there are distinct traces of neuromeres. Toward the cord the cavity is compressed laterally, but there is no sign of a groove between the zones of His.

Fig. 6 shows the wide open anterior neuropore, through which one can look into the hollow optic vesicles, and into the median cavity of the fore brain, which is seen to be a narrow slit, compressed laterally. The edges of the opening are broadly curved or rolling, with no demarcation to indicate where the future line of closure is to be. This is shown in section in Fig. 5.

MODEL OF PHARYNX AND AORTIC ARCHES.

The pharynx, of which the model is a cast, the shape of the cavity being represented, is shown in Fig. 1, in its proper relation to the brain. It consists of a broad body opening anteriorly into the mouth, the lateral extension of which, between the maxillary and mandibular processes, is represented as a cut surface. On the dorsal surface of this body, near the fore brain, are irregularities, chiefly two rounded ridges, one on each side of the median line, representing the out-pocketings for the pharyngeal lobes of the hypophysis, as shown in Fig. 3, and explained above. Behind these, at the angle made by the roof of the pharynx corresponding to the curve made by the brain at the head bend, is a median ridge or point which is continuous with the notochord, and marks the anterior end of the latter. Toward the œsophagus, into which the body of the pharynx merges, the cavity becomes more and more compressed laterally, until it is an antero-posterior slit. From the sides of this main cavity three lateral out-pocketings project, the most dorsal one being divided into two smaller projections near the end. These represent the gill pouches, and diminish in size from before backward. The first two rapidly become compressed antero-posteriorly and end in blunt, more or less vertical ridges, while the third is more tapering, and the fourth ends in two distinct pointed branches.

In the tissue between the opening of the mouth and the first gill pouch, and also between the first and second, and second and third gill pouches, run the first, second, and third aortic arches on each side, joined dorsally by the dorsal aortæ, which make an impression on either side of the median line of the body of the pharynx, and then continue downward, beside and behind the œsophagus. On the model, the aortic bulb is represented as cut off just above the standard; a cup-shaped chamber leads from this forward, and gives off on each side two vessels; one pair of vessels forms the first aortic arches, a second pair divides

into the second and third arches of each side. The connection between these last two pairs of arches and the aortic stem is very slender, compressed laterally, as though the arches had grown from the dorsal aortæ and had only just united with the ventral aortæ. Of the fourth and fifth aortic arches the only trace is the rim of the cup-shaped part of the ventral aorta, shown in the figure below the bend of the third aortic arch. No branches come from the dorsal aortæ in the position of these fourth and fifth arches. The dorsal aortæ are joined in a single median vessel for a considerable distance along the back; there is no trace of carotid branches from the first arches toward the head.

MODEL OF HEART.

An anterior view of the model of the heart is given in Fig. 9, the model is seen slightly from the right side. The most anterior portion is the large rounded aortic bulb (*B. ao.*), cut off just below its division into aortic arches as shown in the model of the pharynx. Continuous with this is the ventricle (*Vent.*) a large single chamber, beginning at a constriction marked by a groove on the outside (*Gr.*), extending downward on the left side, turning on itself to form the apex of the heart, and then tapering upward to merge with the aortic bulb. The walls of the ventricle are thick, smooth externally, but internally very irregular, with deep pockets lined by endothelium leading from the main chamber, representing the sinusoids of the heart. In the aortic bulb the walls become smooth internally; there is no sign of division into right and left ventricles. The left auricle (*Lt. au.*) is placed above the ventricle, marked from it externally by the groove. Its walls are thin and folded, probably by pressure, and internally the endothelium is smooth, with no pockets nor trabeculæ. The left and right auricles are joined near their upper ends anteriorly by a prominent ridge, enclosing a cavity, which extends downward, compressed antero-posteriorly, and opens by a narrow channel into the ventricle to the left of the median line. Above this connection the right auricle rises dorsally to a rounded peak, not quite so high as the top of the left auricle, from which it is separated by the intestine (*In.*), but, unlike the left auricle, the right extends far below the auriculo-ventricular canal as a spacious pouch, reaching almost to the level of the apex of the ventricle, and occupying a position behind and to the right of the aortic bulb and the lower part of the ventricle. Here, as in the left auricle, the walls are thin, smooth on both external and internal surfaces, and thrown into great folds. If this auricle were distended it could certainly contain a larger volume of blood than the

ventricle. This enormous right auricle is not, to my knowledge, figured in any model or drawing of human or other mammalian hearts of any stage, but conforms almost exactly to the Selachian heart.

The blood enters the heart by way of the right auricle, from the sinus venosus, as is shown in Fig. 10. In this figure the heart is viewed from behind and slightly from the left side, and a portion of the model, the

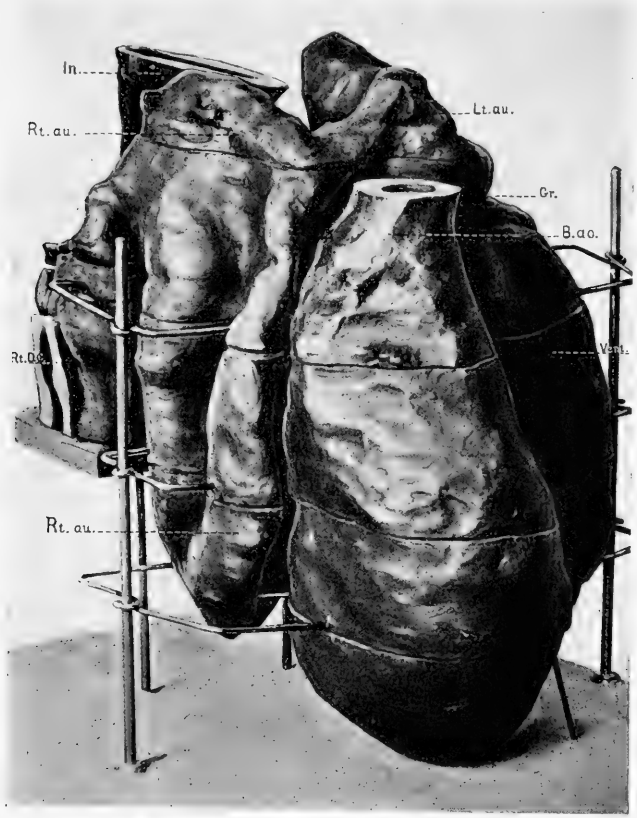


FIG. 9. Model of heart, anterior view. × 90.

dorsal wall of the sinus venosus, has been removed. The jugular veins from the head region and the umbilical veins from the body join on either side, on the left more anteriorly than on the right, and from this junction on each side an irregular sinus extends across the embryo, interrupted in places by bands of tissue (the cut end of one of which is seen in the figure) into which open dorsally the two vitelline veins. From this sinus venosus the blood passes to the right auricle by two sep-

arate openings (*x* and *y*). Here again this embryo differs from those previously described or modelled, in all of which a single channel leads from sinus venosus to right auricle. Dr. Minot has recorded separate openings into the heart for the cardinal and omphalo-mesaraic veins in the chick (Textbook, p. 282), but the double openings just described do not appear to be correlated with those in the bird.

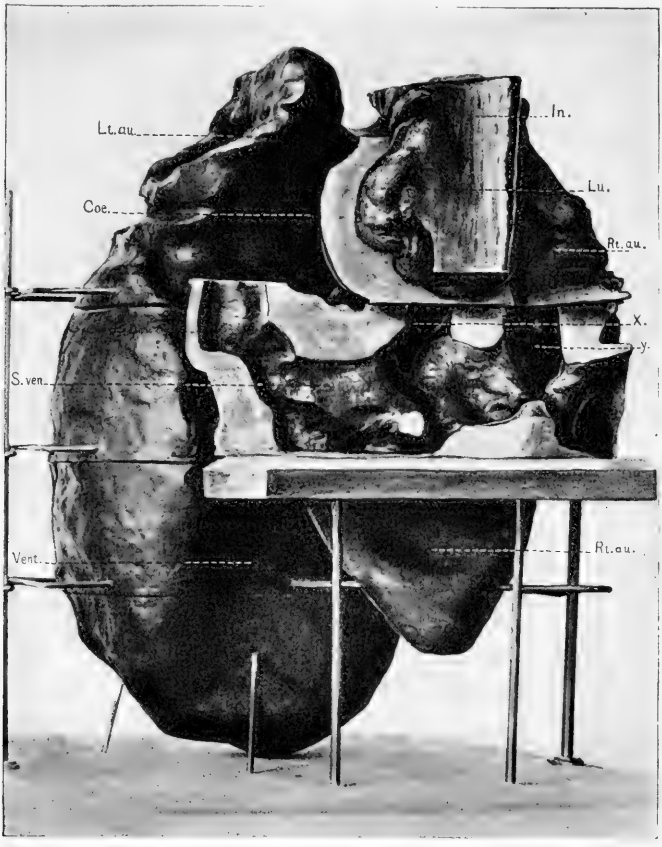


Fig. 10. Model of heart, posterior view. × 90.

The heart lies in the body cavity or cœlum, and is attached to the body wall by the sinus venosus, and to the intestinal tract by a short fold of mesenchyma. Fig. 10 shows also a portion of this intestinal tract; the mesodermal part has been partially dissected away from the entoderm in order to show the outgrowth destined to become the lung (*Lu.*). This outgrowth, situated at the level of the left auricle on the ventral side of

the intestine, where the pharynx is becoming narrowed to form the œsophagus, is rounded, irregular, and extends to right and left and caudally. The right side of the outgrowth is the larger and extends further tailward, leaving the œsophagus at a more acute angle than the left, a reversal of the adult condition. There is no distinct trachea, as the cavity of the œsophagus is continued directly into the cavities of the right and left lobes of this outgrowth.

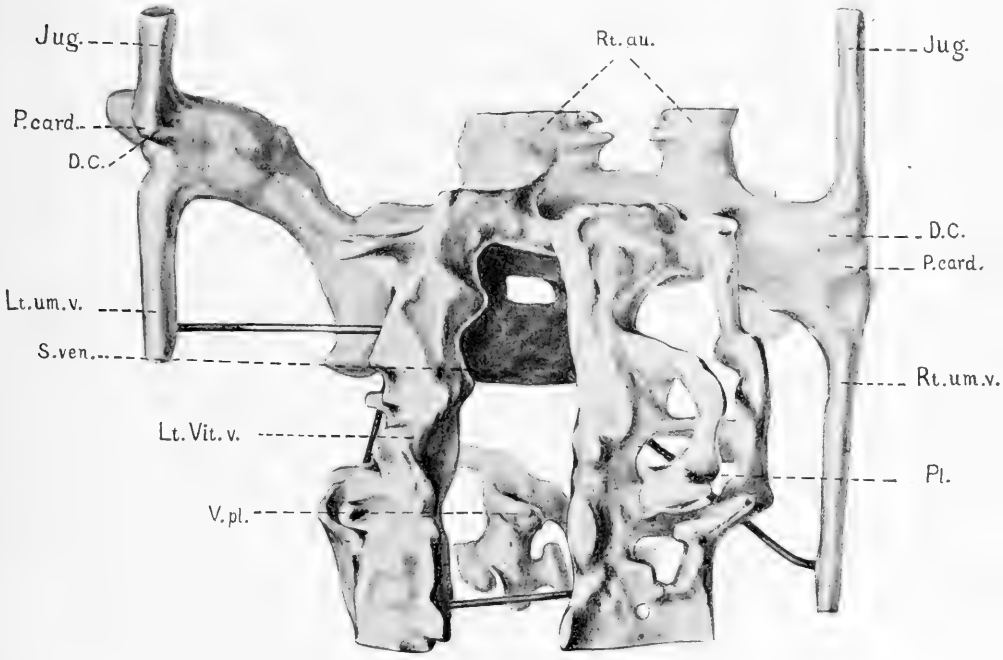


Fig. 11. Model of veins near heart, dorsal view. × 160.

MODEL OF VEINS NEAR HEART.

This model represents the venous channels as solid, and includes the junction in the sinus venosus of the three large pairs of veins, the jugular and the umbilical veins in the body wall, and the vitelline veins in the intestinal wall. The sinus venosus is shown as a cavity in the model of the heart, so that these two models overlap to a certain extent. Fig. 11 shows the model as seen from the dorsum of the embryo; Fig. 12 as seen from the left side; and Fig. 13 is a view taken from the caudal end of the embryo, and a little from the right side.

The umbilical veins, entering the embryo by the body stalk, run in the

body wall forward, at a level ventral to the intestine, varying only slightly in diameter. At points differing slightly on the two sides, more anteriorly and dorsally on the left, they join the two jugular veins and turn inward (on the left side also ventrally) as the sinus venosus. From each jugular vein, nearly at its junction with the umbilical vein, arises a small bud, the future posterior cardinal vein (*P. card.*). The Duct of Cuvier (*D. C.*), later a well marked trunk extending from the junction

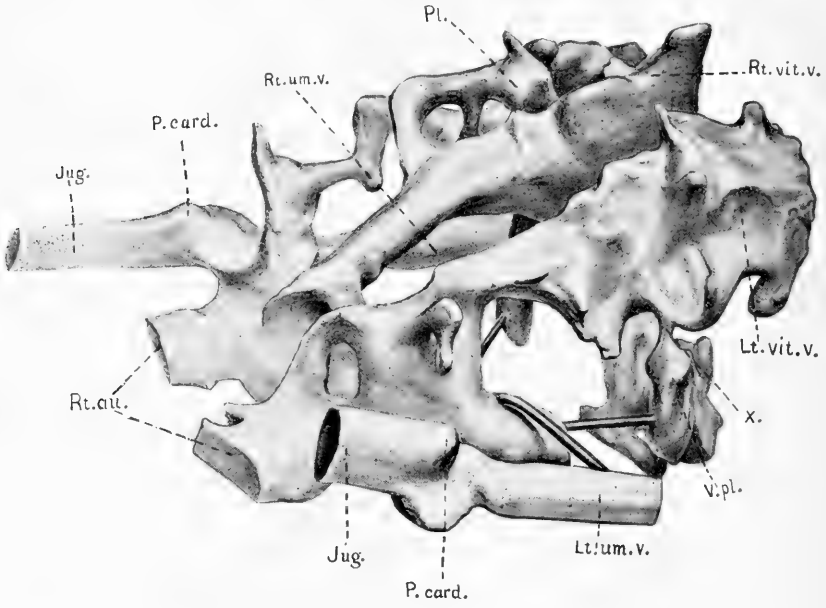


FIG. 12. Model of veins near heart, from left side. × 160.

of jugular and cardinal veins to the entrance of the umbilical veins, can in this embryo, then, scarcely be said to exist, so short and ill-defined is it.

The sinus venosus is seen, as in the model of the heart, extending across the embryo, an irregular cavity subdivided by bands of mesenchyma (represented in this cast as holes), and emptying anteriorly into the right auricle by two channels (*Rt. au.*). Into this sinus empty also the two vitelline veins (*Vit. v.*) one on each side of the intestine, at a level dorsal to the umbilical and jugular veins (Fig. 12). The vitelline veins, unlike the two other pairs modelled, vary greatly in caliber; they are small and rounded at their entrance into the sinus venosus, but more caudally spread into large channels, each with a mesial wall straight,

vertical, fitting closely the sides of the entodermic intestine, which lies between them (Figs. 11 and 13). The lateral wall of each vitelline vein is very irregular, giving off many branches, which join to form new longi-

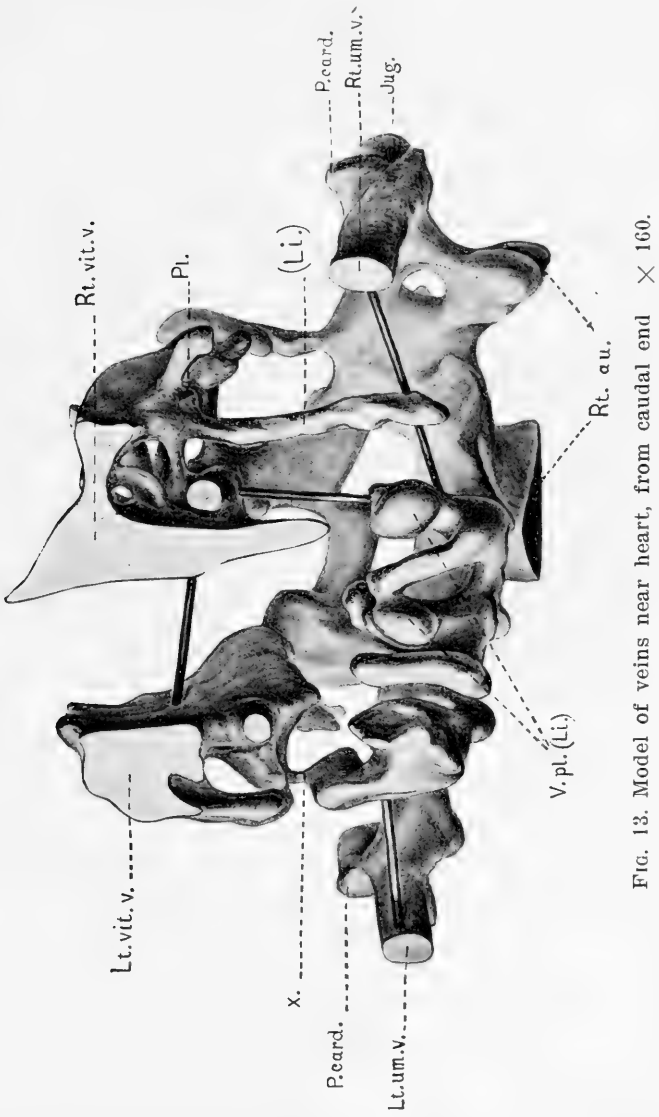


FIG. 13. Model of veins near heart, from caudal end × 160.

tudinal channels, the whole making an irregular plexus. The plexus from the right vitelline vein is more developed than that from the left, and has even acquired an opening of its own into the sinus venosus

(Figs. 11 and 13). From the plexus on each side branches extend ventrally, those on the right ending blindly, but one on the left side joining by a narrow channel (Figs. 12 and 13, *x*) with another venous plexus (*V. pl.*) extending across the median line, ventral to the intestine, and posterior or caudal to the sinus venosus, from which it is entirely free. This ventral plexus is in intimate relation with the liver, and will be spoken of more fully in the next section.

Posterior to the outgrowth of these plexuses the vitelline veins divide into many branches which spread out over the yolk sac, into which the intestine soon opens. Cut ends of some of these branches may be seen in Fig. 13.

MODEL OF THE LIVER.

This model is seen from the caudal end, and a little from the right side in Fig. 14. The intestine is a large tube much compressed laterally, and the vertical right side is shown. From the ventral border of this tube hangs a large outgrowth, extending first ventrally, then laterally, then dorsally, composed of an irregular mass of entodermal cells, which tends to break up into cords, often anastomosing with each other. Only the right side of this mass has been modelled. The cavity of the intestine passes for a short distance into this mass, but without subdivision, so that all the cords and irregularities are solid. In the model a part of the left wall of the intestine has been cut away to allow a view of this cavity.

It has been stated by other writers that the liver arises by cords of cells which grow into the territory occupied by a large vein, pushing before them, and thus becoming invested by the endothelium, and forcing the blood to run in small, anastomosing channels, the sinusoids. This statement would not be true of this embryo, for the liver cords are found growing into mesenchyma, at a level ventral to the vitelline veins; in this same mesenchyma, however, we find the branches of the vitelline veins ramifying, and forming plexuses, and in certain places these plexuses come into intimate relation with the liver cords. These points of contact, where veins and liver actually touch, are marked in the model of the liver (Fig. 14, *V.*), and are seen to correspond with the anterior surfaces of the portion of the plexuses marked in the model of the veins (Fig. 13, *li.*). These two models might be fitted together, in which case we should find the intestine lying between the vertical, mesial walls of the two vitelline veins, the liver spreading in the same mesenchyma that contains the lateral and ventral venous plexuses, between the sinus

venosus in front and the ventral venous plexus behind; but the two models would come into actual contact only at the points marked and already noted. As both the liver and the venous plexuses continue to grow, they will come into contact over more and more of their extent, until finally the result of small, anastomosing channels, the endothelium of which is in close contact with the liver cords, will be produced. The method by which this result is attained is, however, in this embryo different from that usually described; in the one case, the cords of liver cells push against the walls of a pre-existing large venous channel, and

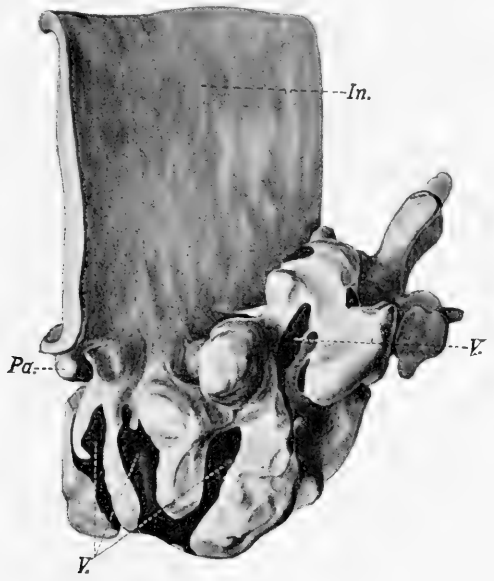


FIG. 14. Model of liver, from caudal end. × 160.

by invaginating these walls, make finger-like trabeculæ within this large space, yet separated from the cavity by the endothelium which invests them. These cords then anastomose until the cavity of the vein is subdivided into many smaller channels, the sinusoids, all lined by the original endothelium, and having for walls, beside this endothelium, the cords of liver cells. In this embryo, on the other hand, the cords of liver cells and the small branches from the vitelline veins are both pushing into the same mesenchyma, both growing rapidly, until, the mesenchyma remaining small in amount, the whole enlarged mass will be made up practically of the veins and the liver cells, which by their continued growth must come into contact, the veins filling practically all the spaces

between the cords, and the thin endothelium becoming wrapped around the cords; and thus the adult condition will be reached.

In this model is also shown the anlage of the pancreas. (Fig. 14, *Pa.*) It is a small, knob-like mass of cells, with no cavity, growing from the ventral border of the intestine just caudal to the liver outgrowth, but distinct from the latter. Further down the intestine (nine sections in the specimen) where it has begun to expand to the yolk sac, is found another, smaller mass of cells, growing also from the ventral border, which probably represents a second anlage of the pancreas; there is no sign of a dorsal anlage. The pancreas also, then, differs from those usually described, as it has probably two outgrowths, neither from the liver stalk, for the duct of Wirsung, and as yet no outgrowth to represent the duct of Santorini. There is no enlargement of the intestine above the liver for the stomach.

MODEL OF THE TAIL END OF THE EMBRYO.

This model has been photographed in two positions, once (Fig. 15) squarely from the right side, and again (Fig. 16) still from the right side, but also a little from behind. The left side of the model shows the surface of the embryo with the umbilical cord attached to it. A portion of the right side, that at the tip of the rump, shows the outer surface also; but for the remainder of this side, the ectoderm of the skin and the mesenchyma have been dissected away to the median line, which in this part of the embryo is distinctly curved on account of the spiral twist of the tail. The curve can be clearly understood by referring to the photograph of a section (Fig. 8) at the level of the two brass handles for moving the model, shown in Fig. 16. The plane of section is shown by the bottom line of the dissected part of the model. The outline of this section will explain the irregularities of the surface at the rump end as seen in the model. Posteriorly, from the median line forward extends a rounded, smooth area overlying the spinal cord; next this anteriorly, another rounded area, broken by transverse grooves into irregular mounds, representing the primitive segments; and still more anteriorly a broad, smooth area, separated from the last by a longitudinal groove, covering the body wall, within which lies the cœlom. These three areas are curved upon themselves at the rump end, the protuberant body wall making a distinct ledge-like projection, as seen in the photographs (Figs. 15, 16, *B. w.*).

Spinal cord.—The spinal cord is shown in both drawings, appearing at the dorsal side of the embryo, directly under the ectoderm of the skin.

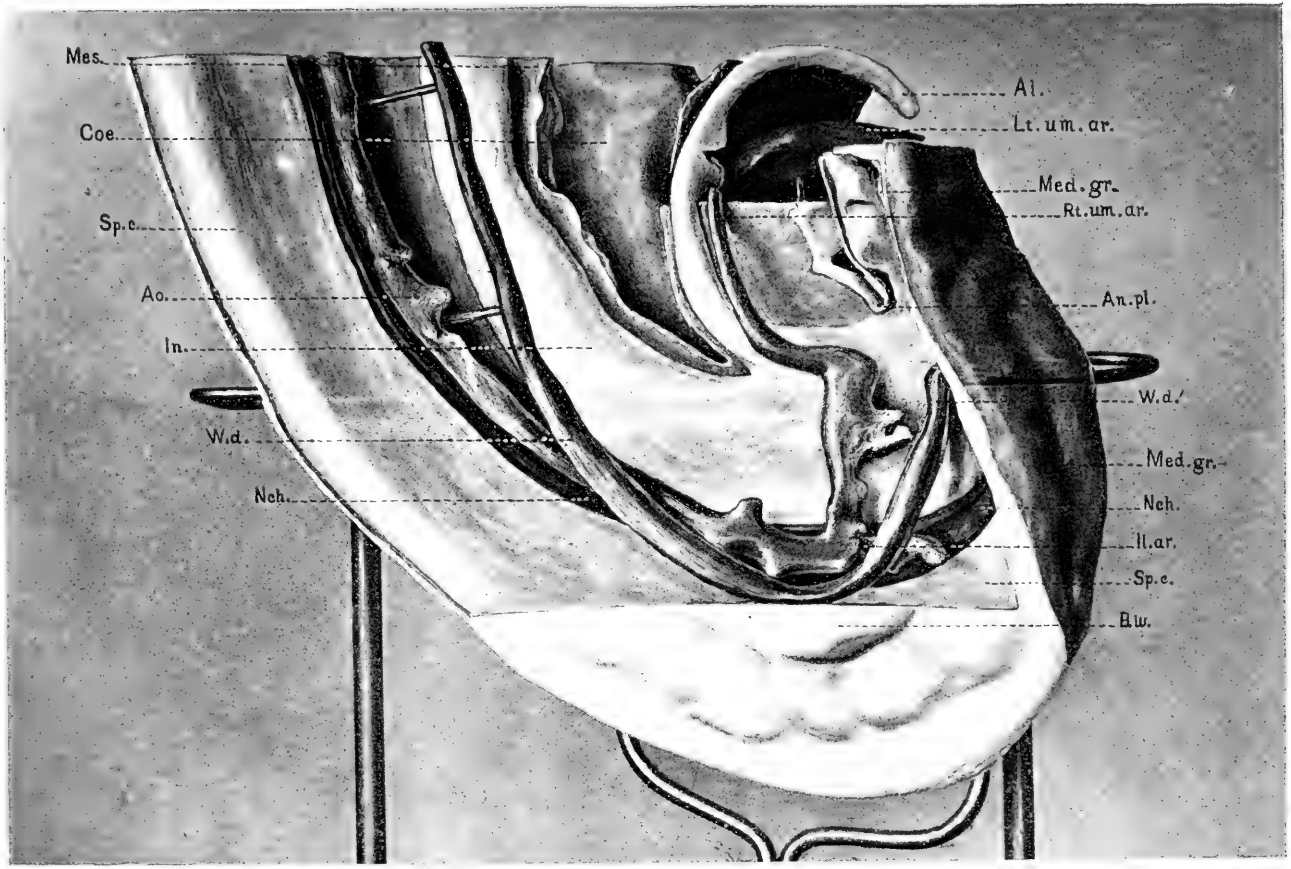


FIG. 15. Model of tail end of embryo. × 90.

The shape in cross section can be seen in Fig. 8; the opening of the canal to the outside is artificial, a tear in the section. The spinal cord follows the curve of the rump, passing nearly out of sight in the undissected part, but reappearing nearer the tail. Here a change has taken place. The ectoderm of the spinal cord and that of the skin have been gradually becoming continuous, and just after rounding the curve of the rump the medullary tube is replaced by an open medullary groove. This opening of the spinal cord to the outside is accompanied, as in the case of the anterior neuropore, by a rolling of the edges, as shown in Fig. 16, and in section in Fig. 8. The groove becomes wider, until the sides lie in the same plane, but the medullary plate is distinguishable from the ectoderm of the skin, almost to the end of the tail, by its thickness. At the tip of the tail, which has been cut off in the model, all the tissues become merged into an undifferentiated mass.

Notochord.—The notochord, shown in the back region in Fig. 15, and in the tail region in Fig. 16, lies just ventral to the spinal cord to which at times it seems attached (Fig. 8). In cross section it is at first rounded, but at the curve of the rump becomes oval, with long axis dorso-ventral, and considerably larger. It follows the curve of the spinal cord, and is lost not far from the end of the tail by merging with the spinal cord (Fig. 16).

Aorta.—The aorta lies ventral to the notochord, separated from it by a narrow strip of mesenchyma. At first, *i. e.,* in the back region, it sends off small buds, not always directed laterally, apparently not segmentally arranged, but presumably representing intersegmental arteries. At the apex of the rump curve the aorta, now entirely separated from its fellow on the opposite side of the body, leaves the notochord and curves sharply forward. At this point a branch is found, extending laterally and toward the spinal cord, mesial to the Wolffian duct; this is the iliac artery (Fig. 16, *Il. ar.*). Beyond this branch the aorta passes, as the right umbilical artery, lateral to the cloaca and the allantois, to the beginning of the body stalk, but here becomes obliterated on the right side (*Rt. um. ar.*). On the left side, however, the left umbilical artery (*Lt. um. ar.*) becomes a much larger channel, and continues along the body stalk. The mesenchyma of the body stalk has been partially dissected away above a certain level to show the left umbilical artery and allantois. Throughout the first part of its course the umbilical artery is of irregular caliber, but soon it becomes smooth.

Intestine.—The entodermic intestine (Figs. 15, 16, *In.*) appears as an oval tube, with long axis antero-posterior. It is situated in front of the

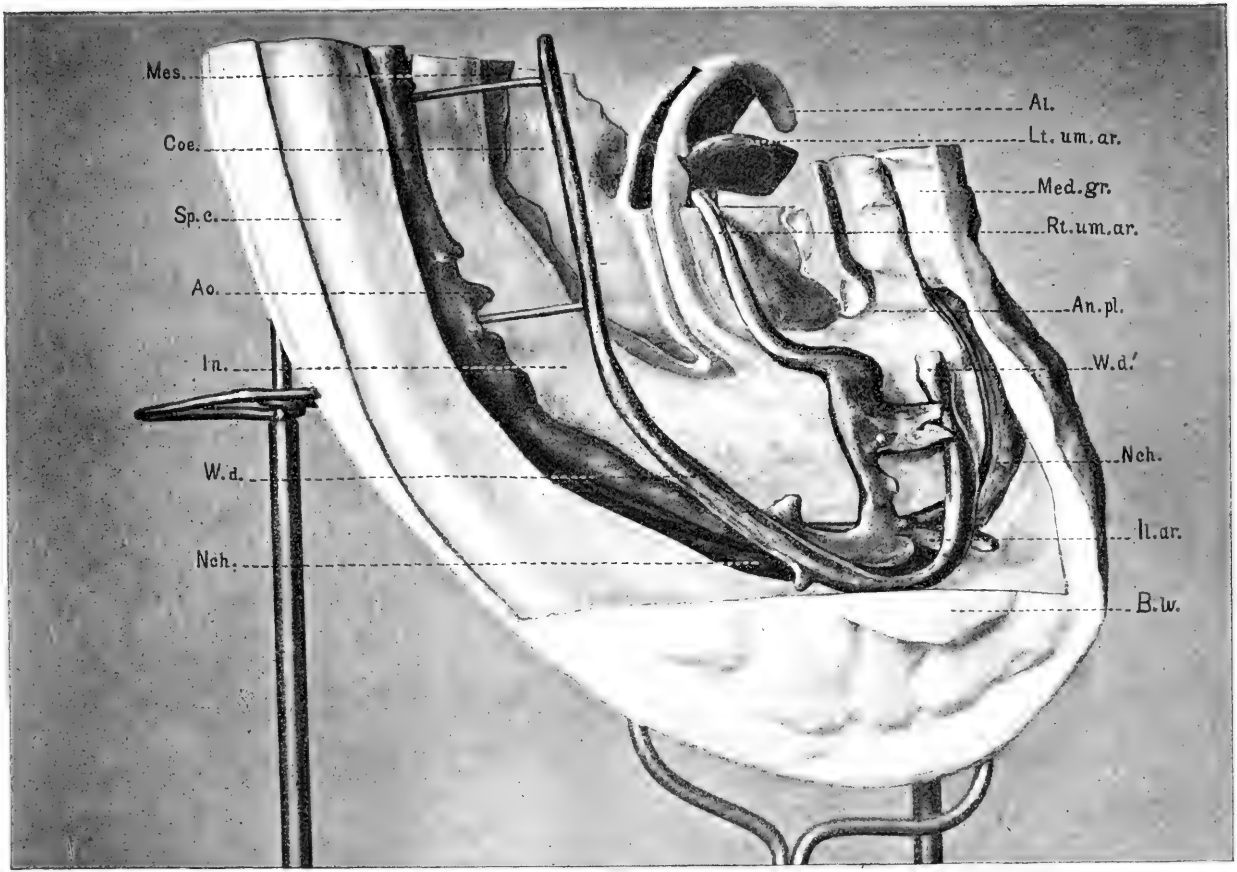


FIG. 16. Model of tail end of embryo. × 90.

aorta, but separated from it by the mesoderm of the mesentery, which has been dissected away to the median line. Ventral to the entodermic intestine, also, the mesoderm of the intestinal wall has been cut away to the median line. The entodermic tube follows the general curve of the embryo, growing gradually larger, and empties into a large rounded pouch, also compressed laterally, the cloaca. In Fig. 8 the section passes nearly through the junction of the intestine and the cloaca, and the compressed shape can be more easily seen. The mesentery becomes gradually shorter and finally disappears entirely. The cloaca, along its greater curvature, lies close to the notochord, and passes into the tail as the tail gut, which soon loses its lumen and ends near the tip of the tail by fusing with the ectoderm in an undifferentiated mass. At the root of the tail the cloaca comes into close contact with the ectoderm of the skin at the anal plate (*An. pl.*). From the upper, posterior corner of the cloaca, just above the entrance of the intestine, passes off the allantois, which, as a slender rounded tube, curves out into the body stalk close to the wall of the cœlom, between and posterior to the two umbilical arteries, and ends blindly. A slight, irregular swelling occurs in its course, marking perhaps the position of the future bladder.

Wolffian duct.—The Wolffian duct on the right side (Figs. 15, 16, *W. d.*) is shown in the model standing out at the side, because freed from the mesoderm. It follows the general curve of the body, just ventral to the plane of the notochord, gradually approaches the median line on its way toward the tail, and ends before reaching the level of the anal plate by joining the cloaca (*W. d.'*). As can be seen in Fig. 8, its position corresponds with the external groove between the area of primitive segments and the body wall. On the left side the Wolffian duct does not extend so far as the cloaca, but ends blindly, and so is cut only once in the section (Fig. 8, *W. d.*). Dorsally the duct is oval in cross section, with long axis parallel with the lateral surface of the Wolffian ridge, which is seen in Fig 8, projecting into the cœlom dorsally; but nearer its union with the cloaca, the duct assumes a rounded form.

The Wolfian tubules, seen in Fig. 8, just mesial to the duct, are not shown in the model. They are short, sometimes with an S-shaped curve, frequently overlapping, as in the section given, where portions of two tubules are shown on the left side (*W. t.*). In all there are twenty-eight tubules, arranged in two groups; one caudal group, consisting of twenty-three tubules, close together, but not definitely arranged in relation to the primitive segments or muscle plates (Fig. 8, *musc.*). This represents the true Wolffian body, and the tubules are all closed at their

distal ends. Beyond this the Wolffian duct can be traced anteriorly for some distance (through twenty sections), and still further anteriorly is found a second group of tubules, consisting of three rather indistinct, scattered units, not connected by a duct. The most anterior tubule lies at the level of the liver and opens by a funnel-shaped mouth into the cœlom. This smaller group of tubules belongs to a rudimentary pronephros.

In conclusion, I wish to point out the chief differences between this embryo and those of the same general stage of growth already described. The most important is the presence of anterior and posterior neuropores, which were supposed to have closed in human embryos of this size, though known to be open in comparable embryos of the pig and rabbit. Next in importance, perhaps, is the large right auricle of the heart, and the double passage into it from the sinus venosus. Next, the growth of liver cells, not into territory occupied by a large vein, but into the mesenchyma surrounding the intestine, where also are growing branches of the vitelline veins; from the intercrescence of cords of liver cells and venous channels, sinusoids would inevitably result. Finally the presence of a few pronephric tubules, and the tardy growth of the left Wolffian duct, which has not yet acquired an opening into the cloaca. Let me add once more that embyro is, histologically and anatomically, in excellent condition, and as far as can be seen normal.

ABBREVIATIONS USED.

Al.=allantois.
An. pl.=anal plate.
Ao.=aorta.
Au.=auricle. (Rt. and lt.)
B. ao.=bulbus aortæ.
B. w.=body wall.
Coe.=cœlom.
D. C.=ductus Cuvieri. (Rt. and lt.)
Gr.=groove.
Il. ar.=iliac artery.
In.=intestine.
Jug.=jugular vein.
Li.=liver.
Lu.=lung.
Med. gr.=medullary groove.
Mes.=mesentery.
Musc.=muscle plate.
Nch.=notochord.

Pa.=pancreas.
P. card.=posterior cardinal vein.
Pl.=plexus.
S. ven.=sinus venosus.
Sp. c.=spinal cord.
Um. ar.=umbilical artery. (Rt. and lt.)
Um. v.=umbilical vein. (Rt. and lt.)
V.=vein.
V. pl=ventral plexus.
Vent.=ventricle.
Vit. v.=vitelline vein. (Rt. and lt.)
W. d.=Wolffian duct.
W. d'.=entrance of Wolffian duct into cloaca.
W. t.=Wolffian tubule.

THE DEVELOPMENT OF THE MOUTH AND GILLS IN BDELLOSTOMA STOUTI.

BY

CHARLES R. STOCKARD.

From the Zoological Laboratory, Columbia University.

WITH 36 FIGURES.

Eminent morphologists (Goette, **75**, Huxley, **76**, and W. K. Parker, **83**) have long since suggested the affinities of the Marsipobranchii and the larval amphibia, and Dohrn, **83**, has even maintained that the cyclostomes are to be regarded as the descendants of highly organized fishes, possibly teleosts. Beard, **89**, furthermore is of the opinion that, "While accepting some degeneration in the Marsipobranchs along with Dohrn, the truth of Balfour's view that they are very primitive forms must also be allowed. They must undoubtedly be added to the gnathostomatous vertebrata. The Marsipobranchii stand between the selacians and ganoids, but far more related to the latter than to the former." The view of Balfour, **85**, here referred to held them to be degenerate but not derived from relatively highly organized fish, as is shown by his following words, "Dohrn was the first to bring into prominence the degenerate character of the cyclostomata. I cannot, however, assent to his view that they are descended from a relatively highly-organized type of fish. It appears to me almost certain that they belong to a group of fishes in which a true skeleton of branchial bars had not become developed, the branchial skeleton they possess being simply an extra branchial system, while I see no reason to suppose that a true branchial skeleton has disappeared. If the primitive cyclostomata had not true branchial bars they could not have had jaws, because jaws are essentially developed from the mandibular branchial bar. These considerations which are supported by numerous other features of their anatomy, such as the character of the axial skeleton, the straightness of the intestinal tube, the presence of a subintestinal vein, etc., all tend to prove that these fish are remnants of a primitive and pregnathostomatous group. The few surviving members of the group probably owe their preservation to their parasitic or semiparasitic habits." Whether Balfour's reasoning regard-

ing the jaws and branchial arches can now be upheld will be discussed in the present paper.

Howes, 91, says, "In view of the admitted importance of (the hypophysis in Petromyzon) it testifies, to my mind, to an enormity in the gap between the Marsipobranchii and the remaining higher Vertebrata which even Balfour's conclusion that the former are the remnants of a primitive group, and Haeckel's famous aphorism that they are further removed from the fishes than are the fishes from man insufficiently expresses."

Max Fürbringer, 00, regarding the relationship between the myxinoids and petromyzontes states, "In wesentlichen Verhältnissen unterschieden sich die Myxinoiden mehr und principieller von den Petromyzonten, als z. B. die Selachier von den Säugethieren; in einzelnen Merkmalen stellten sie sich selbst weiter ab von den Petromyzonten, als diese von den Gnathostomen und zeigten zugleich mancherlei Hinneigungen nach den Akraniern."

A long list of other workers Dean, 99, Johnston, 05, and Worthington, 05, have recognized in these animals very primitive vertebrates coming as it were between amphioxus and the true fishes, and from a study of their embryology and morphology have attempted to arrive at the ancestral or primitive type of vertebrate organs.

Ayers and Jackson, 00, with a few others, are, I think, nearest to the correct position in considering marsipobranchs neither as degenerate parasitic forms nor as altogether archaic types, but rather as primitive animals especially adapted to their peculiar life habits.

Recognizing the chaotic condition of opinion regarding these animals Prof. Dean kindly suggested to me that I undertake a careful study of the development of the organs in the head of Bdellostoma with the hope that the general problem of myxinoid relationship might be more clearly understood. Von Kupffer had in 1900 published the results of a similar study which he made on scanty and defective material, as is repeatedly mentioned throughout his paper. His study was also confined to rather young stages and, as I have found, stopped really before the most interesting points in the development had been reached.

I am under obligation to Professor Bashford Dean not only for suggesting this study and for his criticism during its progress, but also for his kindness in placing entirely at my disposal his unique series of Bdellostoma embryos. I am also glad to express my appreciation of the encouraging interest that Prof. T. H. Morgan has always taken in my work.

General Discussion of Work and Views Relating to the Marsipobranchii.

1. *The morphology of the myxinoids* has been so extensively studied that its literature has become almost cumbersome. The earliest reference to these animals that I have been able to find is that by Gunnerus in 1762. He described Myxine glutinosa under the name "Sleep-Marken." He described the tooth plate as a jaw, and also found the two openings, mouth and nose, leading into the pharynx; the remarkable "tongue" muscle-apparatus he called a wind-pipe or air tube. Gunnerus also described the gills of each side calling them lungs. His work is reviewed in the papers of J. Müller, 1835, and P. Fürbringer, 1875.

In 1790 A. J. Retzius also described the tooth plate as a jaw and the club-muscle as a wind-pipe. He classed Myxine among the fishes instead of with the worms and molluscs as some previous writers had done.

Abildgaard, 1792, mentioned the "wind-pipe" of Gunnerus as the club-shaped jaw muscle and called the tooth plate, Zungen-Zähne, a lower jaw, stating that it was strengthened and moved as such. Abildgaard gave the first correct description of the gills, stating that on each side a canal opened to the outside after receiving the six canals of the gills and these gills through another set of six canals connected with the throat. He also traced the gill vessels.

Bloch, 1789, described two parts of the club muscle, the outer hollow circular muscle, and the longitudinal one. J. Müller states that he gave their connection incorrectly, claiming both to be fastened to the jaw bone, by which Bloch meant the tooth plate or "tongue;" while in fact Müller claims the first to be attached to the tongue cartilage and the second to the tongue itself.

Home in 1815 described rather fully the gill system and compared the organs of Bdellostoma and Myxine. Müller states that in his explanation of figures Home correctly said that the teeth belonged to the tongue, he also described the connected muscle-body as a tongue muscle apparatus. P. Fürbringer says that Home first corrected the error of calling the dental-plate the jaw by recognizing in it a tongue.

In the present paper I shall endeavor to show on the other hand that it was Home and subsequent workers up until 1900 that were in error when they called this organ "tongue," and that the earliest workers were correct in interpreting the dental-plate as a jaw.

I have given this brief historical review for the purpose of showing that the idea that the dental-plate is a lower jaw and not a tongue was the oldest or first interpretation of this organ and not a new

idea originating with Ayers and Jackson as one might conclude from reading their papers of 1900. Those authors, it appears, could not have considered these early views in spite of the fact that they are either mentioned or discussed in the papers of J. Müller and P. Fürbringer which Ayers and Jackson have placed in the literature list of their paper.

As Ayers and Jackson state, morphologists followed for a long time Müller in accepting, not his own or original view, but Home's idea that the dental-plate was a tongue. And even Huxley, in attempting to locate the jaw cartilages of Petromyzon failed to determine that the tongue was really a part of the jaw structures. Ayers and Jackson in their work on the skeleton of Bdellostoma interpreted the cartilages of the tooth plate to be in reality lower jaw cartilages, but this so far as I am able to gather from their paper is all they contribute towards proving the matter. As far as the musculature is concerned they merely note, "The huge club-shaped 'tongue' muscle is made up of the muscles belonging to this arch (meaning the mandibular). These muscles have been in Bdellostoma entirely separated from their attachment to the palato-quadrate, and have been translated to their present position in a manner which will be made clear in a subsequent paper," but no description of this process has since appeared.

It had, however, already been shown by Müller and Fürbringer that the muscles manipulating the tongue are innervated by the ramus mandibularis of the trigeminus nerve, *i. e.*, the true lower jaw nerve. Subsequently Allis, 03, in studies on the adult, and Kupffer, 00, (in his figures) on the embryo, have also shown that the dental plate and its muscles are innervated by the true lower jaw nerve. And during the past year Miss Worthington, working under the direction of Dr. Ayers, has traced the cranial nerves and practically repeated Allis' work. She does not, however, refer to this paper and was evidently unaware that her results had been anticipated in spite of the fact that the admirable paper of Allis' had appeared two years earlier.

I do not wish to appear unsympathetic to the work of Ayers and Jackson: indeed, in the following pages I shall join with them in defending the thesis that the so-called "tongue" or dental-plate in myxinoids is really homologous with the vertebrate lower jaw. I must, however, call attention to some of their errors (which I think have in part resulted from their inadequate knowledge of the literature) in my endeavor to place the results of the present paper in a clearer light. Thus they state, for example, that all anatomists "have apparently overlooked the

fact that were this a tongue *or a tongue-like organ* it would be a great anomoly in vertebrate anatomy; since none of the fishes, even the highest fishes, possess a tongue *or even a tongue-like organ.*" It is difficult to understand how the authors could have gained this impression, for one cannot doubt that fishes have tongues and in some cases very perfectly formed ones (cf. Parker and Haswell's Text-book of Zoology, Fig. 807, in which the tongue of the trout is shown and labeled). They would have been correct in stating that among fishes the tongue is not a conspicuous structure and that no fish has a protrusible tongue readily to be compared with the dental-plate of Bdellostoma.

Again they mention that "The homology of this organ (referring to the dental-plate) with the vertebrate tongue has never been discussed," while as a matter of fact Neal, **97**, three years before, studying the development of the hypoglossus muscles of Petromyzon, made the following statement: "I nevertheless consider it highly probable that the so-called tongue of Petromyzon is not the homologue of the tongue of higher vertebrates. This conclusion is based on the following grounds: 1. The anterior segment of the M. parietalis ventralis remains the same in its relation in Petromyzon as in Ammocoetes, *i. e.,* without relation to a tongue. 2. While the muscles of the tongue of higher vertebrates are derived from the anterior segment of the M. parietalis ventralis, which lies anterior of the hyoid arch, in Petromyzon the muscles of the "piston lingual" extend throughout the length of the branchial region and terminate posteriorly in the cartilaginous pericardium. They are quite separate from the M. parietalis ventralis which lies lateral and ventral to them. I have stated this evidence of the difference in the relations of the tongue of Petromyzon and Gnathostomata lest one not familiar with these relations should think that an organ of the same name in these vertebrates should therefore be an homologous organ. As a matter of fact no comparative anatomist has attempted to homologize these two kinds of tongues." This statement refers particularly to Petromyzon, but since Ayers and Jackson are discussing the Marsipobranchii it seems pertinent in this place. They say that the dental-plate of Bdellostoma is homologous with the two pairs of accessory lingual dentigerous cartilages of Petromyzon. It appears that they had failed to see Neal's work. On the other hand the work of Howes, **91**, is cited in their literature list and indeed quoted on the first page of their paper, yet they have again overlooked or ignored the following remark he makes on pages 134-5: "If, as can hardly be doubted, the 'tongue' of the Marsipobranchii is an organ peculiar to them and

unrepresented in the higher vertebrata, the question how far the above-named median ventral cartilage may be comparable to the basi-mandibulo-hyo-branchials of the latter, as Müller, Huxley, and Parker have together sought to show, must remain in abeyance, until more is known of the development of these fishes."

Howes considers the cartilage which Ayers and Jackson have called the cornual to be closely similar to that which Huxley called the Meckel's cartilage in the lamprey and says "with that I hold it to be homologous notwithstanding Parker's view to the contrary." On this point I must disagree with Howes and accept Ayers and Jackson's idea regarding this cartilage, and further with them I entirely agree that the lower jaw structures are to be found in the so-called "tongue" or dental-plate. This position, as I shall endeavor to show in the following pages is admirably supported by embryological study.

2. As far as Petromyzon is concerned *the development of the head* has been studied by Kupffer, 94 and 95, Dohrn, 83, and others, although it appears that none of these authors have given particular attention to the peculiar development of the "tongue." Kupffer, 99 and 00, also studied the head of Bdellostoma and discussed in detail the development of the mouth, finding as he claims a first or primitive mouth, which is transverse and leads directly back into the gut in a manner similar to the ordinary fish mouth. This primary mouth then becomes closed by a growth of the ectoderm, which Kupffer has called "sekundäre Rachenhaut" the closure persisting until about the time of hatching when the wall is again broken to produce the final or secondary mouth. Max Fürbringer has from this description and for the further reason that the hypophysis always opens into the throat, called the myxinoids Distoma, the petromyzontes Cyclostoma, and the higher vertebrates Gnathostoma.

The general embryology of Bdellostoma has been beautifully worked out by Dean, 99, but he enters only in a general way into a discussion of the head organs. He gives a very correct account of the gill development from a study of cleared total embryos, the finer details being, of course, impossible to determine in this way. Dean stated that the gills were shifted during development from their original position close after the hyoid arch to their final adult place far back along the sides of the body. He also made the remarkable observation, considering his study to be upon cleared totals, that there is a "doubtful" cleft which is early suppressed lying close behind the hyomandibular, therefore corresponding to the "thyroidean" cleft of Dohrn. Dean also, very cor-

rectly, I think, failed to find any correspondence between branchiomery and myomery.

Price, 96, was the first to describe embryos of the myxinoids. He studied three stages in the development of Bdellostoma and in the youngest embryos found a narrow canal extending from the anterior end of the mouth cavity into the cavity common to the nose and hypophysis, while in the older stages this canal was closed. M. Fürbringer saw in this observation very strong evidence in favor of Dohrn's idea that the primitive mouth of vertebrates is represented by the hypophysis.

Price, working with a limited supply of material made the mistake of supposing that a great number of gill pouches appear during development, possibly as many as thirty-five. Of these, he thought the posterior ten or fourteen develop into the gills of the adult while the others entirely disappear. Dean corrected this statement when he found a shifting and not a closing of the gills to take place; and Price himself, in a subsequent paper on the development of the excretory organs of Bdellostoma, based on a more complete series of embryos, has corrected his former statement and accepted Dean's interpretation on this point. Notwithstanding these corrections Price's original idea has since been utilized by Ayers and Jackson, 00, who try to account for the atrophy of the forward gills as due to the enormous development of the "club muscle" in that area. Johnston, 05, also quotes Price's earlier conclusion and states his belief that there is a reduction of the anterior gills on account of the parasitic life of this animal. It has been clearly shown, however, by Howes, 91, Ayers, 93, Jordan and Everman, 96, and Worthington, 05, that these animals are not parasitic but predaceous, attacking disabled fishes. Therefore Johnston's explanation will not suffice. Since, also, there are no forward gills lost we must accept the position that the gills belonged originally to the head region, and owe their extension into the trunk to the shifting forward of trunk myotomes into the occipital region, the view, by the way, which Johnston rejected in favor of his explanation cited above. All of these various disagreements concerning the development of the myxinoids have come within the last few years; Price's initial work on the subject appearing in 1896. It may be mentioned that only a few workers, about half a dozen in all, have been so fortunate as to obtain the material.

This review of the various and contradictory opinions might easily be extended, for no author has seemed to consider his paper complete without taking a new position regarding either the significance of this group of Marsipobranchii or the importance of the evidence furnished

by some organ that he may have studied. Enough, however, has been said to show the need of further embryological observations.

Method and Material.

The material upon which the present results are based was collected in the Bay of Monterey, California, mainly during the summers of 1896 and 1899 by Professor Bashford Dean. This constitutes the most complete series of Bdellostoma embryos that has been procured. The younger stages were fixed for the most part in Graf's picro-formalin, the later embryos being killed, after removal from the parchment-like egg-shell, in sublimate-acetic. In most cases these fixatives have given very satisfactory results.

The sections were prepared by Prof. Dean, Dr. L. Neumeyer, of Munich, and Dr. N. Yatsu, formerly of this laboratory. The embryos are very difficult to prepare, but with such an abundance of sectioned material I have been able to select a complete and satisfactory series, and I am confident that none of my observations are open to the criticism that they have been made upon defective material. The stains used were Heidenhain's iron hæmatoxylin, Delafield's hæmatoxylin, alum and borax carmine, all of which give good results.

The Development of the Mandibular Arch.

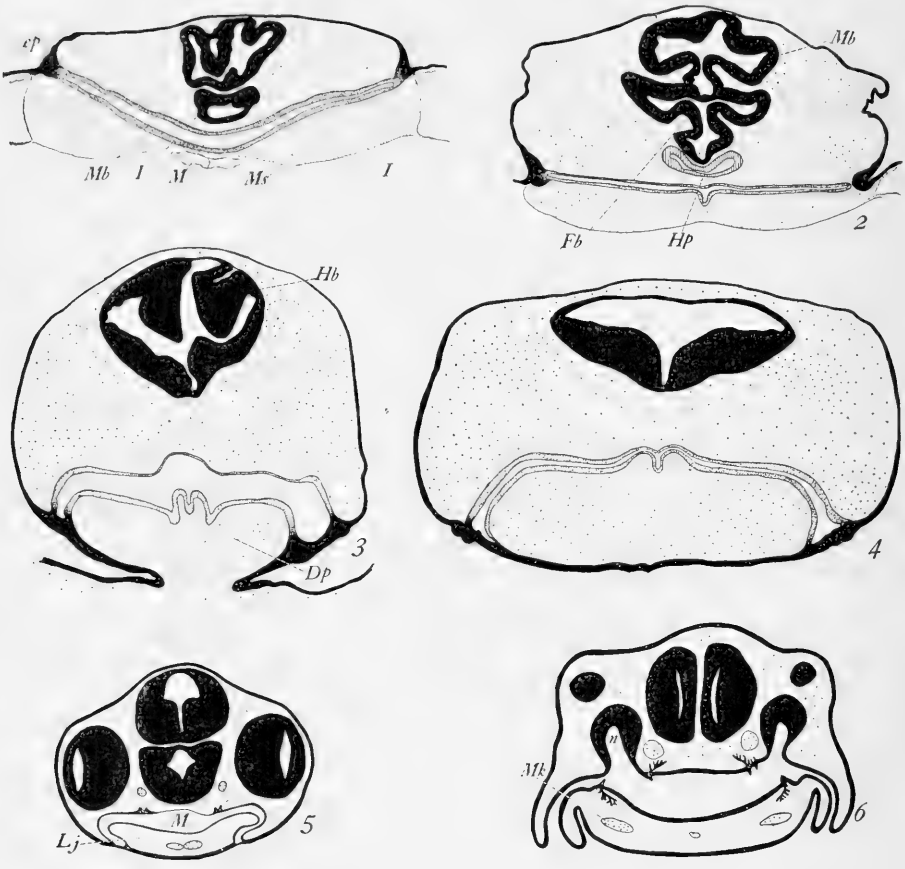
In the young of Bdellostoma one finds, to my mind, a most striking confirmation of Dohrn's view that the mouth of vertebrates is a modified gill arch. In these embryos the mandibular cleft passes through a series of changes during its development which are most suggestive in the light of Dohrn's thesis. Very young embryos, such, for example, in which the nose is still a single tube leading directly into the mouth cavity and in which six or seven gill slits are present on the laterally outspread plates (as illustrated in Dean's Fig. 37, pl. 18, and Fig. 101, pl. 22), will show the mandibular cleft in the following condition: Throughout the greater portion of its extent it is strongly arched with its lateral diverticula directed in an oblique dorsal direction and coming in intimate contact or actually fusing with the ectoderm. This is so strikingly analogous to the way in which a gill cleft meets the ectoderm that no observer could fail to be impressed with the similarity. Fig. 1, a cross section through the widest portion of the mandibular cleft of an embryo at this stage, shows the cleft fusing laterally with the ectoderm and having an arch or curve resembling that of all the following gills. The hyomandibular of the same embryo may be seen for comparison in

Fig. 15. The stippled area of these figures does not necessarily indicate endoderm but merely the epithelial lining of the mouth and pharyngeal cavities. In some figures this, of course, is actually ectoderm. The outer body wall and brain sections are indicated in black.

An embryo slightly older than the former, having almost lost its nasal communication with the throat and having one or two more gill clefts present, shows the mandibular cleft as follows: The arch of the younger embryo has almost entirely disappeared, the ventral mesenchymatous tissue, Ms, has thickened below the throat cavity and seems to have pushed the middle curve of the arch up until in section it appears as a horizontal cleft extending from side to side of the head. In this condition the intimate fusion of the diverticula with the ectoderm still persists. A slight median furrow now runs along the floor of the throat throughout the region of the mandibular cleft. Fig. 2, a section through the widest portion of the mandibular region, illustrates these changes. Somewhat further caudad this arch also has its lateral borders directed slightly dorsal, as a reminiscence merely of the condition of the younger stage. The hyomandibular and other gill clefts of this embryo differ but slightly from those of the younger one.

A somewhat more advanced embryo, measuring about 15 mm. in length, shows the mandibular cleft with its lateral portions curving down ventrally although still fusing with the ectoderm. Fig. 3 shows this condition in cross section; the embryo was slightly shrunken so that the mouth cavity is exaggerated in the figure, yet the increase in the ventral mesenchyme and all points are well shown. The hypophysis, which is seen in Fig. 2 has terminated before reaching this corresponding region in the older embryo. The anterior part of the head has become much longer, a great nose region now extending in front of the forward end of the gut. This section is also far posterior of both the eyes and of the infundibulum while a similar part of the mandibular cleft in Fig. 2 was only one section posterior of the eyes and the infundibulum had not been reached, the fore-brain being shown bent under the mid-brain.

Fig. 4 is a section through the mandibular cleft of an older embryo. In this stage all of the gills have appeared, but are still spread out on the lateral plates, and the nose exists at this time as two parallel tubes, a condition occurring also in the embryo of Fig. 3. The present section shows clearly the ventral inclination of the lateral diverticula of this cleft, as well as the ectoderm pockets, which appear almost ready to break through and form the lateral mandibular gill openings, a process, however, which does not take place: it is only at a later period that the definite



FIGS. 1 to 4 illustrating the development of the mandibular arch in Bdellostoma × 40 diameters, with Figs. 5 and 6 of this arch in Triton for comparison. Fig. 1. Section through the widest part of the mandibular arch in a very young embryo. *ep*, ectodermal mandibular "gill" plates; *I*, infundibulum; *M*, mandibular cleft; *Mb*, mid-brain; *Ms*, ventral mesenchyme. Fig. 2. Widest portion of the arch in slightly older embryo. *Fb*, fore-brain; *Hp*, hypophysis. Fig. 3. Section of older embryo showing ventral turn of the mandibular diverticula. *Hb*, hind-brain; *Dp*, dental-plate anlage. Fig. 4. Section of a still older embryo showing the wide mandibular cleft in actual contact with the ectodermal body wall. Fig. 5. Section through the mandibular arch of Triton, after Greil, compare with Figs. 3 and 4. *Lj*, lower jaw anlage; *M*, mandibular cleft. Fig. 6. An older Triton embryo after Greil, compare Fig. 11 after considering text. *Mk*, Meckel's cartilage; *n*, internal nares.

mouth opening is formed. Here, however, it is seen that the entire portion or mass of embryonic tissue below the mandibular cleft has increased considerably in bulk and that the trough-like furrow still extends along the mouth floor in this region. The furrow deepens during development and finally cuts or separates the ventral tissue into two forward halves, to be described in detail further on. All of this ventral mass is the anlage of the so-called "tongue," it is seen to be enormous and much too far forward for the anlage of such an organ. In all respects, however, it corresponds in position and extent to sections of the lower jaw portions of most embryos.

Two of Greil's, **05,** figures which appeared in his paper on the development of the mouth in Triton are shown in outline, Figs. 5 and 6, to illustrate the identity of the ventral tissue in my figures with the lower jaw tissues. From this identical ventral tissue, as will later be seen, the true lower jaw or so-called "tongue" develops in a remarkable way. Fig. 10 shows the mandibular arch greatly curved, but if the embryo at this age was flattened dorso-ventrally, as it is at the stage from which Fig. 4 was made, then Fig. 10 would give the curve of the mandibular arch similar to that seen in Fig. 4.

The Development of the "Tongue" Shows it to be the Homologue of the Vertebrate Lower Jaw.

It may be said that almost nothing is known of the "tongue" development in myxinoids. This is strange, since one would think that an organ so unique and peculiar as this one is would have been examined by the first workers that procured the embryos of these animals. I wonder particularly that Kupffer in studying the head development in Bdellostoma did not center his attention upon this organ instead of giving it only casual mention.

Göppert, **02,** made in substance the following statement in Hertwig's handbook of embryology, L. 6, p. 36: "The tongue of Petromyzon is first attained when the ammocoete metamorphoses, as the complete change of life is contemporary with the transformation of the structures. The cartilage of the tongue arises according to P. Bujor, **91,** in the massive connective tissue of the ventral wall of the larval mouth. The same author found the tongue muscles evidently first arising from the floor of the mouth in the neighborhood of the velum and thyroid gland. Bujor says that they have nothing to do with the tongue muscles of the higher gnathostomes; and they are controlled exclusively by the vagus according to Neal, **97,** who finds that the so-called *ramus recurrens*

vagi is homologous with the hypoglossus nerve of the higher vertebrates." As cited above in the general discussion Neal has shown that the anterior segment of the *M. parietalis ventralis* is without relation to the tongue in either Ammocoetes or Petromyzon, while the muscles of the tongue of higher vertebrates are derived from this segment. I am sorry not to be able to give at first hand any statement regarding the so-called "tongue" of Petromyzon, but from a study of the literature it seems that we are dealing here with an organ possibly as untongue-like as is the dental-plate of Bdellostoma. Göppert says regarding the myxinoids, that since they fail to have a larval form the tongue is found arising here much earlier than in the petromyzontes. At the termination of embryonic development the tongue has attained its final form and functions from then on as the boring apparatus. He states that Kupffer, 99 and 00, described and figured the massive anlage of the tongue in Bdellostoma as arising from the mesodermal tissue in a stage when the "secondäre Rachenhaut" still existed. Göppert concludes with the pertinent remark that the cyclostome tongue remains a peculiar structure in contrast to the more simple tongue of the gnathostome fishes.

Dean, 99, p. 269, refers but briefly to the tongue, stating merely, that below the opening of the hypophysis into the gut the tongue arises on the ventral wall of the throat as a paired outgrowth, and its rapid development greatly modifies the shape of the mouth cavity.

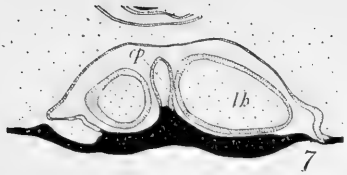
According to my more detailed studies the stages in the growth of the tongue are as follows: The earliest anlage of the dental-plate, as I shall term it from now on, is seen in Figs. 3 and 4 to be represented merely by the thickening of the mesodermal tissue in the ventral mouth region. This tissue composes the entire body of the embryonic head below the throat floor just as the lower jaw tissue does in embryos of Triton, Figs. 5 and 6, Cestracion, and other lower vertebrates. This mesodermal mass continues to increase in size and to become thicker in a dorsoventral direction, while at the same time its anterior end becomes free from the lower body wall and divides into two distinct prongs or forward extensions, Fig. 7 lh. It will be seen that these prongs a short way back from their anterior tips come together in a thin vertical piece, Fig. 7 ep, which now alone connects them with the ventral wall of the head. It may be remarked that Fig. 7 is taken from an embryo in which the first four branchial gills have been drawn in to the head region. In Fig. 8, which is a section through the same embryo taken further tailward, we see the two lateral horns, lh, joined to the median portion, ep, and on the right side of the figure, the section being slightly oblique, the lateral prong has also joined the ventral body wall. Here

again the mandibular cleft turns ventro-laterally; and, as before, the entire tissue below the cavity of the throat is the dental-plate anlage corresponding in position and extent to the lower jaw tissues of all vertebrate embryos. From these figures one might suggest that the median body may still represent a tongue, but it will at once be seen that the sections pass through the region of the hypophysis and are thus anterior ones. They are obviously too far in front of the hyoid cleft to represent the tongue anlage. I have looked with the greatest interest hoping to find some indication of a tongue in these embryos, as I would then be assured that the other apparatus was certainly not such an organ. My failure to find it, however, is not surprising in view of the fact that the tongue is inconspicuous in most fishes, and may even be entirely wanting in some. It may not be out of place to recall here that the fish's tongue is essentially a projection of the forward part of the hyobranchial apparatus with the mucus membrane of the mouth covering the thickened submucosa. Its development is, therefore, intimately connected with that of the skeleton.

Fig. 9 shows the condition of the dental-plate as illustrated by a section passing through the anterior part of the organ. In this embryo the gills are completely drawn into their position on the sides of the neck, *i. e.*, not spread out laterally as in the previous stages. They have shifted a considerable distance back of their original position, so that a long interval now exists between the hyomandibular cleft and the first branchial gill. In none of the embryos mentioned above had any shifting of the gills taken place. The gill pouches it may here be noted are just beginning to be formed, a process which will be described further on. This embryo is also interesting as being the youngest one in which the anlage of the thyroid gland makes its appearance, Stockard, 06. The anterior ends of the fore-gut and of the nasal canal are directed ventrally and they are now more caudad in position than when fully developed.

The two lateral prongs of the dental-plate anlage have grown further forward than the median copular portion and are seen to have become thicker dorso-ventrally, as has, also, the entire head of the embryo. For this reason the mandibular cleft has now a strong curve, but still comes in intimate contact with the ventral body wall, being prevented from opening to the outside only by the secondäre Rachenhaut of Kupffer. Two median tentacles are seen below in cross section showing their cores of mesoderm stippled. As we pass caudally in this same embryo to a place shortly anterior of where the Nasenrachengang, Hp, opens into the throat we find such a section as is seen in Fig. 10. This shows

FIGS. 7 to 14. Sections showing the development of the dental-plate or *lower jaw*. × 41 diameters. Fig. 7. Through the anterior part of the mouth showing the dental-plate anlage in a young embryo. *cp*, the thin median body; *lh*, two large anterior prongs, all of these parts are united further back. Fig. 8. A more posterior section of the same embryo, the median body, *cp*, is here united with the anterior prongs, *lh*. The anterior prong on the right side is seen united also with the ventral wall of the head. *M*, mouth cavity. Fig. 9. Section of the anterior portion of the dental-plate in older embryo, *lh*, the anterior prongs have grown ahead of the median body in their forward extention. *Hp*, hypophysis; *T*, tentacles. Fig. 10. Section through the same embryo in a more posterior region than Fig. 9. The anterior prongs, *lh*, are here joined to the median body, *cp*. The entire dental-plate anlage has its ventral wall in common with the ventral wall of the head just as has the lower jaw of the Triton embryo in Fig. 5. Fig. 11. Section through the anterior prongs, *lh*, of the dental-plate showing the teeth anlage on their median faces. *ht*, teeth; *Bv*, blood-vessels; *C*, mandibular cartilage; *T*, section of tentacle; *T'*, section through base of more posterior tentacle. Fig. 12. A more posterior section than Fig. 11, the two forward prongs of the dental-plate are joined together medio-ventrally, and laterally to the head. Their ventral wall is the ventral wall of the head as would be expected of the lower jaw. *Tc*, throat cavity; *x*, a place where the forward prong is still free from the head not far enough posterior to give the complete union. Fig. 13. Through the longitudinal mouth of an old embryo. *lf*, Fleshy lip folds the anterior lateral extentions of the dental-plate body; *Bs*, blood sinus. Fig. 14. A more posterior section through the same embryo. Shows dental-plate with teeth, *ht*, developing on its dorsal surface; *C*, basal cartilage of dental-plate; *Tc*, throat.



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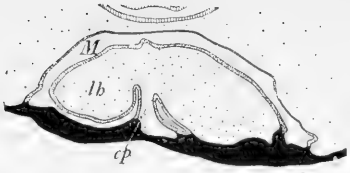

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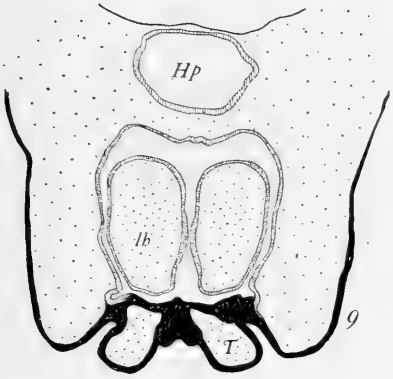

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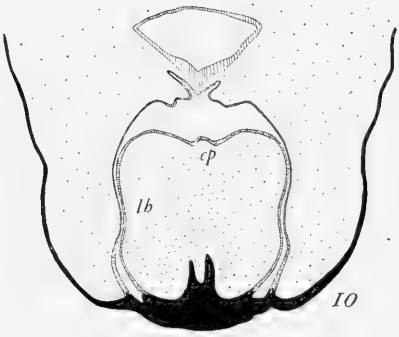

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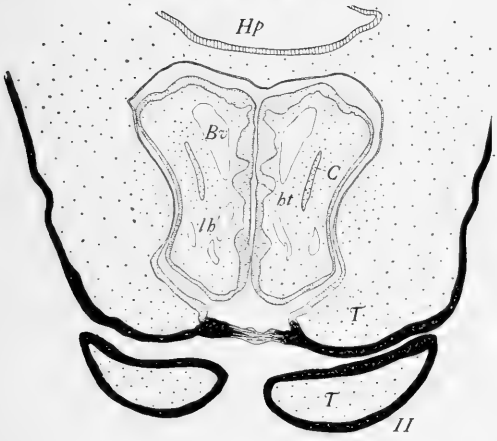

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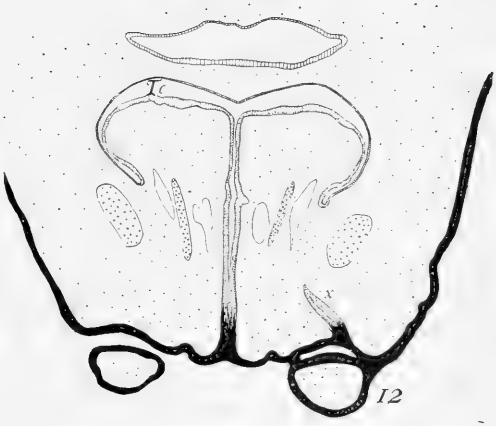

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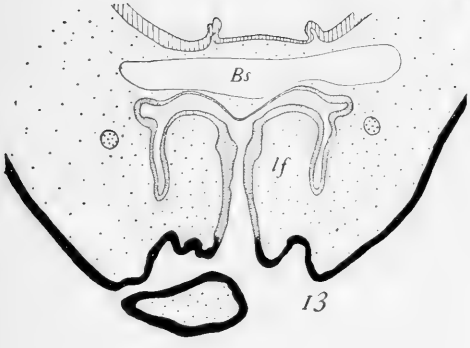

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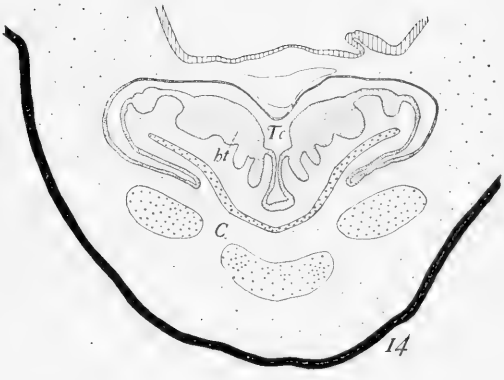

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that the two forward lateral prongs have, as we traced them, posteriorly fused with the middle copular portion, which is shown in the figure as the median ridge on the throat floor and as a ventral triangular protrusion of the median mesoderm. The lateral prongs have also become joined to the ventral wall of the head. Four sections back of this one they join the head laterally also, and thus the vertical side spaces seen in the figure are lost, leaving only the upper space as the throat cavity. It is plainly seen that the entire mass of tissue ventral of the throat cavity and included in the forward mandibular arch is the tissue that should form a lower jaw in an embryo, and in no particular the tissue to form simply the tongue of an animal. Its extreme anterior origin is in itself strongly against such a view.

Development continues without any marked points of interest until we examine an embryo of about 28 mm. in length. This may be described as follows: The mouth opening is almost formed, as the secondäre Rachenhaut is beginning to disintegrate, the gills have shifted backward, being more than twice as far posterior of the auditory vesicles than these are from the anterior point of the head. The gills are well pouched and the cartilage arches or cuffs are forming about their external tubes. A large and well-formed club muscle extends from the velar region to the first gill, this organ only appears after the gills have shifted some distance back of the hyoid cleft. The circular muscle of the club first arises as two muscle bodies some distance apart and dorso-lateral to the longitudinal one, and as it develops the circular muscle migrates ventrally and surrounds the long one.

This embryo has its dental-plate extending forward as two large prongs which may be traced a long way back before the median copular portion is reached. The prongs are thus seen to grow ahead of this piece in their forward development, for we recall how close to their anterior ends the copular portion was found in the embryo of Figs. 7 and 8. The early anlage of the teeth now appear on the median faces of these two prongs. The teeth are here entirely derived from the epiblastic wall. Cartilages are developing one to each prong of the dental-plate and its substance is very vascular, enclosing large blood sinuses. Fig. 11 is a section through the anterior region of the dental-plate and shows two tentacles, T, cross sectioned below, while two others, T′, are cut through their base of attachment. The Rachenhaut is disintegrating along its middle portion. The cartilages of the two prongs are shown coarsely stippled and the numerous blood vessels, Bv, are indicated in outline, two enormous sinuses being located one along each dorso-median border of the prongs. The teeth anlage are represented by

the thickened portions of the epiblast. Tracing the organs caudally through the series we find the two dental-plate bodies becoming joined to the head laterally and joining one another medio-ventrally until we reach a section, Fig. 12, where the lower surface of these dental-plate bodies forms the ventral wall of the head. They are thus in all respects similar to a lower jaw in position and composition, and have as far as I am able to interpret nothing about them suggestive of a tongue. The median furrow left by the incomplete dorsal union of the two prongs runs for many sections through the throat region, then is lost as the thin middle body referred to above comes between the two halves of the throat floor and forms a slight ridge along the median line. The hypophysis, seen in Figs. 11 and 12, runs back for quite a distance before opening into the gut.

As development progresses the two anterior parts of the dental-plate become attached laterally to the head further and further forward until they cease to project as free portions. Studying an embryo 13 mm. long, in which the brain has flattened and become almost solid as in the adult, and in which the nasal opening is now anterior and terminal, instead of ventral as in younger stages, we find that the mouth is a longitudinal opening much as it is in the mature condition. This opening or longitudinal mouth is easily comparable with the ordinary transverse mouth of other vertebrates when we remember that in the myxinoids the forward portions of the lower jaw, which are hardly more than lip folds, have remained separate in the old embryos and that the tooth bearing portion has been carried to the back of the mouth. Now if we should unite medianly the forward projections of the dental-plate, the mouth could only open as an anterior-ventral transverse slit, or a slit in front of the lower jaw border as it does in other vertebrates. Referring to Dean's Fig. 113 of a total embryo, the anterior prongs of the dental-plate are indicated by shaded portions, and the lighter median streak shows their forward separation. The figure makes clear the close similarity to a transverse mouth. Dean writes, p. 263, that, "The mouth cavity suggests closely that of a gnathostome; in ventral aspect it appears as a narrow crescent whose convexity is directed forward."

The longitudinal condition of the mouth will be made clearer by examining sections of the 13 mm. embryo. Fig. 13 is through the mouth opening and the two forward lip-like folds of the dental-plate, lf, are fused laterally with the head. One of the tentacles, T, is sectioned below. As the two lip-like bodies are followed back through the series they are found to be continuous with the dental-plate of which

they are in fact the forward fleshy extensions. Fig. 14 is a section further back in which the two forward parts have fused medianly to form the dental-plate, and on this bilateral body the teeth are developing. These now appear upon its dorsal surface instead of on the two median surfaces as in Fig. 11, for the median surfaces which faced one another when the teeth began to form, now spread apart and become the final upper or dorsal surface of the dental-plate. The thin cartilage of the plate is seen below the dental surface.

From the foregoing account a critic might object that, if we interpret the tongue as the lower jaw, we have a jaw of a most unorthodox type, for it develops in such a way that one portion of it may be moved out between its two other parts; a condition which seems at first sight mechanically impossible. But it must be remembered that the forward attached parts are only the fleshy folds of the jaw, mere lip-like structures, while the dental-plate itself has a sheet-like membrane spreading out from its four sides and loosely folded about its edges. This membrane is continuous with the lining membrane of the mouth cavity. By means of the highly modified muscle system the tooth-plate may be lifted up and carried forward, owing to its loose-folded membranous connection. The movements, moreover, in the living animal have recently been explained by Worthington, 05.

It follows finally from the discussion of the development of the mandibular arch and dental-plate in Bdellostoma that the organ long called the tongue in myxinoids is in no sense comparable or homologous with the vertebrate tongue. In this case, therefore, it should no longer bear so misleading a name, since the word tongue must necessarily imply or suggest a tongue-like structure. The term dental-plate which has been applied by several writers to this organ seems a very appropriate one. It indicates the nature of the organ and in no way interferes with the understanding that it is actually homologous with, though superficially widely different from, the lower jaw of all other gnathostomes. The Marsipobranchii have been so repeatedly placed among the Gnathostomata that the group will scarcely seem new in its correct position.

The Development and Fate of the Hyomandibular Gill Cleft.

This gill cleft in the youngest stages is strikingly similar to the mandibular, both having extensively developed diverticula bending upward in a manner typical for all of the gills. Dean has stated, p. 270, that the mouth is distinctly paired in character, but unlike the hyomandibular pouches, its diverticula are shown in section to bend downward,

instead of upward, and to fuse at their margins with the ectoderm, thus suggesting the gill slit mode of origin of the mouth so elaborately defended by Dohrn. This statement is correct except for the very young stages in which, as I have shown, the mouth diverticula also bend upward, Fig. 1, and suggest, therefore, much more forcibly the gill slit origin of this organ.

The hyomandibular pouch follows close after the mandibular, which in all stages is the most anterior, and there are no premandibular diverticula of the gut, which Dean indicated from his study of surface preparations.

The very young embryo described as having the nose tube leading directly into the gut, and shown in Dean's Figs. 37 and 101, presents the hyomandibular pouch in the following condition. It is situated well in front of the auditory vesicles and is enormous in extent, as is shown in Fig. 15, a section of the same embryo from which Fig. 1, of the mandibular arch, was taken. In this section it will be seen that well-marked ectodermal pockets are present and a corresponding thickening of the endodermal gut wall is in close contact with them. It thus appears as if this gill is about to establish an opening to the exterior, but such is not the case since from this stage onward there sets in a steady retrogressive development.

Fig. 16 shows a section through an embryo in which the mandibular arch has flattened out or become horizontal, no longer curving upward, the condition shown in Fig. 2. Fig. 16 is through that part of the hyomandibular pouch where it comes in closest approximation to the corresponding ectoderm pocket. The gut pouch, therefore, is decreasing in its lateral extent, while in cross section the lateral diverticula become retort-shaped, large bulb-like chambers forming their extremities. The endodermal thickenings in the present stage are not so marked as in Fig. 15. As the embryo continues to develop the ectodermal plates or thickenings become gradually less pronounced until in embryos of about 15 mm. in length there appears no ectodermal evidence of the hyomandibular, Fig. 17. The bulb-shaped extremities of the gut diverticula in Fig. 17 are more definitely marked off than in the younger stage of Fig. 16, here, also, the hyomandibular cleft is behind the auditory vesicles instead of being anterior to them as in the younger embryos. In the matter of position the relation of the hyomandibular to the auditory vesicles is slightly variable in embryos of the same developmental stage, but as development progresses the ear-vesicles always attain a more anterior position. This fact is probably due to the forward shortening or

condensation of the brain, though I shall be better prepared to discuss this subject after a more careful study of the brain development.

The hyomandibular cavity continues to degenerate and there is only a slight indication of it in embryos of later stages, *i. e.,* those in which the branchial pouches and head cartilages are forming. In Fig. 18 through the posterior auditory region of such an embryo the hyomandibular is indicated by the slight lateral diverticulum marked Hm just at the beginning of the throat where the gut wall arches dorsally to map out the velum. Older stages lose even this indication and the gut arches up around the anterior end of the velum without any suggestion of the hyomandibular diverticula.

Thus in Bdellostoma the hyomandibular cleft early attains an enormous development, then gradually decreases in size, and disappears before the embryo has reached a very advanced stage.

The Presence and Fate of Two Pairs of Post-Hyomandibular Gill Clefts.

Dean, **99,** writes (op. cit., page 270) that the question of the disappearance of a pair of gill clefts lying post-hyomandibular has been strikingly revived by his studies. In a foot-note on the same page he also mentions this "doubtful cleft" which lies close behind the hyomandibular as corresponding to the thyroidian cleft of Dohrn. Studying sections of these embryos I have been able to confirm the observations made on cleared totals by Dean and I have further demonstrated a second cleft following close behind the first, which likewise disappears during development.

The existence of these additional clefts in the embryo is of evident interest with regard to the maximum number of gill slits in chordates. Since the adult Bdellostoma often possesses as many as thirteen pairs of gills we may now include from embryonic data a total number of fifteen pairs exclusive of the hyomandibular and mandibular clefts. There seems to be no valid argument against this greater number representing a primitive feature. The tendency in Bdellostoma is to lose its large number of gills as is shown first by the suppression of these two embryonic pairs and further by the observations of Miss Worthington, **05,** on the adult. She has found (p. 633) the number of gills in different species ranging from seven to thirteen pairs, and in the case of the Californian species no number seems to be either normal or fixed. Of more immediate interest is her observation that traces of lost gills are often to be found not at the hinder end of the series of gills, where one would at first suppose, but at the cephalic end.

The two pairs of post-hyomandibular or thyroidean gills, (neither name, unfortunately, designates them accurately) always remain in their original position, not being affected by the shifting process which carries the true branchial gills back into the trunk region. In this connection it is important to note that these two gills are always drawn well into the head region and never occur out on the lateral gill plates where all of the following ones first make their appearance. The thyroidean gills disappear soon after the shifting starts.

The young embryos in which the diverticula of the mandibular arch bend upward show these gills in the following condition. In Fig. 19, which passes through the first of these two gills, the ectoderm forms a depressed pocket and is much thickened where the gut diverticulum comes close to it, the endoderm also thickening. This is in the anterior auditory region. The second thyroidean gill is much the same and is found in the posterior auditory region, one thus following close after the other. The next gill, which is destined to form the first true branchial, follows at an equal distance behind the second and may be seen for comparison in Fig. 23. This is not so well-developed as the thyroidean gills, being further caudad in the series and the developmental stages are gradually less advanced as we pass tailward.

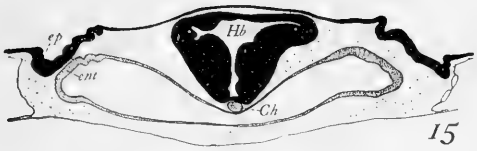
In embryos which have the mandibular arch flattened horizontally and which show seven or eight gill clefts, the first thyroidean gill as shown in Fig. 20 has a well-marked ectodermal pocket and the corresponding gut diverticula is greater in extent than that of any following gill. Fig. 24 shows the first branchial gill in the same embryo. It is situated just at the base of the laterally outspread gill plate. The first thyroidean gill is again in the anterior auditory region and the second stops just as the auditory vesicle fades out posteriorly. In Fig. 20 the slight nick, 2t, just ventral of the first thyroidean is the most anterior tip of the gut diverticulum for the second thyroidean; all of the gills having an obliquely caudal direction from pharynx to body wall.

When the embryos are 15 mm. long (a stage in which the mandibular arch is as seen in Fig. 3 and the hyomandibular in Fig. 17) the first thyroidean gill is in the condition shown in Fig. 21. It is still in the anterior auditory region, but one will note that the ectodermal thickenings and pockets have now disappeared as have the hyomandibular ectodermal thickenings at this stage, Fig. 17. The gut diverticulum will be noticed now to extend little more than one-half of the distance from the gut to the ectodermal wall. The second thyroidean gill presents much the same appearance, the two are therefore undoubtedly degenerating

Figs. 15 to 18 illustrate the degeneration of the hyomandibular cleft × 44 diameters. Fig. 15. Through the widest portion of the cleft in a very young embryo. On the left side the endodermal and ectodermal thickenings are almost in contact. *ep*, ectodermal hyomandibular gill pocket; *ent*, endodermal thickening; *Ch*, chorda; *Hb*, hind-brain. Fig. 16. Section of an older embryo showing increased space between ectodermal and endodermal parts of the gill. Fig. 17. Section of still older one. The ectodermal thickenings have disappeared and the endodermal diverticulum, *Hm*, is less extensive; *v*, anlage of velum. Fig. 18. Section of old embryo, the slight diverticulum, *Hm*, is all that remains of the hyomandibular cleft. *A*, ear; *Ca*, cartilage of the auditory box.

Figs. 19 to 22. Sections showing the degeneration of the first post-hyomandibular gill. Lettered as above. Fig. 19. Section of a very young embryo. Fig. 20. Older embryo showing the gill further developed. *2t*, the anterior beginning of the second post-hyomandibular gill. Fig. 21. Section of older embryo showing degeneration of the ectodermal thickening, and the endodermal diverticulum is less extensive at this age. *p*, pharynx. Fig. 22. Embryo in which this gill is almost lost, there being no ectodermal indication of it, and the throat diverticulum is very small.

Figs. 23 to 25 show sections of the first branchial gill in early stages of its development. Fig. 23. From the same young embryo as Fig. 19. Fig. 24. From the same embryo as Fig. 20; *np*, branchial nerve placode. Fig. 25. Older embryo with the endodermal diverticulum, *ent*, much increased in extent; *g2*, *g3*, anterior edges of the diverticula of the 2d and 3d branchial gills. Fig. 26. Guide to indicate the embryonic areas from which the above sections were taken. *Hm*, region of the hyomandibular ones. *T*, the first post-hyomandibular ones. *Br*, the first branchial gill region.



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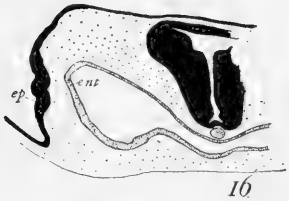

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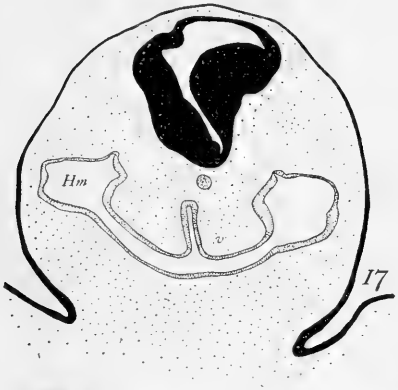

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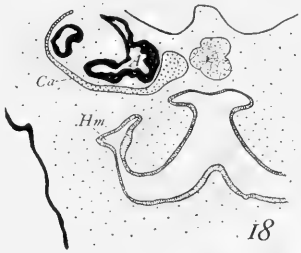

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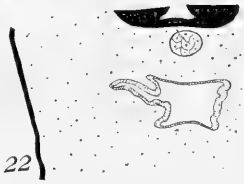
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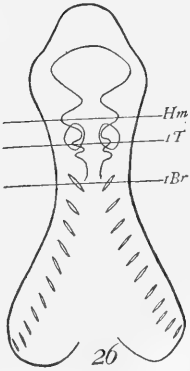

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while the first branchial, seen in Fig. 25, has progressed considerably beyond the condition shown in Fig. 24.

Fig. 22 shows in an older embryo a stage of still further degeneration of the first thyroidean gill. Here again there is no ectodermal indication of the gill, and the gut diverticulum extends somewhat more than a third of the distance from the pharynx to the body wall. Finally in older embryos these gut diverticula become less and less important until they are lost entirely. Thus they cannot be found in a stage when the gills are beginning to form the pouches and have shifted back into the trunk region.

Including the mandibular I have thus far described the developmental changes taking place in the four anterior pairs of gill clefts. The development of the mouth arch is progressive and highly modified; beginning as an ordinary gill cleft extending dorsally, it changes the position of its diverticula into a more and more ventral position, and finally undergoes complicated changes in connection with the development of the dental-plate.

The three following gills, hyomandibular and the two thyroidean, undergo a distinct retrogressive development: in the young embryo they are the most advanced elements of the entire gill series; they then gradually degenerate and in late stages they become entirely lost. On the other hand, the first branchial cleft as we have seen develops progressively, and becomes the first true marsipobranch of the adult. All of the following gills progress in a similar manner and reach the mature condition in a way to be described below.

The Development and Shifting of the True Branchial Gills—Pouches, Arches, Etc.

The development and changes in the relative position of the gills in Bdellostoma are among the most interesting phases in the embryology of a Myxinoid. Dean in his study of cleared embryos described the general processes of the gill development with remarkable correctness, so that one studying the finer details in serial sections is surprised at the manner in which the general points of development were interpreted from the total embryos.

On page 261 Dean says, "In the hinder (head) region we have thus a view of the mode of closure of the fore-gut: and we note the drawing together of the sides of the gill-lappets and the mode of backward extention of the ventral rim of the definitive pharynx, in a way which suggests curiously an analogy with reptiles and birds." This description is just

such as one would give after a study of sections and when the large yolk-bearing egg of Bdellostoma is recalled, resembling so closely the eggs of reptiles and birds, we would expect the mechanics of development to operate in a comparable fashion, *e. g.*, the closing together of the gut walls. We find on page 264 in a discussion of late embryos, that a great increase in the length of the neck region has taken place, the head now advancing around the anterior end of the egg. "With this continued growth the further change in the position of the gill pouches is naturally connected. Contrasted with the preceding stage the interval between the row of gill pouches and the eye has become nearly doubled and in this space the tongue muscle has taken its position. The more rapid growth of the dorsal region of the head and neck, with the accompanying growth of the tongue muscle is, I believe, sufficient to account for the apparent translocation of the line of gills." We shall later see how very close to such an explanation of the gill shifting we are forced after a careful consideration of the evidence gathered from a study of sections. By the "tongue muscle" Dean refers to the large "club muscle" arrangement of Ayers which lies between the end of the velum and the first branchial gill and serves to operate the dental-plate during its vigorous feeding motions.

I fully agree with Dean that, "Both Price and von Kupffer have assumed a correspondence between branchiomery and myomery of which in this form at least I can find no evidence." Since Price's, **96**, idea, which he has corrected, **04**, that a large number of gills disappear during development was erroneous the considerations of Ayers and Jackson, **00**, and Johnston, **05**, over this point must be disregarded or modified so as to apply to the true conditions.

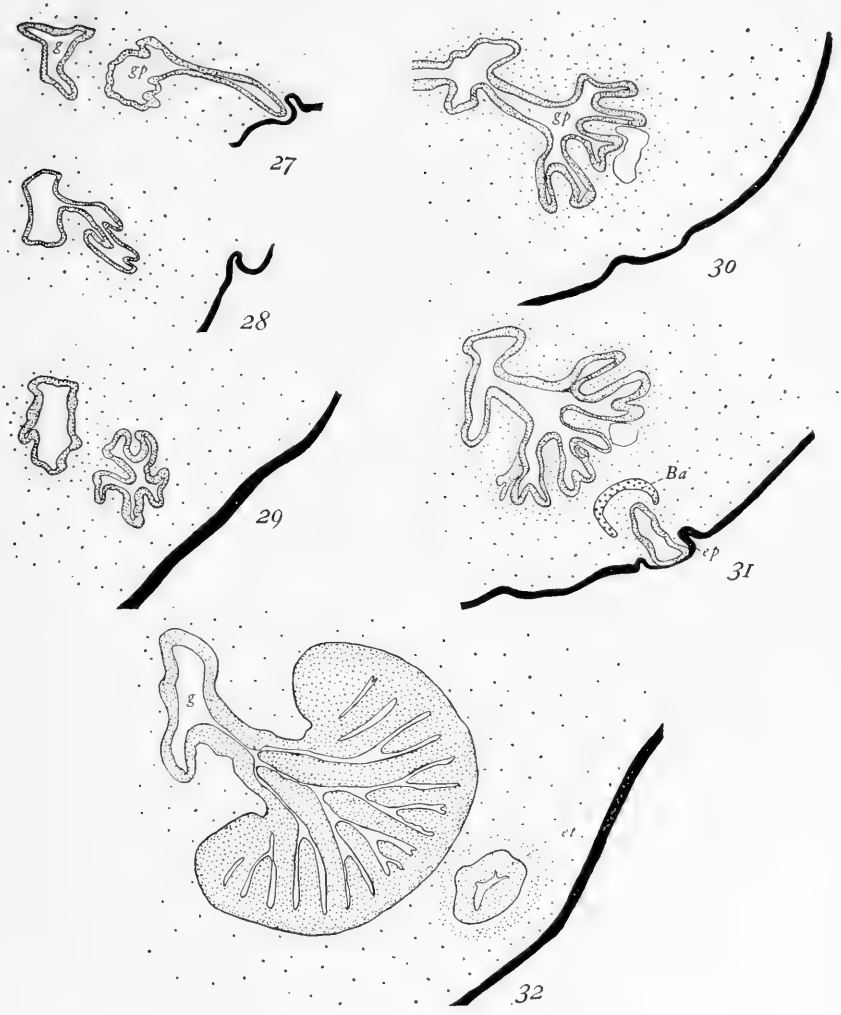
Development of the individual gill.—Nothing prior to this has been contributed to the development of the Myxinoid gill, the formation of its complex pouch or the cartilaginous portions accompanying it.

The entire series of gills develop alike with the exception of the simple œsophago-cutaneous duct which fails to form the pouch and has its external tube leading out in common with the gill next to it. The most anterior gill may be taken as an example since it is better developed than the following ones throughout almost the entire embryonic life. The earliest anlage of this gill consists of a slight thickening of the ectoderm and a corresponding thickening and uppushing of the endoderm, the gut endoderm at this stage being spread out over the yolk, as seen in Fig. 23. As development progresses, Fig. 24, the ectodermal pocket becomes a fold just at the base of the lateral gill lappets and the

endodermal outpushing has become more pronounced, reaching to the ectoderm. At this stage an ectodermal nerve placode appears just dorsal to the gill. Following this, the remaining seven or eight gills are still spread out on marginal lappets. The present figure was taken from the same embryo which furnished Fig. 16 of the hyomandibular cleft and Fig. 20 of the first thyroidean gill.

Fig. 25, from an embryo 15 mm. in length, shows the first gill still at the base of the lateral gill lappets with the endodermal diverticula much greater in extent and the ectodermal thickening but little changed. The extreme anterior parts of the second and third gills are indicated by the two indentations in the endoderm g2 g3. These gills are now beginning to be drawn in from the lateral lappets as the closure of the fore-gut progresses. And at this stage the hyomandibular and first thyroidean gills have already been shown in Figs. 17 and 21 respectively. The fore-gut continues to close by the drawing together of the sides of the gill lappets, and thus the gills are brought in along the sides of the neck in the manner which Dean has already described. The shifting does not begin until this process is almost completed.

Pouching of the Gills.—Before the gills begin to shift backward the endodermal diverticula are coming to form chamber-like cavities a short distance from their proximal ends. Fig. 27 shows a section through a gill at this stage the swollen cavity, gp, is seen near the pharynx, g, the connection of the gill tube with the pharynx is anterior to this section, the gills all having a trend from the gut caudally to the ectoderm as will be seen by referring to the diagram Fig. 35. This swelling of the gill tube is the earliest step in the formation of the complex gill pouch of the adult. The progressive changes in the endodermal gill chamber may be seen by studying the sections shown in Figs. 27 to 32. In Fig. 28 the wall of the chamber is beginning to be folded and in Fig. 29 the pouch is already assuming a rather complexly folded condition. In Fig. 30 the pouch is seen to communicate with the pharynx, the outer tube, *i. e.*, leading from the pouch to the ectoderm, appearing only in a more posterior section. In this section the ectoderm is seen to form a slight fold, ep, margining the end of the endodermal tube. At this time the pouch begins to grow rapidly, as will be noticed by comparing the several figures all drawn to the same scale. The gills at the stage shown in Fig. 30 have shifted far back, almost as far as in the fully developed condition. In Fig. 31 is seen a section of a somewhat further developed pouch from the mid gill region of the same embryo of which Fig. 30 represents the most anterior pouch. The pouch of Fig. 31



FIGS. 27 to 31. The development of the pouch of the first branchial gill × 60 diameters. Fig. 27. *gp*, portion of the gill tube with its walls bulged apart to form a chamber the earliest anlage of the pouch. *g*, pharynx. The gill connection with the pharynx is anterior of this section. Fig. 28. In an older embryo, the walls of the chamber beginning to fold. Fig. 29. A still older stage the pouch walls further folded. Fig. 30. The pouch increased in size and becoming more complex. Fig. 31. The cartilage arch, *Ba*, is forming about the external gill tube; *ep*, the ectodermal pocket. The pouch is larger and more complex than formerly. Fig. 32. Through a gill pouch in the mid-branchial region of a lately hatched embryo. *g*, pharynx; *et*, section of the external gill tube.

is larger and more complex, and the section shows, fortunately, a portion of the tube leading to the ectoderm. The ectoderm itself now forms a slight pocket and is reduced in thickness at this point. Around the external gill tube the cartilage arch, ba, is forming and it is seen to be entirely an extra-branchial arch in all cases except the œsophago-cutaneous duct. And here the cartilage extends entirely to the gut, oftentimes spreading out slightly along its walls, as Ayers and Jackson, oo, have also pointed out. As seen from Figs. 30 and 31 the gills in the mid region at this stage are becoming better developed than the anterior ones. Indeed in the hatched embryo the extreme anterior and posterior pouches are rarely so well formed as those nearer the middle of the line.

Finally, Fig. 32 shows a section through a gill of a lately hatched embryo. The gills now, of course, open to the exterior and are perfectly pouched. In the figure *et* is a cross section through the external gill tube which now leads from the pouch to the skin in an almost posterior direction. Thus the characteristic marsipobranch is entirely derived from the endodermal portion of the gill and reaches its adult complexity by a spreading out and subsequent folding of the walls of the originally simple gill tube. The ectodermal portion of the final gill structure is insignificant, being nothing more than a short rim about the external gill opening.

The Cartilage Arches, in their embryonic condition are, as far as I could judge from a comparison with Ayers and Jackson's description, very similar to these structures in the adult. At some stages they appear to be slightly more extensive. These differences, however, are insufficient to base conclusions upon since all parts of these animals are subject to such wide variations. The branchial skeletons of the Marsipobranchii have long been contrasted with the typical piscian condition as extra-branchial instead of intra-branchial. This distinction in Bdellostoma at any rate, as Ayers and Jackson maintain, is not conclusive for the reason that the cartilage of the œsophago-cutaneous duct, which is a gill tube, extends entirely into the pharyngeal wall. I think that a very plausible explanation may be offered for the condition in the other cartilage arches which will show them to be secondarily extra-branchial. It will be recalled from the preceding description that all of the gills of Bdellostoma are at one time in their development simple tubular structures just as the œsophago-cutaneous duct always is. Now from analogy I assume that if these gills should remain tubular until the stage in development when the cartilage forms they also would have sheaths of cartilage supporting them from their outer ends along their entire length to the pharyngeal wall just

as in the case of the œsophago-cutaneous duct. If such did occur the pouches could scarcely form. But since the pouch is well formed before the cartilage appears, and as the cartilage formations are not so extensive as to extend around these huge pouches so as to become intra-branchial structures they must remain about the external gill tube as extra-branchial. It may also be remarked, not that I think it a very strong point in the argument, that cartilage supports are entirely unessential to the efficient action of these pouched gills, while such supports are very useful in maintaining a fully spread condition of the typical fish gill. Further, when we remember that only the Marsipobranchii have pouched gills and likewise extra-branchial skeletons, while all fishes have intra-branchial arches and unpouched gills, one can scarcely doubt that some correlation exists between the processes that have given the pouches and the causes that have made the skeleton extra-branchial. Of course the various stages of the struggle, if we may so call it, between the pouches and the intra-branchial arches may well have been more complex than the present explanation supposes.

I am inclined to consider the condition of the œsophago-cutaneous duct as one of arrested development, *i. e.*, it remains in the tubular stage through which all of the other gills pass before forming their pouches. This might be called a modification but it is one which has not greatly affected the primitive condition of this structure, and argues only to a slight degree, if at all, against the above position. Further if it be claimed that the extensive cartilage support of this duct was a new or special acquisition, one might then reply that this indicates the fact that in Bdellostoma we have an animal on the road to possessing an intra-branchial skeleton, which is even now partially formed. The extra-branchial condition from this point of view would be, therefore, primary and not secondary. But since these animals possess a mandibular arch they must have also possessed intra-branchial gill arches because the one is to be considered only as a modification of the other. At any rate according to our present knowledge there is clearly no good reason for accepting Dohrn's view that the arches were lost on account of a change in the animal's life-habits.

From such considerations as the above one must at least admit that these animals are contrasted with fishes as having extra-branchial skeletons on rather flimsy grounds. To any one that will study these structures it will, I believe, become clear that marsipobranchs have in part, and probably once had entirely, an intra-branchial skeleton.

The Shifting of the Gills in the embryo of Bdellostoma was correctly described by Dean, 99, as mentioned at the beginning of the discussion

of the branchial organs. My study of the sections has enabled me to add a few further details.

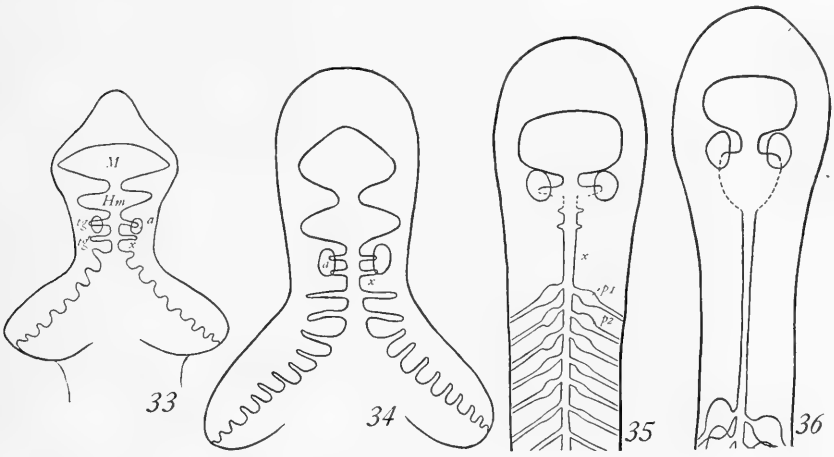
In young stages where the gills are on the laterally outspread gill lappets as illustrated in the diagrams Figs. 33 and 34 no shifting at all has taken place. Each gill follows the preceding one at approximately equal intervals. In diagram 33 M indicates the horizontal outline of the mandibular arch, Hm the same for the hyomandibular cleft, and just following this are the two pair of thyroidean gills. The last, it will be seen, are well within the head region, and are indeed at no time on the lateral gill lappets. After the thyroidean gills, tg, (which are now more advanced in development than any of the true branchial ones) the other gill folds come in regular succession. The auditory vesicles are just over the first thyroidean gills.

In more advanced embryos the gills are being folded in along the sides of the body as the gill lappets are drawn together, Fig. 34 shows the first few already brought in. The ear vesicles have increased in size and now lie over the two thyroidean gills. When the gills are all just about folded in along the neck, a region of rapid growth is formed between the second thyroidean and the first branchial, or in other words just anterior of what was the forward limit of the gill lappets, or at the point marked x in Figs. 33, 34, and 35. This rapid growth of the dorsal region of the head and neck causes the head to advance around the anterior end of the egg, leaving the gills as it were behind. Later the large club muscle of the dental-plate begins to develop in the ventral neck region between the thyroidean and the first branchial gills, thus further facilitating the lengthening of this area and helping to cause the translocation of the gills from their original place in the neck to their final position along the sides of the trunk.

The myotomes in young stages begin behind the gills, the most anterior one being posterior of the last gill, but during development many myotomes come to lie anterior of the first gill. I find no definite relation between myotome and gill arrangement at any stage. Thus one must conclude from a study of Bdellostoma embryos that there is no essential correspondence between myomery and branchiomery.

The exact growth point in the shifting process can be located since the second thyroidean gill undergoes its entire degeneration without changing its relative position, while the next gill which followed it so closely comes finally to lie a long way posterior to its place of origin. Since also the second thyroidean is the most posterior cleft which does not arise on the lateral gill lappet and the first branchial is the first one of the lappet series the idea at once suggests itself that the lappet area may be as it

were loosely associated with the dorsal muscular tissue, consequently in the rapid growth of this dorsal tissue the lappet structure with the gills is left behind. The complex changes connected with the enormous growth of the dorsal neck tissue, the development of the large club-muscle, and the connection of the gill lappet tissue with the dorsal musculature are the principal causes operating to produce the translocation of the gills in the embryo.



Figs. 33 to 36. Diagrams illustrating the change in position relations during the development of the gills. Fig. 33. The condition in young embryos when all of the branchial gills are out on the gill lappets. *a*, ear vesicle; *M*, outline of the mandibular diverticulum; *Hm*, the hyomandibular; *tg*, *tg'*, first and second post-hyomandibular gills; *x*, point where rapid growth takes place causing the head to grow forward leaving the gill area behind. Fig. 34. The first few gills are drawn in from the lateral lappets. *x*, the rapid growth point; *a*, ear vesicle increased in size. Fig. 35. An embryo in which the gills have partially shifted. The two post-hyomandibular ones are in their original position and have decreased in extent. *p*1, *p*2, the beginning of the pouches in the branchial gills. Fig. 36. The gills shifted far back. The ear vesicles are partially anterior to the hyomandibular. The indefinite posterior border of the hyomandibular cleft is dotted.

Fig. 35 shows the gills partially shifted back: the pouches, p, are forming near the communications of the gill tubes with the pharynx, the thyroidean gills have greatly degenerated and the auditory vesicles are seen to have moved forward in their relation to the mandibular arch. This forward change at such a period is probably due to a shortening or forward condensation of the embryonic brain. Fig. 36 indicates the further change in the gill position. In Figs. 35 and 36 the posterior limit of the hyomandibular clefts are dotted to indicate that they fade out over the velum in a very indefinite manner as above referred

to. The club muscle develops entirely between the dotted lines and the first gill, being thus confined to this area of rapid growth.

As stated in a previous paper on the development of the thyroid gland in Bdellostoma, this gland and the other organs of the gill region develop entirely *in situ* not shifting or moving during their development. For the gill shifting process is not an actual moving of the gills as will be clearly understood from the explanation which has just been given.

Conclusions.

My studies up to this point have not yet made it possible for me to satisfactorily interpret Kupffer's, **00**, "primary mouth" in Bdellostoma, a structure he describes from very young embryos. But I must admit that I am extremely skeptical regarding this earliest mouth. At times I am led to believe that this was really the nose which in the case of very early embryos, as I find, leads directly into the throat. The most convincing thing in Kupffer's argument is the following paragraph regarding the "secondäre Rachenhaut," a structure which undoubtedly exists.

"Bei dieser Sachlage warfen sich verschiedene Fragen auf. Zunächst die, ob die Epithelplatten, durch welche beide Canäle äusserlich geschlossen werden, primäre Bildungen sind, oder ob es sich hier um einen secundären Verschluss vorher klaffender Oeffnungen handelt. Im ersteren Falle wäre die den Munddarm verschliessende Platte als Rachenhaut aufzufassen und am Hypophysencanal fände sich eine analoge Vorrichtung. Dann aber wäre das Epithel beider Canäle ein endodermales und es ergabe sich hieraus der paradoxe Schluss, dass das Epithel der Nase nur an das Emdoderm Anschluss hätte. Viel einfacher würde sich aber die Deutung vorliegender Verhältnisse gestalten bei der Annahme, dass beide Canäle erst secundär sich geschlossen haben."

Dean, **99**, also states on page 270, referring to an old embryo that, "It seems clear to me, however, that the mouth and nasal openings in the present instance are certainly to be looked upon as of secondary acquisition." I must express my conviction as to the truth of the two above statements, and must regret that I have not been able in the young embryos to find the satisfactory stages showing the primary mouth opening and its subsequent closure by the "secundäre Rachenhaut."

There is much in these embryos to suggest the correctness of Dohrn's idea that the nasal tube, naso-hypophyseal canal, in myxinoids is the homologue of the ancient vertebrate mouth.

Regarding the relationships of the myxinoids I should say that it is

evident from the foregoing facts that they are gnathostomatous vertebrates, and therefore the name cyclostomes should no longer be used in reference to them. The term Marsipobranchii proposed by Bonaparte in 1846 and adopted by Huxley, Parker, Beard, and others may very fitly be used to designate this group. This suggestion has been so frequently made in the literature of the subject that it sounds like a very old cry, nevertheless it is one that should be heeded as it will eliminate from our classification a term whose meaning is undoubtedly misleading, as well as one which will hinder the recognition of these animals as true jaw-bearing vertebrates.

As to the position of the Marsipobranchii in the gnathostome series I have no definite opinion. These animals are, however, undoubtedly primitive as is indicated by the development of their mandibular arch and the presence of the additional anterior gill clefts along with other anatomical features such as a simple ear, straight digestive tract, etc. They are in other respects decidely specialized, as is indicated by the development of the dental-plate with its musculature, which is in reality the modified lower jaw apparatus. The peculiar pouched gills must also be regarded as specialized. The only point that I know of in the Bdellostoma embryo to suggest degeneracy is the fact that a lens-like thickening of the ectoderm forms over the optic cup and later disappears. I should rather, however, interpret this as a case of arrested development since the adult eye is embryonic in its appearance, as was shown by Allen, 05. Lewis, 04, has also shown that when the optic cup of the amphibian eye is removed, or does not continue its development, the lens will degenerate in the embryo. All of those features in Bdellostoma that have been accounted for as due to its parasitic or degenerate condition must be, in my opinion, explained on some other grounds.

SUMMARY.

1. In the development of the mandibular cleft of Bdellostoma the lateral diverticula at first turn dorsally, fusing with dorsal ectodermal plates in the same manner that all gills do. The diverticula later extend horizontally, and finally, bending ventrally, come to resemble the mouth arch of most other vertebrates. The fusion of the ectoderm and endoderm continues through all the stages of development.

These conditions suggest forcibly Dohrn's idea of the origin of the vertebrate mouth from a pair of gill slits.

2. The so-called "tongue" of myxinoids is really the homologue of the gnathostome lower jaw.

a. Its earliest anlage comprises the entire mass of tissue ventral of the mandibular cleft, and from similar tissue in all other gnathostomes the elements of the lower jaw are differentiated during development.

b. The "tongue" should better be called dental-plate since its muscles are not comparable with the tongue muscles of other vertebrates, and since it is innervated by the true lower jaw nerves, r. mandibularis of the trigeminus, as shown by many workers.

c. The dental-plate originates too far forward in the embryo to be a tongue, it is not associated with the hyobranchial apparatus, and its bilateral manner of development with its paired cartilage is jaw-like.

d. The development of the horny teeth on its surface and many other features of its later growth are jaw-like.

e. The ventral wall of the dental-plate of old embryos forms also the ventral wall of the head; and the later development of this organ makes it clear that the longitudinal mouth of myxinoids is only a slightly altered transverse mouth.

3. The hyomandibular cleft is enormously developed in young embryos, having well-formed ectodermal pockets in direct contact with the endodermal diverticula. A retrogressive development soon commences and the gut diverticula gradually decrease in size and finally disappear. Similarly all traces of the associated ectodermal pockets are finally lost.

4. Two post hyomandibular gills occur in the early embryos and are for some time in advance of the true branchial gills in their state of development. They then begin to degenerate, and rapidly disappear. One of these gill pairs may correspond to the thyroidean gill of Dohrn. They are interesting since the maximum number of gills in chordates appears thus increased from fifteen to seventeen.

5. The large and complex gill pouches of the adult are derived from the simple endodermal gill tube of the embryo by a spreading apart and subsequent folding of its walls in the region near its communication with the pharynx.

6. The cartilaginous branchial arches of the embryo are scarcely different from those of the adult. There are reasons for believing that the branchial skeleton of the Marsipobranchii is not essentially different from that of fishes: the extra-branchial condition is not entire and is probably secondary.

7. A great change in relative position appears during the development of the gills. The gills originate in the posterior head or neck-region of the embryo and come finally to lie far back along the sides of the trunk. A region of rapid growth can be located between the second thyroidean

and first branchial gills. The complex changes connected with the enormous growth of this neck tissue, together with the development of the large "club muscle," and the loose connection of the gill lappet tissue with the dorsal body musculature are the main causes operating to produce the translocation of the gills.

8. From this study of the head organs in embryos of Bdellostoma I am led to believe this animal in many respects primitive, while in others it is no doubt specialized. There are no reasons for believing it parasitically degenerate.

Since it is a true jaw bearing vertebrate the term cyclostome should no longer refer to it, the less objectional one marsipobranch should be used entirely.

Zoological Laboratory, Columbia University, June 4, 1906.

LITERATURE.

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I

PROCEEDINGS OF THE ASSOCIATION OF AMERICAN ANATOMISTS.

NINETEENTH SESSION.

August 6-10, 1905.

The Nineteenth Session of the Association of American Anatomists was held in *Geneva, Switzerland, August 6-10, 1905,* in conjunction with the Anatomische Gesellschaft, the Anatomical Society of Great Britain and Ireland, L'Association des Anatomistes of France and the Unione Zoologica of Italy; the joint meeting of the societies constituting the First International Congress of Anatomy.

The Executive Committee of this Congress considered an invitation extended by the Executive Committee of the Association of American Anatomists to hold the Second International Congress of Anatomy in Boston, Mass., in 1907, in conjunction with the International Zoological Congress. The Executive Committee of the First International Congress of Anatomy expressed its regret at being unable to accept this invitation, inasmuch as it had been previously decided to have the meetings of the International Congress of Anatomy occur not oftener than once in five years.

Of the members of the American Association of Anatomy, there were present at the First International Congress of Anatomy:—Edward Phelps Allis, Jr., (Milwaukee, Wis.), Elexious Thompson Bell (University of Missouri), Frederic Henry Gerrish (Portland, Me.), Francis John Shepherd (Montreal, Canada), George L. Streeter (Johns Hopkins University).

The members of the Association of American Anatomists participated in this Congress as follows:—

CONCERNING THE DEVELOPMENT OF THE ACOUSTIC GANGLION IN THE HUMAN EMBRYO. By G. L. Streeter, *Department of Anatomy, Johns Hopkins University.*

A study based on a series of wax models of the ear vesicle and acoustic ganglion, reconstructed from human embryos measuring respectively 4, 7, 9, 11, 14, 20, and 30 mm. in length. It was found that in the early

stages the acoustic ganglion consists of an upper and lower division. On the ventral border of the latter a portion of the ganglion cells of this division become massed together into a separate bud, and form the primitive ganglion cochlearis; in other words, the acoustic ganglion at this stage consists of an upper division entirely vestibular, and a lower division partly vestibular and partly cochlear. As the differentiation proceeds the group of cells forming the cochlear ganglion continues to become more distinctly separated from the rest of the acoustic mass, and gradually assumes the spiral form, seen in the adult.

The nervus cochlearis sprouts out from the ganglion cochlearis comparatively late, and passes up along the median side of the acoustic mass, without joining with the nerves to the sacculus and the posterior ampulla. Contrary to the younger His, the author concludes that the nerves to the sacculus and posterior ampulla belong to the vestibular rather than the cochlear division of the acoustic nerve.

PROCEEDINGS OF THE ASSOCIATION OF AMERICAN ANATOMISTS.

TWENTIETH SESSION.

In the Medical Building, Laboratories of Anatomy and Histology and Embryology, University of Michigan, Ann Arbor, Michigan,

December 27, 28 and 29, 1905.

At its business sessions the Association took the following actions:

The minutes of the Secretary as printed in the American Journal of Anatomy, Vol. IV, No. 2, pages I-II, were approved.

The Association received a communication from Prof. Burt G. Wilder, Chairman of the Committee on Brain Requests and Methods, stating that this committee had held one meeting, but were as yet not prepared to make a formal report. The committee asks to be continued.

On motion this report was accepted and the committee continued.

The permanent secretary of the American Association for the Advancement of Science, Doctor L. O. Howard, states that this association would be pleased to have the Association of American Anatomists meet in affiliation with the A. A. A. S. during convocation week, 1906, in New York City. It was informally agreed that if the Association of American Anatomists should meet during convocation week (1906), they would in all probability meet in New York City.

In response to a request from the secretary of the Committee of Ar-

rangements for the International Congress of Anatomy, the Association, on motion, appointed Dr. Charles C. Minot, as member—from the Association of American Anatomists—of the Committee of Arrangements of the International Congress of Anatomy for 1910, with Dr. Franklin P. Mall, alternate.

At the suggestion of the Executive Committee, the following distinguished European anatomists were elected honorary members of this Association:

Professor Ramon Y. Cajal, Professor of Normal Histology, Mádrid, Spain.

Professor Oscar Hertwig, Professor of Anatomy and Embryology, Berlin, Germany.

Forty-six new members were elected.

The Committee on Nominations (Dr. Charles R. Bardeen, Prof. Simon H. Gage, Dr Robert J. Terry) made the following report:

For President.....FRANKLIN P. MALL.
For First Vice-President.....GEORGE A. PIERSOL.
For Second Vice-President.....ROBERT R. BENSLEY.
For Secretary and Treasurer.....G. CARL HUBER.

For Members of Executive Committee.

CHARLES S. MINOT.....Term expiring in 1908.
JAMES PLAYFAIR MCMURRICH.....Term expiring in 1910.

On motion the Secretary was instructed to cast a ballot for the officers recommended. Carried.

TREASURER'S REPORT FOR THE YEAR 1905.

Balance on hand December 27, 1904	$48.04	
Total receipts for 1905	932.10	
	$980.14	$980.14
Expenditures for 1905:		
Expenses of Secretary for Philadelphia meeting, 1904	24.00	
To American Journal of Anatomy	794.50	
To Ramon Guiteras, Secretary American National Com. International Med. Congress	25.00	
Envelopes and postage	11.00	
Printing	17.00	
To 1 check returned and charged to the Association	5.00	
	$876.50	876.50
Balance on hand December 27, 1905		$103.64

The Treasurer's report was audited by a committee consisting of Dr. Robert R. Bensley and Dr. William S Miller. This committee reported: "We have examined the Treasurer's accounts for 1905 and found them correct."

On motion the report of the Auditing Committee was accepted and adopted.

ABSTRACTS OF PAPERS PRESENTED.

CONTRIBUTION TO THE GENETIC INTERPRETATION OF THE MAMMALIAN INTERNAL SPERMATIC VEIN. By George S. Huntington. *Columbia University, New York.*

Read by title.

ON THE PAROTID OF PROCAVIA. By George S. Huntingdon. *Columbia University, New York.*

Read by title.

(Owing to illness Dr. Huntington was unable to attend the meeting.)

THE HOMOLOGY OF THE VERTEBRATE LIMBS. By James Playfair McMurrich. *University of Michigan.*

A HITHERTO UNRECOGNIZED FEATURE IN THE DEVELOPMENT OF THE REPTILIAN POSTCAVA. By Charles F. W. McClure. *Department of Biology, Princeton University.*

Read by title.

ON THE PRESENCE OF A TYPE OF POSTCAVA IN THE ADULT CHEVROTAIN, TRAGULUS MEMINA (ERXLEBEN), WHICH IS UNUSUAL IN RUMINANTS. By Charles F. W. McClure. Department *of Biology, Princeton University.*

Read by title.

PECULIARITIES OF THE NEGRO BRAIN. By Robert Bennett Bean. *Department of Anatomy, University of Michigan.* *See* American Jour. of Anatomy, Vol. V.

THE DISTRIBUTION OF THE BRONCHIAL BLOOD-VESSELS. By William S. Miller. *Department of Anatomy, University of Wisconsin.*

The bronchial arteries, after entering the hilus of the lung, follow the bronchi buried in their fibrous layer. Two, sometimes three, main branches accompany each bronchus. The branching of the bronchial ar-

teries corresponds to the branching of the bronchi, along which numerous small rami continually spread themselves out to form an irregular plexus, in general arranged at right angles to the muscular layer. In a collapsed lung the bronchial arteries have a tortuous course, but when the lung is inflated and the bronchi correspondingly lengthened their course is quite straight. The main trunks can be followed as far as the bronchioli respiratorii where they terminate in a capillary network which extends to the distal end of the ductuli alveolares.

From the arterial plexus in the fibrous layer small twigs are given off which penetrate through the muscular layer and having reached a position beneath the epithelium lining of the bronchi they turn and run for a short distance parallel with the muscular layer, giving off capillaries which run parallel to the long axis of the bronchus. These capillaries unite to form a network of venous radicles situated at first side by side with the arterial radicles, then passing obliquely through the muscular layer they form a second plexus, with a coarse mesh, along the boundary line between the fibrous and muscular layers.

From this second venous plexus small veins arise which form one of the sources of origin of the pulmonary vein. These small veins, which may be designated as broncho-pulmonary (Le Fort), arise at various intervals along the larger bronchi; along the smaller bronchi and the bronchioli respiratorii they usually arise, one on either side, at the place where the bronchi divide; from the ductuli alveolares they arise one on either side of their distal end and carry off from the bronchial tree the last trace of blood which is found in the terminal capillary network into which the main trunks of the bronchial arteries break up.

By a careful study of the vascular network in the bronchial muscosa one can recognize small areas composed of an arterial radicle, its capillaries and a loop of the venous plexus. I have found this area in the bronchi of man, the dog and the cat. It may be called the unit of distribution.

The veins arising from the first two or three divisions of the bronchi do not join the pulmonary vein, but form true bronchial veins which empty into the azygos or into the vena cava. This has already been noted by several authors.

No anastomoses were found between the bronchial and pulmonary arteries. In many sections quite large branches could be seen passing from the bronchial arteries to the walls of the pulmonary artery where they became the vasa vasorum, but in no instance was the differential injection mass found in the lumen of the pulmonary artery.

A NOTE ON METHYLENE VIOLET AS ONE OF THE NUCLEAR DYES IN THE ROMANOWSKY STAIN.

By Ward J. MacNeal. *Department of Histology and Embryology, University of Michigan.*

Bernthsen in 1885 showed that decomposition of methylene blue by alkalies yields methylene violet, which he obtained pure, methylene azure which he was unable to prepare in pure condition, and the leukobases of these substances and of methylene blue.

Michaelis and Giemsa have recognized the nuclear dye of the Romanowsky stain as methylene azure and Giemsa has prepared a pure methylene azure by a secret method, and this is on the market. These observers regard methylene violet as of no value.

Unna has compared the staining properties of Giemsa's methylene azure with his own polychrome methylene blue solution, and finds the older preparation much more active and therefore concludes that azure is not the only staining principle of polychrome methylene blue. By adding methylene violet and alkali carbonates to azure solutions he approximated very closely polychrome methylene blue.

My first experiment was an attempt to isolate Nocht's " roth aus methylene blau " by extraction with ether. By means of a mechanical extractor 0.4 gram of this substance was obtained and identified by its chemical reactions as nearly pure methylene violet (Bernthsen).

Methylene violet was next prepared by Bernthsen's method and its reactions compared with the ethereal extract of Nocht's solution.

The next step was to combine methylene violet into a suitable staining mixture. It is insoluble in water, but soluble in aqueous methylene blue solution.

Methylene violet	0.5 g.
Methylene blue (med. pure)	0.5 g.
Sod. carbonate (cryst.)	0.25 g.
Glycerin	50.00 cc.
Distilled water	50.00 cc.

The solid ingredients should be finely divided by trituration. CO_2 is given off for some hours after preparation of this solution. It is used by mixing a few drops with dilute aqueous eosin and floating fixed preparation on the mixture for 2 to 30 minutes.

A solution of the dye in methylic alcohol is useful for rapid work. The following formula has been tried:

Methylene violet	0.12 g.
Methylene blue	0.12 g.
Eosin	0.12 g.
Methyl alcohol (pure)	100.00 cc.

Grind the solids thoroughly, dissolve in the warmed alcohol, and filter. This is used the same as Leishman's solution.

Methylene violet may be prepared by Bernthsen's method. A fairly rapid method of making it is as follows:

Methylene blue	5.00 g.
Na_2CO_3 crystals	3.00 g.
Distilled water	2000.00 cc.

Dissolve separately, mix and boil for five hours, replacing the water lost by evaporation, filter and dry the ppt., which is fairly pure methylene violet. Recrystallization (repeated 8 to 10 times) from 95% alcohol purifies it, yielding beautiful green crystals insoluble in cold water. The crude violet may be used for staining, however.

DEVELOPMENT OF MEMBRANOUS LABYRINTH AND ACOUSTIC GANGLION IN THE HUMAN EMBRYO. By George L. Streeter. *Department of Anatomy, Johns Hopkins University.*

A report supplementing a paper, which was read at the Anatomical Congress at Geneva (Verhandl. d. Anatom. Gesell. 1905), and in which it was shown by a series of reconstructions that the nerves to the saccule and posterior ampulla belong to the vestibular rather than the cochlear division of the acoustic nerve. The additional observations included in this paper concern features in the development of the ear vesicle, being additional early stages of differentiation, and the correction of certain errors in descriptions previously published. It is shown that the saccule instead of developing as a pocket from the edge of the cochlea, as described by His, Jr., is a portion of the vestibule and is partitioned off from the utricle. The saccule is not developed from the cochlea, but the cochlea is developed from the saccule, though this process occurs before the separation of saccule from utricle is complete. It is further shown that each semicircular canal has only one ampulla.

ON THE EPITHELIAL CELL PROCESSES OF THE SULCUS SPIRALIS EXTERNUS. By George E. Shambaugh. *Hull Laboratory of Anatomy, University of Chicago.*

Continuous with the epithelium covering the prominentia spiralis, covered over in part, at times, by the cells of Claudius, and lying directly in the bottom of the sulcus spiralis externus is a group of epithelial cells which possess certain marked peculiarities that distinguish them from epthelial cells found elsewhere in the labyrinth.

In the labyrinth of the pig it was found that these cells first began to differentiate from the remainder of the epithelium of the sulcus ex-

ternus in the embryo measuring 12 cm. in length. In the embryo 15 cm. long processes had formed from the cells throughout the coils of the cochlea and the bunching up of the cells in the sulcus externus began to appear preliminary to the actual invasion of the spiral ligament by these cells. In the fœtus at full term groups of these epithelial cells from the sulcus externus had invaded the spiral ligament, especially in the basal coil, to such an extent that their nuclei were found buried in the spiral ligament as deep as midway between the sulcus externus and the osseous capsule, while protoplasmic process from these cells could be traced out to the loosely arranged connective tissue of the spiral ligament lying next to the osseous capsule.

In sections cut parallel to the coils of the cochlea a continuous line of these cell groups would be found. These groups were arranged at regular intervals, and it was noted that the free surface of the epithelium in the sulcus externus showed a distinct depression marking the site where each group of cells penetrated the spiral ligament. A close study of these cell groups showed that where the section cut such a group through the center of its axis a small but distinct duct could be distinguished. Perpendicular sections cut through the edge of the cochlea would often cut these cell groups at right angles. In such sections it was noted that these cells were usually slightly separated from the surrounding connective tissue probably due to shrinking. In these sections the central duct could often be made out.

THE NERVE SUPPLY TO THE LEG OF THE FROG AFTER COMPLETE DEGENERATION OF THE MOTOR FIBERS. By Elizabeth H. Dunn.
Department of Neurology, University of Chicago.

After severing the anterior roots of the 8th, 9th, and 10th left spinal nerves (Gaupp's nomenclature) in a frog, Rana virescens Cope; female, length 229 mm., weight about 55 grams, a period of eight months was allowed for the completion of degeneration. On comparison of the osmic stained nerves at various levels the following conclusions seem justified.

A large afferent suply to the muscles, about 50% of the total normal supply, is present at all levels.

On each side the findings as to the size of the largest fibers innervating the various segments corroborate the earlier statement that the largest fibers run the shortest distance, a marked diminution appearing in the average areas of the largest fibers given off successively to the thigh, shank, and foot.

A comparison of the muscular and cutaneous afferent fibers as to size shows that the largest are distributed somewhat evenly in both instances, but that many more very small fibers pass to the skin than to the muscles.

A study of the fibers on the operated side shows a swollen condition with increased relative area of the axis cylinder in the thigh, although in the main the normal one to one relation of axis cylinder to medullary sheath is maintained.

In the intact leg the distribution of the fibers to the various segments is in accordance with the law of distribution previously formulated in this laboratory.

ON THE HISTOGENESIS OF SMOOTH MUSCLE. By Caroline McGill. *Department of Anatomy, University of Missouri.*

Read by title.

TOTAL FOLDS OF THE BRAIN TUBE IN THE EMBRYO AND THEIR RELATION TO DEFINITE STRUCTURES. By Susanna Phelps Gage. *Department of Histology and Embryology, Cornell University.*

Continuing the investigations reported last year concerning the total transverse folds of the brain tube, human specimens from the Cornell University and Johns Hopkins Medical School collections have been examined. These range from the 3d to the 9th week of development. The present report covers only the findings in the oblongata.

The total folds forming so prominent a feature of the 3-week brain, begin to be obliterated on the outer surface by the formation of white matter. Before the end of the 4th week the outer surface shows only slight indications of total folding, but the inner surface is sharply divided and the multiplying cells are masses along the lines of the original folds. The folds show a tendency to form pits at about their middle. At 5 weeks these pits deepen and are emphasized by the bulging longitudinal bundles in the floor of the oblongata. The pits are here clearly associated with increasing cell group and nerve roots. At 7 weeks, though masked by greater growth of longitudinal bundles and longitudinal spreading of cell groups, the same essential arrangements can be traced. At 9 weeks the inner surface of the oblongata retains at its greatest depth of folding, remnants of the 2d, 3d and 4th oblongata folds which still show relations through cell groups with the Vth, VIIth and VIIIth Nerves which are characteristic from the earliest stages.

This feature was traced through a series of cat brains until the pons and cerebellum were well developed and all showed characteristic remnants of these same folds in the depth of the pons bend.

In an especially favorable specimen, the folds, each in its turn were seen to contribute fibers to the longitudinal bundles of the region, thus show-

ing that these folds not only are associated with serial nerve roots, but with the longitudinal association tracts.

From the above it seems probable that segmental relations in the adult brain can ultimately be traced with greater accuracy than hitherto.

THE ORIGIN OF THE GERM CELLS OF THE TURTLE. By Bennet M. Allen. *Department of Anatomy, University of Wisconsin.*

EARLY STAGES IN THE DEVELOPMENT OF THE NASAL SKELETON OF AMBLYSTOMA. By Robert J. Terry. *Washington University, St.-Louis.*

THE MUSCLES OF THE FEMALE PELVIC FLOOR. By William Keiller. *Medical Department, University of Texas, Galveston.*

Read by title.

THE FIFTH AND SIXTH AORTIC ARCHES AND THE RELATED PHARYNGIAL POUCHES IN THE RABBIT AND THE PIG. By Frederic T. Lewis. *Harvard Embryological Laboratory. Presented by Charles S. Minot.*

FURTHER EXPERIMENTS ON THE DEVELOPMENT OF THE PERIPHERAL NERVES OF VERTEBRATES. By Ross G. Harrison. *Department of Anatomy, Johns Hopkins University.* American Journal of Anatomy, Vol. V.

EXPERIMENTAL EVIDENCE IN SUPPORT OF THE OUTGROWTH THEORY OF THE AXIS CYLINDER. By Warren Harmon Lewis. *Associate Professor of Anatomy, Johns Hopkins University.*

The nasal pit in amblystoma when transplanted, at a stage before its nerves appear, in such a manner that its deep surface lies in contact with the inner layer of the ectoderm, will send out its nerve fibers between it and the ectoderm at which place there is no mesenchyme.

If the anterior end of the brain is removed before the olfactory nerves are sent out, mesenchyme fills in the region more or less and the nerves from the nasal pit grow out into this mesenchyme in various directions, and as in the transplanted pit they take paths which are in no sense predetermined.

The optic nerve from transplanted eyes, in the majority of instances, runs for long distances in among the pigment cells of the outer layer. In a few experiments where the transplanted eye is in contact with the medulla, the optic nerve may pass into the medulla and run for some distance in the latter. In a few instances the fibers of the optic nerve pass directly out through the pupil into the mesenchyme surrounding the transplanted eye.

Transplanted pieces of the medullary plate possess the power of self-differentiation, send out nerves into the strange mesenchyme surrounding them or when in contact with the wall of the pharynx may send axis cylinders for some distance in among the epithelial cells of the wall of the pharynx.

Injuries to the brain made shortly after the closure of the neural fold may form a point of exit for new nerves which may run out into the mesenchyme in various directions. Such nerves may arise even anterior to the optic nerve.

The only adequate explanation of such phenomena seems to me to lie in the acceptance of the outgrowth theory of the axis cylinder.

EXPERIMENTS ON THE REGENERATION AND DIFFERENTIATION OF THE CENTRAL NERVOUS SYSTEM IN AMPHIBIAN EMBRYOS. By Warren Harmon Lewis. *Associate Professor of Anatomy, Johns Hopkins University.*

A portion of the dorsal lips of the blastopore transplanted into the mesenchyme of a much older embryo of Rana palustris will differentiate into spinal cord, notochord and muscle, indicating that the cells of the rim of the half-closed blastopore are already predetermined. Small pieces of the medullary plate when transplanted into the mesenchyme of older embryos likewise possess the power of self-differentiation, and they are also able to regenerate a considerable portion of the brain corresponding to the region which would normally have developed about the piece so cut out. As for example, a small piece from one side of the mid-line and not extending more than half way to the neural fold of that side, will regenerate a perfectly bilateral medulla with ventricle, roof and typical arrangement of the white and grey substances of the normal medulla. Such pieces may also send out nerves along new paths in the strange mesenchyme surrounding them.

Embryos from which various portions of the medullary plate are taken will regenerate the lost part and a perfectly normal brain will result. After closure of the neural folds lost parts of the neural tube are as a rule only imperfectly regenerated.

EXPERIMENTS ON THE ORIGIN AND DIFFERENTIATION OF THE LENS IN AMBLYSTOMA. By Wilbur L. Le Cron. *Student of Medicine, Johns Hopkins University.*

The work of Spemann and Lewis has shown conclusively that lens formation is dependent upon the optic vesicle for its initial stimulus. Although the lens is not a self-originating structure, the question arises,

is it capable of self-differentiation or is the continued influence of the optic vesicle necessary for its normal development?

At the suggestion of Dr. Lewis, I undertook in the Spring of 1905 an experimental study of the question of self-differentiation of the lens in Amblystoma punctatum. It seemed possible that by removing the optic vesicle, or optic cup, at various stages before and during lens formation, one would be able to determine whether continued influence of the optic vesicle is necessary for the perfect development of the lens.

The operation of removing the optic vesicle without injury to the developing lens or surrounding ectoderm is a simple one. The embryos were operated upon under the binocular miscroscope. A semicircular incision was made a little caudal to the eye region, and the skin flap turned forward, thus exposing the rounded optic vesicle. The latter was cut off close to the brain, and then readily removed, without injury to the lens-forming ectoderm or lens-bud. On replacing the skin flap into its original position, healing was rapid, requiring but a few hours. The embryos were killed in Zenker's fluid 2 hours to 30 days after the operation. In all 63 experiments were made.

When the optic vesicle was removed from an embryo of a stage shortly after the closure of the neural folds, but before there were any signs of lens formation, the latter did not take place, unless the eye regenerated. In Amblystoma then, as in Rana, the lens is not a self-originating structure.

When the optic vesicle is removed during the time of formation of the lens-plate, the latter is found to possess very little power of self-differentiation and may remain as a mere thickening of the ectoderm.

If the optic vesicle is removed at a later stage, when there is a well developed lens-bud, we find that it possesses more power of self-differentiation and will progress to the formation of a lens vesicle having walls one or two layers in thickness, but beyond this stage it does not seem to be able to differentiate, and even after many days, it remains in this condition, without the formation of lens-fibers.

If the optic cup is removed at a still later stage, as when the lens vesicle has pinched off from the ectoderm, but still in contact with the same, we find that a still greater amount of self-differentiation is present, there being enough to carry the lens vesicle on at the normal rate for a few days, and lens-fibers will form. But its growth and differentiation ultimately become retarded and stopped, and certain changes set in. The lens-pole disappears, and new lens-fibers cease to originate, the anterior epithelial layer grows over this pole thus enclosing completely the lens-fibers, which begin to degenerate and finally become a mere vacuolated mass.

The lens, then, is not a self-originating structure, but is evidently dependent upon the stimulus of the optic vesicle for its origin. It seems that this influence of the optic vesicle upon the overlying ectoderm must be of considerable duration, and that even after lens formation has begun, a continued influence of the optic cup is necessary for the normal differentiation and growth of the lens.

ON THE RELATION OF THE NERVE ENTRANCE TO THE INTERNAL ARCHITECTURE IN MAMMALIAN MUSCLE. By Charles R. Bardeen. *Department of Anatomy, University of Wisconsin.*

SOME NEW FACTS TOUCHING THE ARCHITECTURE OF THE SPINAL GANGLION IN MAMMALS. By S. Walter Ransom. *Neurological Laboratory, University of Chicago. Presented by H. H. Donaldson.*

There are in the second cervical ganglion of the white rat about three times as many cells as there are medullated afferent fibers (8500 cells, 2500 fibers). Nevertheless, section of the nerve caused the disappearance of half of the cells from the ganglion; that is, about 4500 cells dropped out following the section of 2500 medullated afferent fibers. This result was constant in the nine cases enumerated. In spite of this large and very constant reduction in the number of the ganglion cells there was but a slight and inconstant decrease in the number of medullated fibers in the dorsal roots.

It is clear that the present conception of the architecture of the spinal ganglion does not furnish an adequate basis for the explanation of these results.

GLYCOGEN IN A 56-DAY HUMAN EMBRYO AND IN PIG EMBRYOS OF 7 TO 70 MM. By Simon H. Gage. *Department of Histology and Embryology, Cornell University.*

A 56-day human embryo preserved in 95% alcohol showed abundant glycogen in the tissues where it is most prominent in the lower mammals of corresponding stages. It was especially marked in the developing epidermis, in the cartilages and in the skeletal muscles. It was also abundant in the epithelium of the main air passages, but scanty in the heart.

For comparison pig embryos ranging from 7 to 70 mm. were preserved in 95% alcohol and cut into serial sections. As they were placed in the alcohol before any changes had supervened, the glycogen was more satisfactorily preserved than in the human embryo which had macerated somewhat before the alcohol penetrated the chorion.

In tracing the appearance and disappearance of glycogen in the different series it was found that at the earliest stages of an organ or tissue no glycogen is present, but in a sort of middle stage when the organ or tissue is taking on something of its definitive structure, glycogen is very abundant, and somewhat later as the organ becomes perfected the glycogen gradually disappears. For example, in the youngest pigs the glycogen is so large in quantity in the heart muscle that the organ stains almost black with Gram's iodin solution; but in the largest pig (70 mm.) the glycogen had partly disappeared. In the alimentary canal the glycogen passes as a kind of wave along the tube commencing at the mouth, esophagus and stomach, and as the villi commence to be formed, passing along into the small and large intestines.

No glycogen was found in the liver of any of the pigs studied. This agrees with the statement of Bernard that the liver is relatively very late in assuming the glycogenic function.

The presence of glycogen in the endymal cells covering the choroid plexuses of the brain (Creighton) was verified, but as with the other organs the glycogen is not present during the first stages. Late it is very abundant and is present in the endymal cells not only of the relatively free plexus, but for a considerable distance upon the ventricular brain surface. This endymal glycogen was found in the plexus of the lateral ventricles of pigs from 40 to 70 mm. It does not appear in the endymal cells of the 4th ventricle until the pigs are between 50 and 75 mm. in length.

Contrary to the statements of Bernard, much of the cœlomic epithelium is very freely suplied with glycogen (pigs of 35-45 mm.); and contrary to the statement of all authors (Bernard, Barfurth, Creighton, Pflüger and those to whom these authors refer), that no glycogen is present in the nervous system in any stage of its development in vertebrates, abundant glycogen was found in a part of the cells of the ganglia of the dorsal roots of the nerves. This persists up to about 15 mm. pigs. While the ganglion cells possess the glycogen the outgrowing nerves seem to be wholly free from it and appear as almost transparent spaces, but in older pigs 30 to 70 mm. and perhaps older, the nerves beyond and within the ganglion are so densely surrounded by glycogen that they stain a deep brown by iodin. So far as at present known, the glycogen is not in the fibers but in the surrounding tissue.

Abundant glycogen was also found in the olfactory as well as the respiratory epithelium of the nose and its presence is very striking in the epithelium of the cochlear canal opposite the embryonic organ of Corti. Glycogen is, therefore, normally present in parts, at least, of the central

nervous system and the organs of sense in mammals. This is in accord with the findings of glycogen in the cells of the spinal cord of Amphioxus and Asymmetron and in the brain and cochlear canal of Ammocœtes which were reported last year (American Journal of Anatomy, Vol. IV, p. XII of Proceedings of Association of American Anatomists).

AN EXAMINATION OF THE METHODS FOR THE MICHROCHEMICAL DETECTION OF PHOSPHORUS IN TISSUES. By Robert R. Bensley. *Hull Laboratory of Anatomy, University of Chicago.*

Experiments were described which seemed to show that the results obtained by the method of Macallum are in reality due to the formation of molybdic acid compounds with the tissue-substances. The reasons for this conclusion are briefly as follows:

Phenylhydrazin hydrochloride reacts with solutions of molybdic acid prepared by the methods of Ullik and Graham to produce the blue oxide of molybdenum. Sections treated with solutions of molybdic acid containing 5% of nitric acid, then reduced by a solution of phenylhydrazin hydrochloride, give a reaction similar in all respects to the so-called phosphorus reaction, except that a strong reaction is obtained in the collagenic fibrils as well as in the nuclear chromatin.

Nitric acid affects the reduction of molybdic acid, ammonium molybdate and ammonium phosphomolybdate by phenylhydrazin hydrochloride in the same way, but to different degrees, inasmuch as low concentrations of nitric acid serve to prevent the reduction of molybdic acid and of ammonium molybdate to the blue oxide, while high concentrations (e. g. 36%) of nitric acid only retard the reduction of ammonium phosphomolybdate. Accordingly, solutions of phenylhydrazin hydrochloride containing a high percentage of nitric acid should detect ammonium phosphomolybdate in sections, while not affecting in any way molybdic acid compounds or ammonium molybdate mechanically held by the tissue. Tests made on sections impregnated with molybdic acid and on sections into which ammonium phosphomolybdate has been artificially introduced show that this is the case. Sections treated with molybdic acid solutions show no reaction with solutions of phenylhydrazin hydrochloride containing a low percentage of nitric acid, while sections treated with phosphoric acid solutions and then with warm nitric molybdate reduce rapidly in a solution containing 0.1% of phenylhydrazin hydrochloride and 15% of nitric acid. Sections treated with Macallums's nitric-molybdate reagent behave like sections treated with solutions of molybdic acid. That is to say, low concentrations of nitric acid suffice to prevent their reduction by phenylhydrazin hydrochloride. For example, a solution contain-

ing 0.1% of phenylhydrazin hydrochloride and 3.27% of nitric acid produced no reaction with sections treated with pure molybdic acid or with Macallum's nitric-molybdate reagent after three minutes' treatment, although similar sections containing ammonium phosphomolybdate artificially introduced reduced to a maximum extent in the same solution in less than one minute. Clearly, the sections treated with the nitric molybdate reagent contained no phosphomolybdate of ammonium. Otherwise the reduction would have been obtained in the presence of a high content of nitric acid. The experiments show that the reducing agent employed does not discriminate between ammonium phosphomolybdate formed at the expense of the phosphorus of the tissue and other molybdenum compounds which are likely to be present, inasmuch as it produces like colored compounds with molybdic acid and ammonium molybdate.

They show further that the phosphorus, if liberated as phosphoric acid, is not precipitated at the point of liberation as ammonium phosphomolybdate. Thus the essential conditions of a successful phosphorus reaction by this method are not fulfilled.

ON THE STRUCTURE OF THE HARDERIAN GLANDS IN MAMMALS. By John Sundwall. *Hull Laboratory of Anatomy, University of Chicago. Presented by Robert R. Bensley.*

ON THE SO-CALLED TRANSITIONAL CELLS OF LEWASCHEW IN THE ISLETS OF LANGERHANS. By M. H. Lane. *Hull Laboratory of Anatomy, University of Chicago. Presented by Robert R. Bensley.*

A report was made of observations on the structure of certain cells in the islets of Langerhans which may have been among those observed by Lewaschew and interpreted by him as transitional in nature between the islet cell and the acinus cell. Comparatively few references to these cells appear in the literature. Diamare described them as large deeply staining cells occurring on the borders of the islets of the rabbit and guinea-pig, and Schultze makes a similar brief reference to their occurrence in the same animal. By means of Bensley's neutral gentian technique it has been possible to stain these cells distinctively and to determine their characters. It has been found that they are much larger than the ordinary islet cells, and in preparations stained by the neutral gentian method, appear intensely blue, while the other cells of the islets are pale orange. Under high powers it is seen that the deeply stained cells owe their appearance to the fact that they contain innumerable minute granules which are stained deeply with the violet component and stand out clearly on an orange stained background of the ordinary protoplasm of

the cell. In many cells a small space on one side of the nucleus is found to be free from the granules and probably contains the centrioles, although the large number of granules present in the cell makes the actual observation of them difficult. The granules do not correspond in their chemical properties either with the zymogen granules of the acinus or with the prozymogen. Unlike the zymogen granules they do not dissolve in 70% alcohol, and they differ from the prozymogen by not staining in toluiden blue or polychrome methylene blue. It seems probable that we have to deal here with a specific element of the islets of Langerhans differing both from the cells of the acinus and from the ordinary islet cell. By analogy with the hypophysis, to the chromophile cells of which these cells bear a remarkable resemblance, they may be called the chromophile cells of the islet of Langerhans. They have been found in the cat, dog, rabbit, white rat and guinea-pig.

EXPERIMENTAL STUDIES ON THE NATURE OF THE CELLS COMPOSING THE GASTRIC GLANDS OF THE DOG. By B. C. Harvey. *Hull Laboratory of Anatomy, University of Chicago.*

The changes which take place in the bodies of the fundus glands of the dog as a result of gastro-enterostomy were described and discussed. After gastro-enterostomies the cells in the bottoms of the fundus-glands at the site of operation which normally are ferment-forming cells are replaced by mucous cells. After 6½ months, however, the cells in this region are all ferment cells again, as in the normal stomach. Similar changes occur after simple incisions into the gastric mucous membrane. The new mucous cells which are formed in the first two months could not be formed by the division of previously existing cells of the same kind because mitoses are extremely infrequent in this location. They could not arise from parietal cells because no transformation stages can be observed. They must, therefore, have arisen by the transformation of ferment-forming chief cells, the number of which is inversely proportional to that of the mucous cells. The direct proof that the ferment-forming chief cells become mucous cells can be found in the fact that transition stages have been observed. The new ferment-forming chief cells found at 6½ months after the operation were shown to have been formed by transformation of mucous cells. The conclusion was drawn that these cells are not specifically distinct but may change from one type to the other, and later resume their original condition, in response to the action of varying external conditions. Also, mucous transformations of ferment cells are not to be regarded as necessarily either degenerations or final differentiations.

ON THE ARTERIÆ RECTÆ OF THE MAMMALIAN KIDNEY. By G. Carl Huber. *University of Michigan.*

In corrosion preparations of the blood-vessels of the kidney of the dog, cat, rabbit, and guinea-pig it was observed that all the straight arterioles and capillaries passing to the medulla were branches of efferent vessels of the glomeruli.

ON THE STRUCTURE OF THE AMPHIBIAN AND REPTILIAN KIDNEY. By G. Carl Huber and Ward J. MacNeal. *University of Michigan.*

The presentation consisted in a brief description of models of the uriniferous tubules of the frog and the turtle, Chrysemys marginata. The models were made after the Born method of wax-plate reconstruction. The duct systems of these kidneys were studied by the corrosion method.

ON A MODIFICATION OF THE OBREGIA-GULLAND SUGAR-DEXTRIN METHOD. By G. Carl Huber and Clarence Snow. *University of Michigan.*

DEMONSTRATIONS.

1. Robert R. Bensley: *a*, Paneth and goblet cells in the villi of the opossum; *b*, Mucous staining in the glands of Brunner. (Hull Laboratory of Anatomy, University of Chicago.)
2. Benson A. Cohoe: Preparations of the submaxillary gland of the rabbit. (Hull Laboratory of Anatomy, University of Chicago.)
3. Simon H. Gage: Preparations showing glycogen in embryos. (Laboratory of Histology and Embryology, Cornell University.)
4. Susanna Phelps Gage: Models reconstructed from blotting paper to illustrate the total folds in the brain tube.
5. Ross G. Harrison: Specimens showing the development of peripheral nerves. (Department of Anatomy, Johns Hopkins University.)
6. G. Carl Huber: *a*, Wax reconstructions of the uriniferous tubules of frog and turtle; *b*, Corrosion preparation showing the arteriæ rectæ of the mammalian kidney. (Department of Histology and Embryology, University of Michigan.)
7. Clarence M. Jackson: Model of the thoracic and abdominal viscera of a human embryo aged six weeks. (Department of Anatomy, University of Missouri.)
8. William Keiller: Casts demonstrating the muscles of the female pelvic floor. (Medical Department, University of Texas, Galveston.)
9. Thomas G. Lee: Early stages of mammalian embryos. (Laboratory of Histology and Embryology, University of Minnesota.)
10. Warren H. Lewis: *a*, Preparations from experiments on the origin and differentiation of the lens in Amblystoma; *b*, Preparations on the regeneration and differentiation of the central nervous system in amphibian embryos; *c*, Preparations from experiments on the outgrowth theory of the axis cylinders. (Department of Anatomy, Johns Hopkins University.)

11. James Playfair McMurrich: Muscle anomalies. (Department of Anatomy, University of Michigan.)
12. William S. Miller: *a*, Models, drawings and preparations showing the distribution of the bronchial blood-vessels; *b*, The lymphatics of the lung of Necturus. (Department of Anatomy, University of Wisconsin.)
13. A. G. Pohlman: Muscle anomalies. (Department of Anatomy, University of Indiana.)
14. D. G. Revell: *a*, Preparations from the human stomach, showing the distribution of mucus and zymogen in the glands; *b*, Intestinal epithelium, including goblet cells and Paneth cells from the human stomach in the neighborhood of the oesophagus. (Hull Laboratory of Anatomy, University of Chicago.)
15. George E. Shambaugh: Preparations to illustrate cell processes in the sulcus spiralis externus. (Hull Laboratory of Anatomy, University of Chicago.)
16. G. L. Streeter: Wax models of the membranous labyrinth and acoustic nerve complex reconstructed from human embryos. (Anatomical Laboratory, Johns Hopkins University.)
17. Robert J. Terry: Wax reconstructions of the nasal skeleton of Amblystoma. (Department of Anatomy, Washington University, St. Louis.)

CONSTITUTION AND LIST OF OFFICERS AND MEMBERS.

Constitution.

ARTICLE I.

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

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

ARTICLE II.

The officers of the Association shall consist of a President, two Vice-Presidents, and a Secretary, who shall also act as Treasurer. The officers shall be elected by ballot every two years.

ARTICLE III.

The management of the affairs of the Association shall be delegated to an Executive Committee, consisting of seven members, including the President and Secretary, ex-officio. One member of the Executive Committee shall be elected annually.

ARTICLE IV.

The Association shall meet annually, the time and place to be determined by the Executive Committee.

ARTICLE V.

Section 1. Candidates for membership must be persons engaged in the investigation of anatomical or cognate sciences and shall be proposed in writing to the Executive Committee by two members, who shall accompany the recommendation by a list of the candidate's publications, together with the references. The election shall take place in open meeting, a two-thirds vote being necessary.

Section 2. Honorary members may be elected from those not Americans who have distinguished themselves in anatomical research.

ARTICLE VI.

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

ARTICLE VII.

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

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

Section 3. The ruling of the Chairman shall be in accordance with "Roberts' Rules of Order."

ORDERS ADOPTED BY THE ASSOCIATION.

The election of delegates to the Executive Committee of the Congress of American Physicians and Surgeons shall take place every three years.

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

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

The Secretary and Treasurer shall be allowed his traveling expenses

and the sum of $10 toward the payment of his hotel bill, at each session of the Association.

That the Association discontinue the separate publication of its proceedings, and that the AMERICAN JOURNAL OF ANATOMY be sent to each member of the Association, on payment of his annual dues, this journal to publish the proceedings of the Association, including an abstract of the papers read.

Contributors of papers are requested to furnish the Secretary with abstracts within a fortnight after the meeting.

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JAMES PLAYFAIR MCMURRICH.....Term expiring in 1910.

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CHARLES S. MINOT.

Alternate.

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For addresses of officers, see list of members.

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