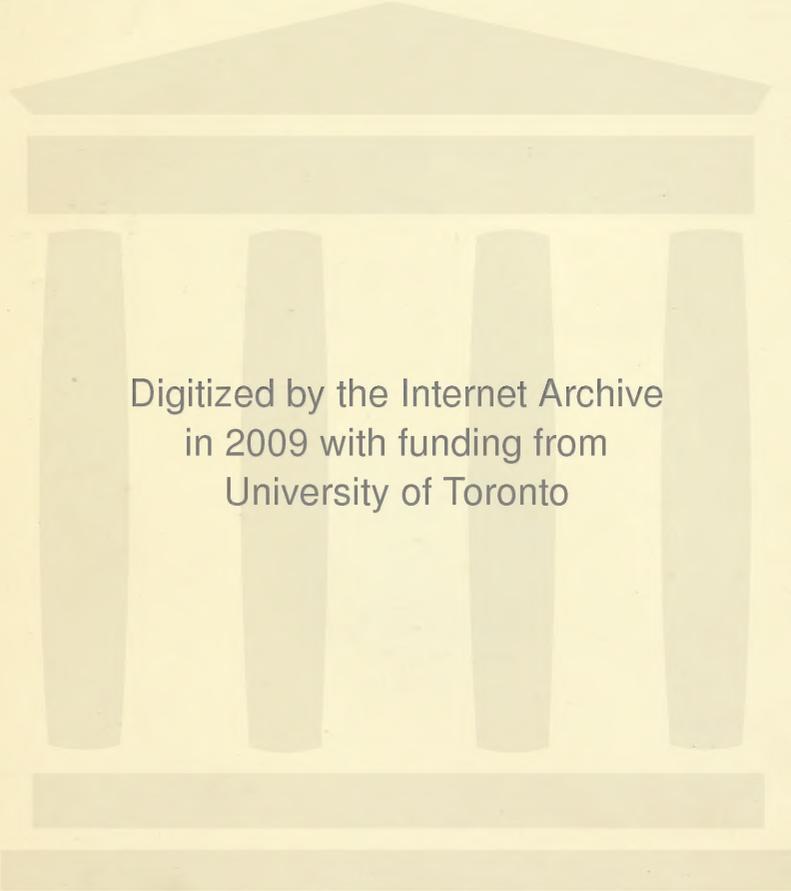


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CONTRIBUTIONS TO EMBRYOLOGY

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CONTRIBUTIONS TO EMBRYOLOGY, No. 10.

THE HUMAN MAGMA RÉTICULÉ IN NORMAL AND IN
PATHOLOGICAL DEVELOPMENT.

BY FRANKLIN P. MALL.

With three plates.

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THE HUMAN MAGMA RÉTICULÉ IN NORMAL AND IN PATHOLOGICAL DEVELOPMENT.

BY FRANKLIN P. MALL.

INTRODUCTION.

Students of embryology are familiar with the jelly-like substance found in the human exocoelom, which varies much in appearance in different specimens. Sometimes this substance is gelatinous, with delicate fibers; at other times it is mixed with granules; and, in extreme cases, it forms quite a solid body. I think it was Giacomini who pointed out definitely that the morphological appearance of the magma determines, with considerable certainty, whether or not the contained embryo is normal or pathological. We are indebted to him for about a dozen papers on pathological embryology, a summary of which he published in Merkel-Bonnet's *Ergebnisse*. In this summary the following statement is made:

"In the early stages of development we can determine by the extent of the exocoelom and its contained magma whether or not the embryo under consideration is normal. A large coelom, containing a rich magma, with its meshes sufficiently filled with a flaky precipitate to mask the embryo, is a certain sign of pathological development."

It is well known that the magma is least conspicuous in fresh specimens and becomes more pronounced after being hardened in alcohol or other preservative fluids. In recent years it is found that magma shows to the greatest advantage in specimens hardened in formalin; the fibrils are somewhat tougher, but the magma has usually the same appearance as in the fresh state. However, the experience of embryologists has been that the magma is more pronounced in pathological specimens, and for this reason it has been suspected that it does not exist in normal development. In fact, the illustration of the magma given by Velpeau in his monograph—in which he first uses the term "magma réticulé"—is undoubtedly of a pathological specimen. A glance at the other plate which accompanies this handsome monograph shows clearly that most of the specimens he describes are decidedly pathological. During the 80 years which have elapsed since his time, embryologists, through comparative study, have been able to separate normal from pathological embryos with considerable precision; and in the abortion material, as collected in various laboratories, far over one-half of the specimens of the first 2 months of pregnancy are pathological, and in them we usually find a highly differentiated magma. However, if normal specimens are studied with care, we find that they, too, contain some magma; therefore, magma must be viewed as a normal constituent of the human ovum.

It has been shown by Keibel that there is marked magma within the exocoelom of monkey embryos. In specimens containing embryos 1.3 mm. and 5 mm. in length, he describes it as a flaky, reticular mass outside the amnion, and speaks of it

as coagulum composed of reticular magma which had to be removed before the embryo could be seen. Undoubtedly he was dealing with normal specimens, thus showing quite conclusively that a delicate magma must be viewed as a normal constituent of the exocoelom. According to Keibel's figure 7, the magma appears to be denser in monkeys than is usually the case in normal human specimens. However, a very dense magma in human specimens invariably indicates, as was first demonstrated by Giacomini, that the ovum is pathological.

The best account of magma réticulé is given by Retzius, who brought the subject up to 1890, and left it with the conclusion that magma réticulé is a normal constituent of the human ovum. The statements of the earlier embryologists, from the time of Haller, are mainly of historical interest: but these investigators were at times inclined to view magma as a "middle embryonic layer" of the ovum, and, again, they believed it to represent the allantois of lower animals. Retzius re-investigated the subject, taking into consideration normal as well as pathological embryos, and his conclusion is that magma is present in both kinds. His own words are as follows:

"Bei der Öffnung des Chorionsackes der Eier des ersten und zweiten Monats sah ich, wie in der Einleitung erwähnt wurde, und dies oft, am besten nach kurzer Erhärtung in Ueberosmiumsäure (von $\frac{1}{4}$ Proc.) oder in Müllerscher Lösung (gewöhnlich nach doppelter Verdünnung), in dem schleimigen Inhalt, welcher zwischen dem Chorion und dem Amnion, also im subchorionischen Raume vorhanden war, dünnere oder dickere Fäden und Stränge, die mehr oder weniger dicht von der äusseren Fläche des Amnion zur inneren Fläche des Chorion hinüberliefen, um sich dort mit ihren Enden an den beiden Häuten zu befestigen, indem sie sich oft an ihnen verbreiterten und in ihre bekleidende Schicht übergingen. Diese Fäden und Stränge, welche im frischen Präparate kaum sichtbar waren, traten nach der Behandlung mit den erwähnten Flüssigkeiten deutlich hervor. In der Fig. 15 der Taf. XVIII habe ich ein solches Ei abgebildet. Das stark zottige Chorion (*ch*) ist geöffnet, und man sieht im subchorionischen Raume den Amnionsack (*a*) an weisslichen Strängen (*m*) aufgehängt liegen.

"Die Stränge, welche im unerhärteten Zustande eine schleimigfaserige Consistenz haben, sich aber ohne Schwierigkeit Stückweise ausschneiden lassen und dann in Mikroskope eine deutlich faserige Structur darbieten, zeigen nach der Erhärtung einen ausgeprägt fibrillären Habitus. In einer homogenen, structurlosen Grundsubstanz treten Züge echter bindegewebiger Fibrillen hervor, welche oft eine Haupttrichtung einschlagen, also ziemlich parallel verlaufen. Jedoch kommen auch viele sich kreuzende Fasern vor. Hier und da bemerkt man dickere Bündel verschiedenen Calibers welche aus dicht gedrängten Fibrillen bestehen. Es sind also fibrillär-bindegewebige Balken, welche durch eine homogene, zahlreiche einzelne Fibrillen enthaltende Intercellularsubstanz ziehen. Zwischen den Balken und Fibrillenzügen sieht man recht zahlreiche Zellen, welche theils und am meisten rundlich oder oval, theils auch spindelförmig sind und in ihrem oft reichlichen Protoplasma grössere glänzende Körner enthalten. Diese Zellen liegen in der Grundsubstanz ohne besondere anordnung zerstreut, bilden also keine Scheiden o. d. um die Fibrillenbündel.

"Man hat es hier offenbar mit einem unreifen Bindegewebe zu thun, einem embryonalen mucösen Bindegewebe, welches indessen in der Entwicklung zum fibrillären Bindegewebe schon weit vorgeschritten ist."

THE MAGMA IN NORMAL DEVELOPMENT.

We have now in the literature a detailed description of a number of young human ova, and, according to their clinical histories, some of them, at least, must be normal. The classic specimen is that described by Peters, which came from a woman who had committed suicide. The specimen was hardened *in situ* in an approved manner, and was worked up and described under the best possible conditions. In it the coelom is filled with a gelatinous substance, through which radiate

delicate bands of fibrils, among which appear scattered nuclei. Near the embryo there is a small space, the interpretation of which was very difficult at the time the specimen was described.

Since Peters studied this specimen, the sections have been carefully reworked and discussed in a critical way by Grosser, who gives a new interpretation in two figures and states that the cavity of the ovum contains reticular magma which is partly made up of heavier strands of tissue accompanied by nuclei. In the neighborhood of the embryo there are two large spaces, lined with cells, which appear to be the primitive body-cavities. In his work on the comparative development of the embryonic membranes, Grosser describes this space in great detail and also gives us two new illustrations of the embryo in his plates 3 and 4. According to this authority these two body-cavities communicate by means of a slit-like canal just behind the umbilical vesicle (Grosser's figure 31, plate 4). This interpretation of the Peters specimen shows that the cavity of the ovum is first filled with a free mass of reticular magma, after which the coelom begins to form near the body of the embryo. As this cavity expands subsequently, it probably first destroys the more delicate strands of magma, leaving the heavier ones; thus in a short time the cavity of the ovum is lined by the endothelium of the coelom, which also must cover the stronger bands of magma radiating as trabeculae throughout this cavity (Grosser, pp. 78 and 79).

Keibel explains the formation of the human coelom as follows:

"It is, however, not quite clear how the cavity traversed by scattered strands of mesoblast and lying between the yolk-sac and the chorion in the Peters ovum is to be interpreted. It may be supposed to represent the extraembryonic coelom; but it may also be imagined that it has arisen from an extensive loosening up of the tissue, and not by a splitting of the mesoderm, and that the triangular space between the caudal extremity of the embryo, which is lined with flat cells having an epithelial arrangement, is the first primordium of the coelom."

A condition similar to that found in the Peters specimen has been observed by Lewis in the Herzog specimen, which is of about the same stage of development. Lewis says (see his paper, p. 300) that there are occasional clefts in the mesoderm of the chorion of the Herzog embryo, but that they are of doubtful significance. His reconstruction shows a strand of mesoderm, more pronounced than in the Peters ovum, extending from the yolk-sac to the chorion and circumscribing a space on the ventral side of the embryo.

Eternod has written several papers in which he describes the formation of the exocoelom and the fate of the magma réticulé. He says that it first fills the entire space between the primordium of the embryo and the chorionic wall. Later, larger spaces appear within the substance of the magma, leaving denser strands of magma fibrils to support the embryo within the gradually expanding chorion. In general this coincides with the opinions just cited.

The relation of the exocoelom to the magma is strikingly shown by Waterston in a section of a small embryo *in situ*. The space between the embryo and the chorion is filled with a dense mass of fibrils, into which the exocoelom is burrowing. Waterston's figure 1 shows the relation of this cavity to the magma, and only near the embryo is the exocoelom lined with a layer of cells. When this figure is compared

with Grosser's figure of the Peters ovum, it becomes clear that the two spaces in the latter are in reality the beginning of the exocoelom.

The studies referred to above indicate that the space near the embryo is the primitive exocoelom and that the remainder of the so-called cavity of the chorion is simply the young normal ovum filled with delicate fibrils which communicate freely with the fibrils of the chorionic membrane. We have in our collection a young normal specimen, No. 763, containing an embryo anlage 0.2 mm. in length, which in general confirms the observations in the Peters ovum. A list of the normal specimens in our collection discussed in this paper is given in table 1.

TABLE 1.—List of normal embryos.

Cat. No.	Length of embryo	Dimensions of chorion.	Menstrual age in days.	Condition of magma.	Cat. No.	Length of embryo.	Dimensions of chorion.	Menstrual age in days.	Condition of magma.
763	0.2	4 × 2.2	60	Some reticular.	588	4	19 × 15 × 8	49	Strands of magma.
591	2	16 × 14 × 12	14 (?)	Do.	136	4	14 × 11 × 6	56	Reticular excessive.
779	2.75	16 × 14 × 12	40	None.	836	4	22 × 18 × 11	36 (?)	Delicate reticular.
164	3.5	17 × 17 × 10	...	Few strands	148	4.3	17 × 14 × 10	31	Small amount of magma around cord.
463	3.9	17 × 12 × 7	48	Much reticular.					
486	4	22 × 22 × 22	41	Do.	576	17	30 × 30 × 25	...	Small amount of magma.
470	4	20 × 13	34	Very few fibrils.					

Specimen No. 763 was removed from a woman who was the mother of 6 children, the oldest being 10 years old. She had had one miscarriage. During the year before the operation she suffered much from headache and backache, but otherwise her health appeared to be normal. When she was admitted to the hospital she complained of abdominal enlargement and there was some urinary disturbance. At the operation for rupture of the perineum the uterus was scraped out; subsequently the ovum was found in one of these scrapings. The fragments both of the mucous membrane and ovum appear to be normal.

Unfortunately we have only a few of the sections of this valuable specimen, but these show that we are undoubtedly dealing with a normal ovum of the same stage of development as that described by Peters. The chorionic cavity is partly filled with mother's blood, but there are some strands of reticular magma, with nuclei and protoplasm radiating through the blood. The specimen has been stained in hematoxylin and eosin, which is not especially favorable for defining magma fibrils.

The specimen described by Herzog is also undoubtedly normal, as it was obtained from a woman who was killed by a stab-wound through the heart. The large colored plate published by Herzog shows the specimen to be quite identical with that of Peters. It shows free cells in the coelom, which contains no other foreign substance, but a photograph (figure 24, published by Herzog) shows that the coelom is filled by a very pronounced substance, reminding one very much of reticular magma. The same is true of a specimen recently described by Johnstone. A colored photograph which he published shows quite distinctly a pronounced magma throughout the coelom. (See, for instance, his figure 3.) This establishes definitely the presence of reticular magma in ova the size of the specimen of Peters. We have, however, the valuable specimen of Bryce and Teacher, which also shows the condi-

tion of the magma in an earlier stage. In this specimen the chorionic cavity is filled with a dense mass of fibrils, throughout which are scattered numerous nuclei, as shown in their plates 3 and 4. The specimen was not perfectly hardened and there is a small cleft between the chorionic wall and the mass of magma. As yet there is no exocoelom, showing that it is younger than the Peters specimen.

More advanced stages of the condition of the magma are represented in the specimens described by Jung and by Strahl and Beneke. In the Jung specimen the cavity of the ovum is filled with a very pronounced magma, running together in stronger bands, as in our own normal specimen, No. 836, to be described later. The larger cavity Jung marks "exocoelom," but it is not clear that this is lined with endothelium. From his large illustration one gains the impression that the specimen is somewhat pathological, for it is of the same type as numerous specimens in our collection with embryos that are usually found to be pathological. Taking the illustrations given in Jung's plates 1 and 2, the specimen again appears to be pathological, and I should be inclined to pronounce it such did not his plate 6, figure 17, show this same section on an enlarged scale, which gives a very sharp outline of different embryo structures and scattered through them are numerous cells undergoing division. It would be impossible, with our present knowledge, to accept such sections as coming from a pathological embryo. The specimen described by Strahl and Beneke is of about the same stage as the Jung specimen, although the magma does not seem to be so well pronounced. It is unequal in mass and has scattered through it delicate strands, as shown in their figure 63. In fact, the above-described specimen underlies also the diagram on the form of the coelom given by Strahl and Beneke on page 18 of their monograph.

Magma of uniform consistency, as seen in the Bryce and Teacher specimen, soon arranges itself in bands, which gradually become more and more pronounced in older specimens. Between these bands are spaces filled with fluid, and those spaces near the embryo become lined with endothelium to form the exocoelom. There are other spaces between the exocoelom and the chorionic wall. The sharper bands of magma fibrils—well shown in our embryo No. 836 (plate 1, figs. 3 and 4)—apparently support the embryo and the wall of the exocoelom within the chorion.

We have in our collection an excellent embryo, No. 391, which is a little larger than that described by Strahl and Beneke. This specimen came to us in formalin and was opened with great care. It was found that the embryo and appendages were suspended by means of numerous delicate fibrils which radiated from them to the chorionic wall. As the sections were stained with cochineal, the fibrils do not show in them, so that this description is based entirely upon the appearance of the uncut specimen. In general the specimen appears to be normal.

Our specimen No. 779, somewhat older than the one just mentioned, apparently contains no magma. It also was hardened in formalin. The ovum is entirely covered with villi, which branch twice, are of uniform size, and appear to be normal. In the main chorionic wall there is a pronounced fold. The specimen was bent along the line of the fold, but the chorion was gradually dissected away with the aid of direct sunlight. The chorion is entirely lined by a smooth membrane, and contains a cavity which is filled with a clear fluid and which apparently contains

no magma. Within there is a clear, worm-like body, which is bent upon itself, with another body arising from the middle of the bend. Apparently this is a flexed embryo with the umbilical vesicle attached to it. The body is of uniform diameter, measuring less than a millimeter. We are probably dealing here with a normal embryo. In opening this specimen great care was taken not to touch the embryo, so as to avoid injuring it. The embryo was taken out and cut into serial sections. It contains 14 somites and is without limb-buds. The sections give the impression that the embryo is pathological. There are no data in the history of the case which bear upon this point; therefore, for the present we may view it as a normal specimen without magma—or, if the embryo is taken into consideration, as a pathological specimen with dissolution of the magma. Usually in pathological specimens the magma is greatly increased in quantity.

No. 164 is a somewhat older specimen. It came to us from an autopsy, with the entire uterus, and the sections of it indicate that the embryo is undoubtedly normal. The only record of the magma which we now have is given by several photographs which were taken at the time we received the specimen. These show a few strands of reticular magma, without any granular magma, radiating from the embryo. The photographs were taken while the specimen was in formalin.

The next specimen, No. 463, is somewhat more advanced in development and contains a flexed embryo, 3.9 mm. in length. The ovum is covered completely on one side, and partly on the other, with villi 1.75 to 2.75 mm. long. On the partly covered side the villi leave relatively bare one area, centrally situated, measuring 8 by 4.5 mm. Over it the villi occur only here and there, about 2 mm. apart, and are branched and apparently normal. On opening the ovum the reticular magma is found to fill the exocoelom. By carefully exploring with fine tweezers, an apparently normal embryo is seen with a yolk-sac measuring 3.5 by 4 mm. The embryo has anterior limb-buds and at least three gill-slits which are visible externally. No note was taken at the time regarding the condition of the magma, but sections of the entire chorion show that there is a very decided reticular magma between the embryo and the chorionic wall. There is no granular magma. The magma is composed mostly of fibrils, of much the same appearance as those of mesenchyme. Between the network of magma fibrils are denser strands accompanied by cells. In the fresh state undoubtedly the denser strands would appear as fibrils, while the rest would be transparent and jelly-like. The specimen came from a woman who was perfectly healthy and had given birth to 2 children during the last 4 years. This was her first miscarriage, and there was no indication of uterine disease.

Specimen No. 486, of the same degree of development as the one described above, is in a perfect state of preservation, but there is no history which would indicate whether or not the specimen is normal. However, the chorion is covered with villi about 3 mm. long, with a bare spot on one side about 4 mm. in diameter. The sections of the embryo do not show any attached fibrils of magma, but the chorionic wall, after hardening in alcohol, shows a decided layer of magma attached to it.

No. 470 is an interesting specimen, as it was found floating in a mass of blood-clots, which were sent to the laboratory in formalin. The ovum is covered with

normal villi and contains a well-formed embryo within the amnion. It is apparently normal in every respect. No magma could be seen at the time, but drawings of the embryo subsequently made show delicate strands of fibrils forming a fuzzy layer around the umbilical cord and extending over the umbilical vesicle: undoubtedly these are magma fibrils. This seems to be the normal condition for this stage and is verified in specimen No. 836, to be described later. Sections through the mass and the chorion, stained with carmine, show the magma as a granular mass: only at points is there any indication of fibrils. However, this mass resolves itself into the most definite fibrils when colored with Van Gieson stain, in Mallory's stain, in hematoxylin, aurantia and orange G., or in iron hematoxylin. With Van Gieson stain the fibrils take on fuchsian color about as intensely as do the fibrils of the chorionic wall, with which they are continuous. The contrast obtained with Mallory's stain is quite marked, as the endoplasm of the mesenchyme of the chorionic wall stains slightly blue, while the exoplasm and the fibrils of the magma réticulé remain unstained. This difference is not shown in sections stained in iron hematoxylin, as all fibrils are colored intensely black. However, it does not come out with the Oppels-Biondi method or with hematoxylin and eosin or aurantia. As the fibrils of the magma are continuous with those of the exoplasm of the chorionic wall, which do not stain in Mallory's connective-tissue mixture, they can not be considered as white fibers, and from their failure to stain in Weigert's elastic-tissue mixture they are not elastic. As will be shown subsequently, they give the reactions of embryonic connective-tissue syncytium: and this is Retzius's opinion regarding their character. In specimen No. 486 the fibrils of the magma are not accompanied by any nuclei; so they must be viewed as belonging to the cells of the chorionic wall, from which they extend to bind the chorion with the primordium of the embryo.

Specimen No. 588 came from a woman who had 2 children living, aged 14 and 20 years respectively. Since the last birth she had aborted 11 times, and in the opinion of her physician all the abortions were due to mechanical means. This indicates that the specimen is normal. A figure of this embryo with strands of magma radiating from the umbilical cord and vesicle is shown in plate 3, figure 2.

Specimen No. 136 is of about the same stage of development as No. 588, although the chorion is covered with poorly defined villi. For an embryo of this stage it is unusually small, and I have therefore listed it with the pathological specimens in my paper on monsters. A photograph of the ovum after it had been cut open shows that the chorion is completely filled with reticular magma, so that the embryo is practically obscured. A block of the whole ovum encircling the embryo was cut in serial sections. These show that there are strands of tissue accompanied by cells which form partitions in the exocoelom. The quantity of the magma appears to be somewhat excessive for a normal ovum of this stage of development.

No. 836, a perfect specimen containing an embryo 4 mm. in length, settles definitely the condition of the magma at this stage of development (plate 1, figures 3 and 4). In this ovum the exocoelom, measuring 9 by 4 mm., contains a delicate spiderweb-like reticular magma, several of the strands being considerably larger than the others. Most of this magma occurs between the yolk-sac and the amnion

and the adjacent chorionic wall where the fibrils are unusually abundant. This specimen was obtained from a hysterectomy upon a woman, 25 years old, for a fibrous tumor of the uterus. She had been married 4 years, this being her first pregnancy. There were no special symptoms bearing upon the case, excepting the discomfort which accompanied the tumor of the uterus. Her last menstrual period had been delayed, and as it had been more profuse than usual she believed that she had had a miscarriage; otherwise, everything appeared normal. This was confirmed by a careful examination of the specimen, which showed it to be normal in every respect. The uterus was opened by the surgeon at the time of the operation, but fortunately the site of the ovum was not injured. The specimen was sent to the laboratory immediately, where it was fixed by Dr. Evans, who made the following record:

"The specimen consists of a myomatous uterus which has been opened (apparently in a midline anterior incision) so as to disclose an abundant deciduous endometrium thrown into large folds. At the upper posterior surface of the uterus an oval mass, about 25 by 20 by 20 mm., projects. It is a sac and is covered with a rather smooth membrane (decidua reflexa), beneath which tortuous vessels are apparent. On one side the sac (the implanted chorion) is adherent to the uterine mucosa (decidua vera). With a sharp scalpel the entire mass was dissected away from the uterus and brought under a binocular microscope in warm salt solution. The middle portion of the free surface was opened carefully, beautiful villi being found, and then the delicate wall of the chorion was divided. Within, a transparent young embryo and its umbilical vesicle were seen, the embryo appearing to be about 5 mm. in length. Through this opening in the chorion, warm (40° C.) saturated aqueous solution of HgCl₂, containing 5 per cent glacial acetic acid, was gently introduced and the entire mass placed in 500 c.c. of this fixation fluid. The main body of the uterus was dissected from the myomatous nodule and fixed in 10 per cent formalin, the site of the implanted ovum being marked by a short wooden rod."

The fixed and hardened specimen had undergone a readily appreciable shrinkage from the condition seen in warm salt solution. All of the tissues were beautifully preserved. The implanted ovum, covered with the decidua capsularis, measures approximately 22 by 18 by 11 mm. The adjacent decidua parietalis is thrown into large folds, which are themselves marked by numerous tiny elongated crack-like depressions, as well as by more circular pit-like apertures. The relatively smooth but irregular surface of the decidua capsularis is marked here and there by very conspicuous, small, oval pits, which may attain 0.5 mm. in diameter. The four flaps of this coat at its highest point, where it was opened directly over the middle of the ovum, are rather smooth on their inner surface and stand apart from the subjacent chorionic villi (intervillous space) to which they were originally adherent. The villi are about 2.5 mm. in length and possess one or two large branches and many "stub-like" tiny bulbous ones on the main stem. The villi are uniformly distributed in the small area exposed. With a slender scalpel the ovum was carefully divided under the dissecting microscope, the embryo and yolk-sac being visible. The yolk-sac appears to be almost 2 cm. in diameter and the embryo is surrounded by its amnion, its head (visible from above) being about 3 cm. in length and showing the fourth ventricle covered by a transparent ependyma. Two gill-arches are visible. The yolk-sac surface presents an exquisite picture of irregular, clear vascular channels and a uniform pattern of small, opaque, white blood-islands. The preservation seems perfect.

After the embryo had been carefully removed, the ovum was cut into blocks which included its implantation. A block 1 mm. thick, which included the largest circumference of the embryo, was embedded in celloidin, the sections being stained in various ways. A photograph of this block is represented in plate 1, figure 4, which shows strikingly the extent of the magma. Sections which have been stained in hematoxylin and aurantia show the magma much as it appears in the other embryos that have just been considered. There is a denser magma just under the chorionic wall, and heavy strands radiate in every direction, with a fine network resembling spider-web, among the main strands. A number of loose nuclei accompany these strands, but they do not have the appearance of the nuclei of the main wall of the chorion. They are mostly round and are of unequal thickness, simulating very much the blood-cells. Occasionally there is a large nucleus. Sections which have been treated by the Weigert fibrin method do not show these fibrils. This confirms a previous experience which I have published elsewhere in my paper on monsters, namely, that magma fibrils do not give the reaction of fibrin, nor do these fibrils stain well in Van Gieson's mixture; however, they take on color similar to the mesenchyme of the chorion.

At points it appears as though these fibrils arise directly from the chorionic wall. They stain intensely blue by the Mallory method, and in sections treated in this way the nuclei of the mesenchyme of the villi look much like the accompanying nuclei of the magma fibrils. On one side of the ovum a denser mass of the magma is directly continuous with the mesenchyme of the chorionic wall. However, just in this region the magma contains no nuclei. It, therefore, appears that the magma fibrils must be associated, at least partly, with the nuclei of the chorionic wall. Exceedingly good histological pictures were obtained from sections stained by Heidenhain's method, which show all the transition stages between magma containing no nuclei and magma very rich in nuclei. It would seem that there is quite a free wandering of the nuclei along the magma fibrils, and whenever they come in contact with the chorionic wall the fibrils enter it, showing direct continuity. The most instructive specimens are obtained by the Weigert elastic-tissue stain, which gives a slight blue-black tinge to the mesenchyme fibrils of the chorionic wall, as well as to those of the centers of some of the villi. The magma itself takes on a very light stain, but where it is in contact with the chorionic wall it grades over into its blue network. It appears, then, that the centers of the villi, which represent their older portion, stain somewhat with elastic-tissue stain; and, if we view the chorionic wall as the more differentiated portion of the chorion, we must conclude that the older mesenchyme fibrils behave more like elastic-tissue fibrils than do the younger. At any rate, the magma fibrils do not take on elastic-tissue stain.

From all that has been said it is clear that the mesenchyme of the chorionic wall and the magma fibrils are continuous and, as I have pointed out elsewhere, they together form a common syncytium. I have already demonstrated that very young connective tissue arises directly from the mesenchyme, the earlier stages of which I have designated as the connective-tissue syncytium. Towards digestive reagents the connective-tissue syncytium gives somewhat the reaction of yellow elastic tissue, just as do the mesenchyme and the magma of No. 836 when treated with Weigert's

elastic-tissue stain. I have also shown that the younger the connective-tissue syncytium is, the more difficult it is to digest it in pepsin. Frozen sections shrink but little when treated with acetic acid, while white fibers become transparent. The syncytium itself is somewhat elastic, as shown by pressure upon the cover-glass over a frozen section. If treated for 24 hours with pepsin, the fibrils disintegrate. They are therefore much more resistant to the action of pepsin than are white fibrils.

The action of pancreatin is, in a measure, the opposite of that of pepsin. When the main mass of syncytium is formed by exoplasm, it digests readily in pancreatin. The more the syncytium is developed, the more resistant it is towards pancreatin. Very young syncytium fibrils, therefore, react towards pancreatin and pepsin much like elastic fibers and this is confirmed in a measure, by tinctorial methods, when applied to sections of the chorion and magma, in specimen No. 836.

I have discussed the denser strands of tissue within the main mass of the magma. In the fresh state it appears that these are distinct fibrils, as shown in plate 3, figure 2. They are, also, observed in plate 1, figure 3. It is not quite so clear that there are fibrils in the magma as shown on plate 1, figure 4. In fact, it appears as though we have compartments separated by membranes, and that at the junction of several of these membranes the fibrils become denser, and therefore often appear as distinct fibers. It would be more appropriate than to state that the exocoelom is broken up into compartments the walls of which are composed of membranes, and that where several of the membranes come together the increased amount of tissue gives the point of juncture the appearance of fibers to the naked eye and under the enlarging lens.

I have taken great pains to follow the cells which mark the stronger bands of magma, and it is difficult to arrive at any conclusion, for, in a measure, they seem to be related to the endothelial lining of the exocoelom. In the Peters ovum the spaces near the embryo are lined by a distinct layer of cells, but otherwise there is no indication of endothelial lining in any other portion of the chorionic cavity, nor is there any indication of such a lining in the figures given by Herzog, Johnstone, Jung, or Strahl and Beneke. It would seem that what corresponds to the exocoelom of the chorion in the later stages is represented by a diffuse mass in the specimen of Bryce and Teacher where the nuclei are scattered through it. The mode of the destruction of the mesenchyme is well indicated in figures on page 18 of a monograph by Strahl and Beneke. These irregular cells are first of all attached to the heavier strands of magma, and they must, therefore, correspond to the endothelial lining of the exocoelom. For the present, however, it appears as if the exocoelom of the human chorion is lined only in part by a layer of endothelium; these cells also accompany the magma fibers and line the inner side of the chorion near the embryo.

As the amnion expands, it naturally pushes these strands of magma up against the chorion, and in a short time we can recognize only a few fibrils in the exocoelom which encircle the umbilical cord. These are well seen in specimen No. 148, and their remnants are shown in No. 576, of which I give an illustration on plate 2, figure 2. No. 148 is undoubtedly normal, for it was obtained by mechanical means, and No. 576 is also a normal specimen obtained from a tubal pregnancy.

The conclusion regarding the condition of the magma of normal development is that the cavity of the ovum is filled with delicate fibrils which are interspersed with numerous nuclei and which form one continuous network, extending from the embryo to the chorionic wall, and blending with its connective-tissue network. It forms one continuous syncytium, and as the ovum grows the magma réticulé differentiates somewhat. Stronger bands of membranes soon form, breaking the cavity of the chorion into compartments. This process continues until the amnion begins to expand, and then these fibrils are pushed up against the chorionic wall. The exocoelom begins as two larger spaces near the embryo, and in this portion of the ovum its cavity is lined with a layer of endothelium. It is quite certain that this sharply defined cavity does not extend to include the whole cavity of the ovum, but the cells lining it arise in common with those which accompany the magma fibrils. The exact extent and the fate of the two small spaces near the embryo in the Peters specimen is still undetermined, but Waterston's specimen indicates that they do not extend to fill the entire chorionic cavity. The examination of numerous specimens, however, indicates very definitely that the exocoelom of the ovum at 2 months does not contain a complete endothelial lining.

THE MAGMA IN PATHOLOGICAL OVA.

Since the publications by Giacomini it has become well known that an increased quantity of magma within the coelom indicates with certainty that the embryo is pathological. When the magma is pictured or described, it is quite easy to determine whether or not the embryos and ova published in the literature are normal or pathological. This is demonstrated in the plates accompanying Velpeau's work. His was able to separate most of the normal from the pathological embryos, but he relied mainly upon the external form of the specimens, which he compared with other mammalian embryos. Unless an embryo appeared much like those of other mammals and was not transparent and sharply defined, he decided that it was not normal but pathological. The work of Hochstetter, who limited his study to embryos obtained through hysterectomy, has been used to advantage by Keibel and Else in the preparation of their *Normentafel*, so that now we have adequate tables and plates which enable us to recognize with considerable certainty whether or not an embryo is normal, without paying much attention to the magma or the chorion. However, embryologists are well aware that they can predict whether a specimen is normal or pathological by the quantity of the magma which masks the embryo when the ovum is opened.

By the contents of the exocoelom it is quite easy to classify pathological ova into three chief groups. In the first group, which includes most pathological specimens, the magma is changed into an organized mass of reticular fibrils, intermingled more or less with granular substance.

To the second group belong specimens in which the exocoelom is large and contains only a fluid mass—that is, a liquid substance which does not coagulate in either formalin or alcohol. I have pictured a number of specimens of this sort in my paper on monsters. Specimen No. 512, of which I give an illustration on plate 2, figure 1, belongs to this group. The embryo is atrophic, and it is questionable

whether or not it is encircled by the amnion. In these specimens the cœlom is usually enlarged and sometimes it is greatly distended. Often there is a small granular precipitate in older specimens, but this is not of sufficient quantity or density to form a continuous mass. The histories of these specimens show that they are considerably older than their sizes indicate, and I am inclined to view them as having once had a dense mass of magma within the cœlom, which subsequently underwent dissolution, leaving a more or less flaky deposit that finally disappeared altogether.

In the third group, the cœlom is greatly distended, the amnion is usually absent, and the ovum is filled with a gelatinous substance. This is well illustrated by specimen No. 604, plate 1, figures 1 and 2.

TABLE 2.—List of specimens containing pathological magma.

Cat. No.	Length of embryo.	Dimensions of chorion.	Menstrual age.	Contents of ovum.	Cat. No.	Length of embryo.	Dimensions of chorion.	Menstrual age.	Contents of ovum.
	<i>mm.</i>	<i>mm.</i>	<i>days.</i>			<i>mm.</i>	<i>mm.</i>	<i>days.</i>	
278		512	10	30×27×18	No magma.
660	40×55×30	67		636	10	28×28×22	56	
813	80×50×25	200	Hyaline magma.	605	[10.5]	45×40×25	
78	1	36×33×13	87	Fluid.	104	12	35×35×15	35	Granular-hyaline ; also no magma.
531	1.5	19×19×19	45	Flaky.					
250	2	10×9×8		211	12	
12	2.1	18×18×8	41		1117	14	Hyaline magma.
318	2.5	20×18×11	42		270	14	40×30×20	Granular; hyaline also.
513	3	70×30×25	75						
651g	3	35×30×30	Granular.	991	17	73	
244	4	25×15×15	Fluid.	604	17	70×50×50	Hyaline magma.
402	4.5	40×25×20	42		94	20	14	
122	5	20×16×6	65	Do.	1189	22	50×50×50	91	Granular; hyaline.
533	5	35×30×30	56		581a	25	50×42×40	
545	5	12×9×9	53		79	33	50×50×50	
21	5.5	12×9×5		230	57	75×60×50	Do.
560	7	49		261	90	120×70×70	Do.
135	9	105×65×65	Hyaline magma.					

I will now review several specimens illustrating these three varieties of pathological magma. The specimens considered are arranged in table 2. The list could easily be increased to several hundred, but as the specimens with catalogue numbers below 403 have been published in detail with illustrations in my monograph on monsters, I will allude only to some of them. The pathological specimens with numbers over 402 are being prepared for publication, so that a few selected specimens with higher numbers are illustrated. Pathological specimens from tubal pregnancy with numbers up to 1,000 will be found described in detail in my monograph on tubal pregnancy.

The first specimen which I shall consider (No. 278) consists of an entire ovum, measuring 6 by 4 mm. It was sent me by Dr. Stanton, of Albany, New York. The specimen might be viewed as normal, but it contains no embryo, and as it was obtained from a diseased uterus, it is probably pathological, the magma having undergone minor changes.

This ovum was found accidentally in curettings from a woman supposed to have chronic endometritis following pregnancy. There is nothing in the history from which the age of the specimen could be estimated. Part of the specimen had been

cut into sections before it was received at the laboratory, with the statement that no embryo had been found, it having fallen out. I found that the half sent contained a cœlom, 3 by 2.5 mm., filled with magma, in which there was a cavity about 1.5 by 1 mm. Sections showed that the cavity was natural and not sharply defined, with nothing to indicate that it had contained an embryo. On the contrary, it was found that the magma réticulé was composed of a loose network of mesoderm cells, which bound one side of the chorion with the other. These cells are directly continuous with those of the mesoderm and resemble them in every particular. At one point there is a small group of epithelial cells, which may represent what was originally the embryo. Otherwise, the chorion and its villi are normal in appearance, being encapsulated in decidua which has in it some uterine glands. All in all, this specimen reminds one very much of the Peters ovum. There are some leucocytes in the decidua, but no accumulation of them indicating inflammation of the uterus. Several figures, illustrating this specimen, may be found in my monograph on monsters.

Specimen No. 531 is in many respects similar to the one just described (No. 278). It came from a woman who had been pregnant 6 times, her periods having been 17 days overdue before this abortion. The ovum is spherical, 19 mm. in diameter, and is covered only by a mass of villi, which appear normal. The cœlom within contains many magma fibrils, the meshes of which are more or less filled with dense granules, as is shown in plate 1, figure 8. Within this mass there is a detached vesicle, 1.5 mm. in diameter, which no doubt represents the umbilical vesicle.

A specimen intermediate between the two just described is No. 250, of which several illustrations are published in my paper on monsters. The specimen came embedded in a mass of decidua and was obtained by scraping the uterus. When opened it was found filled with magma réticulé just beneath the chorion, in which could be seen a small embryo, and farther away towards the center of the cœlom was the umbilical vesicle. The whole ovum was cut into sections. The chorion and the villi are apparently normal in shape and structure, being also rich in blood-vessels, which are filled with embryo blood. The villi are bathed in mother's blood and covered with an active trophoblast. The decidua is somewhat infiltrated with leucocytes, but there are no abscesses. The front end of the amnion is absent, and its free edge and the embryo are embedded in reticular magma, indicating that the amnion was destroyed before the abortion took place. The general shape of the embryo and its degree of development are practically normal. The heart is well formed and, including the blood-vessels, is filled with blood. The alimentary canal, brain, spinal cord, otic and eye vesicles, myotomes, and branchial arches are much like those of embryo No. 12, to be described presently. The septum transversum is well marked and the thyroid gland is just beginning. The tissues of the embryo, however, and the cavity of the front end of the brain are filled with numerous small round cells with fragmented nuclei. All stages of fragmentation are seen, just as may be observed in the leucocytes in small abscesses. Most of the red blood-cells are within the blood-vessels, but those within the tissues appear perfectly normal. On account of the diminished number of mesoderm cells, which, in fact, diminish in proportion as the fragmented cells increase, the con-

clusion must be drawn that the fragmented cells arise from the mesoderm cells. The epidermis covers the whole embryo. The primary change in this specimen is no doubt in the mesoderm, for all the rest of the embryo appears normal. That the equilibrium was overthrown is indicated by the necrotic amnion and the great amount of reticular magma in the exocoelom. What is especially interesting in this specimen is the partial destruction of the amnion, which brings the embryo directly in contact with the pathological magma of the coelom.

Embryo No. 12, which has been just referred to, may also be discussed in this connection. It was questionable for a long time whether or not the embryo was normal, as the villi and contents of the coelom and embryo are beautifully preserved and show no pathological change. However, more careful consideration of the specimen shows that there are a few fibrinous masses between the villi, with every indication of uterine inflammation and infection. The extent of the reticular magma is more pronounced than usual, and it was necessary to dissect it away before the embryo could be isolated sufficiently so that it could be well seen. The head is no doubt atrophic, and I am fully convinced that this part of the embryo must have undergone pathological changes a short time before the abortion.

Specimen No. 318 is much like No. 250. The ovum, measuring 20 by 18 by 11 mm., is covered with villi which appear to be perfectly normal. Upon opening, it was found to be filled with stringy magma, on one side of which was embedded an embryo 2.5 mm. in length. The head is sharply outlined, but the embryo seems to continue directly with the umbilical vesicle, leaving an atrophic tail. Sections show that the amnion over the head has dissolved, leaving a picture very much like that shown in No. 250. We have here a small embryo with a very large coelom, the ovum being moderately filled with reticular magma and a small embryo only partly covered with the amnion. No. 543 is another embryo of the same type. The magma is a little denser than in No. 318. The chorionic villi are developed, but markedly pathological, as the photograph shows. The embryo within is 3 mm. long, lying quite free within the mass of magma. It is covered by a ragged amnion; that is, the amnion is partly destroyed.

An interesting specimen in this connection is No. 402, which is partly described in my paper on monsters, since the issue of which the embryo and chorion have been cut into serial sections. The villi of the ovum are not well developed, and they are distributed irregularly over the surface. The coelom is filled with reticular magma. The embryo is club-shaped, the head being much too far advanced for the body. The umbilical vesicle is normal in size; the heart is well outlined, and the extremities are just appearing. Sections show that the amnion is greatly distended. Sections of the chorion were stained with cochineal and Van Gieson, and show beautifully the fibrillated structure of the chorionic membrane. These fibers take on red stain, as do those of the reticular magma. The two are continuous, as shown by the illustration on plate 3, figure 3. In fact, this continuity is much more pronounced in pathological than in normal specimens.

Specimen No. 533 (plate 2, figure 3) shows a more advanced stage of an extensive development of reticular magma. The villi of the ovum appear to be normal and the reticular magma is very dense. Between the meshes there are a number of

opaque nodules about 0.5 mm. in diameter. With much difficulty the embryo was teased out, but it was practically impossible to clear it entirely of the magma fibrils. The embryo is long and slender, looking more like that of a dog than a human specimen, the head being unusually small and thin for a human embryo of 0.5 mm. long. The fibers are irregularly stuck together by small granules, and there is a gap in the center which represents the place in which the embryo was located. The illustration shows this condition beautifully. The specimen was sent me by Dr. Fewsmith, of Trenton, New Jersey, who obtained it from a woman whose menstrual period had been a month overdue.

An extremely interesting specimen is No. 545, well illustrated in figure 1, plate 3. The magma is not extensive, but it is pronounced. The embryo is atrophic, and the chorion is only partly covered with villi. The specimen was sent me by Dr. Rand, of New Haven, Connecticut. It was obtained from a woman who is the mother of one healthy child. The last menstrual period began on September 2. Bleeding began on October 22 and ended with the abortion on October 25. The ovum was found embedded in the clots of blood attached to the cervix of the uterus.

An extreme case of degeneration of the magma is shown in No. 660, also well illustrated in figures 4 and 5, plate 3. There is a tendency towards membrane formation, tough strands of fibrils, spaces, and clumps of granules. The chorionic wall is hemorrhagic and degenerated: within there is a collapsed amnion containing a cheesy granular mass.

I shall use two more specimens to illustrate the nature of granular mass in more advanced stages. The first is No. 605 and the second is No. 584c. No. 605 is a white transparent specimen, covered with a uniform layer of villi which branch two or three times. The entire specimen measures 45 by 40 by 25 mm.; a small patch of decidua adheres to the outside. The interior is partly filled with coarse strands of reticular magma, having numerous granules attached. On one side of the specimen the umbilical cord is seen, surrounded by a ragged amnion. The tip of the cord has a piece of intestine and stomach hanging from it. The larger masses of tissue which are intermingled with the reticular magma must be the remnants of the embryo, parts of which appear to be normal, and judging from the form and size of the arms and legs the embryo is about 10.5 mm. long. The second specimen is unusually interesting because it contains a normal embryo with hernia of the liver. The exocoelom is unusually large and is filled with a more extensive layer of reticular magma than should be found in an ovum containing a normal embryo of this size.

The remaining three specimens are given because they well illustrate various degrees of reticular magma within the ovum.

No. 560 (plate 1, figure 6) shows very pronounced reticular magma intermingled with much granular. Two stages of somewhat later development are given in Nos. 636 and 991. In the former (plate 1, figure 10) the magma is more pronounced than in normal development, and in the latter (plate 1, figure 7) it is in an extreme amount.

Finally, a unique specimen (No. 1189) throws some light upon the formation of the reticular magma. The ovum came to us within the uterus, having been removed by an operation. At first it seemed to be normal, but on opening it the

embryo was found encircled by a large mass of transparent, tough, stringy reticular magma, which was removed only with great difficulty. It behaved much like the vitreous humor of the eye. On account of its great quantity we at once suspected that the specimen was pathological, and after the embryo was removed it proved to be so. Although quite advanced in development, its head was found to be smaller than normal, the tissues of the face were dissociated, and the borders of the eye were not sharp but ragged. No doubt the specimen had continued to develop normally until shortly before the operation, and the magma increased in quantity and became tough and fibrous. It is an interesting specimen, showing changes in the magma late in development. Sections of the implanted ovum have not yet been made. The specimen is from a negress, 45 years of age, who had had 9 previous pregnancies. Her last menstrual period was 67 days before the operation. Pregnancy was suspected before the removal of the uterus, but a hysterectomy was performed because her periods had become very severe, lasting 8 days and causing faintness and weakness.

The two types of degeneration which the reticular magma undergoes have been considered above. The magma becomes granular and denser as it lessens and becomes liquid. The liquid again either coagulates or remains fluid when the specimen is fixed in formalin. The two fluid types may be related to the destruction of the amnion, but as yet I have been unable to reach a conclusion regarding this point.

The beginning of the formation of granular magma is shown in specimens No. 560 and 991 (plate 1, figures 6 and 7) as well as in Nos. 533 (plate 2, figure 3) and 660 (plate 3, figure 5). It appears to extend into the cavity of the amnion, and often forms great crusts, which surround the embryo, as shown in several specimens pictured in my monograph on monsters (*e. g.*, Nos. 79, 94, 104, 230, and 261). An extreme specimen of granular magma within the exocoelom is shown in specimen No. 651*g* (plate 1, figure 9).

It is extremely difficult to determine with certainty the structure of the granular magma, but in studying pathological ova (especially those obtained from tubal pregnancy) I have frequently observed that there are large masses of granular magma which take on hematoxylin stain. These granules are mixed with a slimy mass which also takes on hematoxylin stain. My attention was called to these granules because they have a characteristic circular stratification and contain within their centers small granules which also stain intensely. I am by no means certain whether all granular magma stains in this way with hematoxylin, and what I have just stated may apply only to a portion of the granular magma.

Specimen No. 531 (shown in figure 8, plate 1) has its coelom filled with a liquid mass in which there is a granular deposit that surrounds the embryo anlage. Such specimens are numerous and, without opening them, they may frequently be recognized by the transparency of the chorionic wall, which is covered with but few atrophic villi. A more advanced embryo, showing the same condition, is shown in specimen No. 512. In it the embryo is atrophic and macerated, without the presence of an amnion. The chorion is thin and is fully covered with delicate degenerated villi. Other specimens which come within this group are Nos. 21, 78, 122, and 244*a*. These are all illustrated in my monograph on monsters.

Sometimes the entire specimen is filled with a gelatinous mass, which becomes firmer when fixed in formalin and separates into a more solid mass, and into a liquid when preserved in formalin. This mass appears to lie within the amnion in most specimens, as in cases where it fills the whole ovum the amnion is missing. Specimen No. 604 (plate 1, figures 1 and 2) is quite typical, as is also No. 135. In both the embryos are atrophic and necrotic, and the jelly-like fluid fell out with ease when the ovum was cut open. The chorion is atrophic in both of them and is covered only with a few atrophic villi. Specimen No. 604 came to me without a history, and measures 70 by 50 by 50 mm. It is fully covered with fibrinous clots, between which there are few large villi, as the picture shows. The chorionic wall is 3 to 4 mm. in thickness, and its interior is entirely filled with jelly-like magma of uniform consistency. On one side of the specimen, lying free within the hyaline magma, is a straight embryo, 17 mm. in length, with atrophic head, arms, and legs. The same description applies equally well to No. 135. Specimens like these are quite numerous in our collection of human ova, but usually the jelly is lost when the specimen is opened. Figures illustrating embryos of this sort may be seen in my paper on monsters, under the description of embryos Nos. 79, 94, 230, 261, and 270.

No. 1117 (plate 1, figure 5) contains an embryo well packed in the jelly-like magma. The cavity of the ovum is small and its wall is very hemorrhagic. The specimen came from a woman, age 26 years, who was married at 15. She had two births at term and one previous abortion. She believed she became pregnant about 3 months before the operation, although she had not missed her regular periods.

Another specimen belonging to this category is No. 813. It consists of a fleshy mole, well filled with tough, jelly-like magma. All the villi are destroyed and its surface shows ulceration. Further study of this magma is necessary before it can be related to the granular magma which forms with the reticular magma in the exocoelom. I am inclined to believe that the hyaline substance which is so often found within the amnion of pathological specimens arises from the amniotic liquid, which has become richer in albumen, and therefore congeals into a jelly-like mass when preserved in formalin.

CONCLUSION.

The fibrils forming reticular magma are always in direct continuity with those of the mesenchyme of the chorionic wall. This can easily be demonstrated by means of Van Gieson stain, and reticular magma must therefore be viewed as embryonic connective tissue extending into the cavity of the ovum. The stronger strands are accompanied more or less by mesenchyme nuclei, showing that the magma itself must be viewed as independent connective tissue identical with the mesenchyme of the chorion. As the amnion extends these strands are pushed aside, their final remnants being seen in that portion of the exocoelom which encircles the umbilical cord.

In pathological specimens the reticular magma increases in quantity in the earlier stages of development, continuing for a number of months of pregnancy. Frequently the meshes between the reticular fibrils are filled with peculiar stratified granules which take on an extensive hematoxylin stain. Often the amnion is

destroyed early in development, in which case the magma may dissolve, but sometimes it increases greatly in quantity, forming a gelatinous mass. Frequently pathological ova are encountered in which the development of the embryo is retarded, and the amnion is often found filled with a flaky deposit that, as time goes on, increases greatly in quantity and finally forms large crusts which invest the embryo. In other cases there is marked hydramnios, and in certain instances, where the amnion is destroyed, the magma dissolves, leaving only the embryo floating in the fluid encircled by the chorionic wall. Specimens are also found in which the cavity of the amnion is greatly enlarged and is filled with a jelly-like substance, which in later stages may form crusts encircling the embryo. The true relation between the pathological changes of the contents of the exocoelom and of the cavity of the amnion remains to be determined.

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EXPLANATION OF PLATES.

PLATE 1.

- FIGS. 1, 2. Photographs of the two halves of ovum No. 604. Natural size. The cavity of the ovum is filled with a jelly-like substance in which a pathological embryo is embedded.
- FIG. 3. Section through a normal ovum No. 836, encapsulated in the decidua. $\times 31$. Drawn by Mr. Didusch. The embryo lies within the coelom, and bands of magma fibrils radiate from the amnion to the chorionic wall. The head of the embryo shines through the more transparent portion of the amnion.
- FIG. 4. Photograph of a block of the ovum, No. 836, *in situ* after the embryo had been removed. $\times 2\frac{1}{2}$. The supporting strands of magma are strikingly shown.
- FIG. 5. Pathological embryo No. 1117, embedded in hyaline magma. $\times 4$. From a tubal pregnancy following gonorrhoea (?).
- FIG. 6. Pathological ovum No. 560, containing a great quantity of reticular magma. $\times 2\frac{1}{2}$. The embryo is normal in form. From a case of retroversion of the uterus.
- FIG. 7. Pathological ovum No. 991, with the cavity completely filled with reticular magma. Natural size. The embryo is normal in form. From a negro woman. Sections of the embryo indicate that it is macerated.
- FIG. 8. Pathological ovum No. 531, containing a granular deposit around a nodular embryo. $\times 1\frac{1}{2}$.
- FIG. 9. Pathological ovum with a nodular embryo (651g). $\times 2$. The exocoelom is gorged with granular magma.
- FIG. 10. Specimen No. 636. $\times 2\frac{1}{2}$. The embryo and chorion are normal in form, but the reticular magma is markedly increased in quantity.

PLATE 2.

- FIG. 1. Pathological embryo No. 512, lying free within the ovum. $\times 6$. The villi are thin and scattered and the embryo is atrophic. There is no formed magma.
- FIG. 2. An ovum, No. 576, obtained from tubal pregnancy, showing a delicate layer of magma fibrils around the attachment of the umbilical cord to the chorion. $\times 3$.
- FIG. 3. Ovum No. 533, showing very extensive magma. $\times 6$.

PLATE 3.

- FIG. 1. Ovum No. 545. $\times 7$. There is a delicate network of fibrils below the amnion and the chorion.
- FIG. 2. Embryo No. 588. $\times 8$. Delicate strands are shown radiating from the umbilical cord and yolk-sac. This figure is given to show the appearance of magma in vesicle development. From a woman who has had numerous mechanical abortions performed upon herself. Uterus badly inflamed.
- FIG. 3. Section through the chorion and magma of No. 402. $\times 280$. The specimen was stained with Van Gieson stain and shows that the fibrils of the magma are continuous with those of the mesenchyme of the chorionic wall. It came from a case with subinvolution and symptoms of endometritis.
- FIG. 4. Outline of the ovum of No. 660. Natural size. The diagram indicates the part of the specimen shown enlarged in figure 5.
- FIG. 5. No. 660, showing very extensive changes in the magma. $\times 6$. The upper tip of the amnion is shown. The magma is fibrillar and granular, and at places the fibrils seem to form membranes. The chorionic wall is very hemorrhagic.

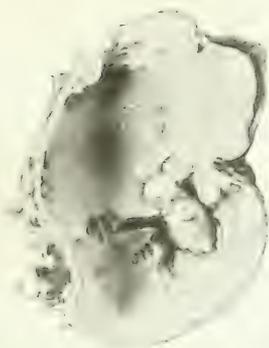


FIG. 1 (100)



FIG. 2 (100)



FIG. 3 (100)



FIG. 4 (100)



FIG. 5 (100)

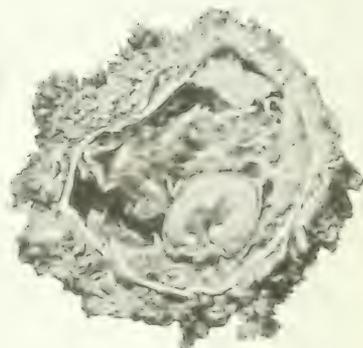


FIG. 6 (100)



FIG. 7 (100)



FIG. 8 (100)

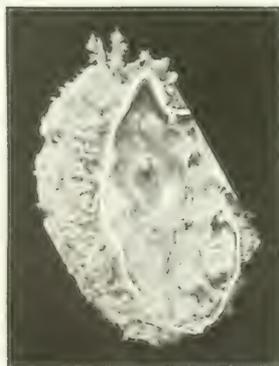


FIG. 9 (100)



FIG. 10 (100)



Fig. 1 (512)



Fig. 2 (520)



Didusch fec.

Fig. 3 (533)

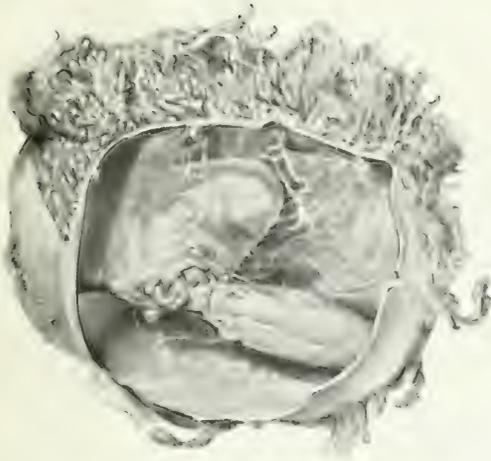


Fig. 1 (545)



Fig. 2 (558)



Fig. 3 (402)



Fig. 4 (600)



Collapsed amnion.

Fig. 5 (660)

Didusch fec.

CONTRIBUTIONS TO EMBRYOLOGY, No. 11.

THE STRUCTURE OF CHROMOPHILE CELLS OF THE NERVOUS
SYSTEM.

By E. V. COWDRY.

Anatomical Laboratory, Johns Hopkins University.

With one plate.

THE STRUCTURE OF CHROMOPHILE CELLS OF THE NERVOUS SYSTEM.¹

BY E. V. COWDRY.

INTRODUCTION.

It has long been known that certain peculiar nerve-cells, well characterized by their structural appearance, occur in the normal human brain, and indeed in the brains of all the vertebrates which have been examined. In fixed preparations they are slightly shrunken, they stain deeply with both acid and basic dyes, and their nuclei are obscure and hard to define. Flesch (1887, p. 196) called them "chromophile" cells. Nissl (1896, p. 1154) thought at first that they were artefacts of some sort, but Cajal (1909, p. 210) and others brought forward strong evidence against this view. Cajal (1909, p. 211) concluded that they were resting cells. On the other hand, in the light of Dolley's (1910, p. 333) work, they would seem to be in the initial stages of fatigue, as evidenced by the increase in the amount of Nissl substance in them and by their obscure, deeply-staining nuclei. Our knowledge of their structure is incomplete so far as the mitochondria and the canalicular apparatus are concerned. Busacca Archimede (1913, p. 332), alone, has observed that the mitochondria in certain cells in the brain of *Testudo graeca* stain particularly intensely with iron hematoxylin, and in some cases seem to lose their definite outlines and to form homogeneous masses. Rina Monti (1915, p. 39) has made a comprehensive study of the canalicular apparatus ("apparati di Golgi") in nerve-cells, but she does not mention cells in the chromophilic condition. I shall consequently venture to present in this paper my observations on these two structures in the chromophile cells in the brain of the white mouse.

MATERIAL AND METHODS.

White mice were employed because they are the smallest mammals which can be conveniently used in the laboratory for experimental purposes. The small size of their nervous system permits the study of the distribution and the arrangement of chromophile cells in serial sections. All the mice were of known age and care was taken that they were perfectly normal.

A modification of the methods of Altmann (1890, p. 27), Galcotti (1895, p. 466), Regaud (1910, p. 296), Bensley (1911, p. 309), and Shirokogoroff (1913, p. 523) was devised for the study of mitochondria. The method has many advantages. In the first place, the use of a mixture of formalin and potassium bichromate as a fixative (Regaud) gives a much more uniform preservation of mitochondria than the osmic acid containing fixatives in general use. The application of the fixative by

¹The work was aided by the Department of Embryology of the Carnegie Institution of Washington, and part of it was done at the Marine Biological Laboratory, Woods Hole, Massachusetts, where, through the kindness of the Director, Dr. Lillie, a room was placed at my disposal.

injection through the blood-vessels (Shirokogoroff) eliminates many very objectionable artefacts due to faulty penetration. The use of permanganate and oxalic acid (Bensley) facilitates the staining of the mitochondria with the anilin fuchsin (Altmann), and the counterstaining with methyl green (Galeotti) permits of the demonstration of the Nissl substance in the same cell with the mitochondria. The fact that the method gives good results in the case of other tissues where the mixtures of Altmann, Flemming, and others are useless on account of their poor powers of penetration, justifies the following detailed statement:

Fixation:

Chloroform the animal. Inject warmed 0.85 per cent NaCl solution into the aorta through the ventricle. If the brain alone is to be studied clamp the descending aorta. If the entire nervous system is to be fixed, clamp the cœliac, the renals, the superior and inferior mesenterics, the iliacs, and the brachials. Continue the injection until the salt solution is returned uncolored through the jugulars. During this time lay bare the arch of the aorta and the carotids from connective tissue, so that they may expand easily and carry more fluid to the brain. Gravity pressure of not more than 6 feet may be employed. Cut the inferior vena cava and the jugulars so that the salt solution may run through easily.

Follow the salt solution with the formalin and bichromate mixture: 3 per cent potassium bichromate, 4 parts; neutral formalin, 1 part. The potassium bichromate acts best when freshly prepared. Neutral formalin is made from the commercial variety by the addition of magnesium carbonate, a deposit of which should always remain at the bottom of the formalin bottle. It is important that the pressure should be at the maximum when the mixture is first injected, so that the blood-vessels may be fixed in a state of dilation. If the pressure is low when the fixative comes in contact with the vessel-walls they will be fixed in a condition of collapse. It will then be difficult, or even impossible, to obtain a complete injection. The injection should be continued for about an hour.

The brain is then dissected out and immersed in the fluid. In the case of the mouse's brain it is sufficient to divide it longitudinally into halves. The fixative must be changed every day for 4 or 5 days, otherwise it undergoes a change evidenced by a darkening in color. This change is accelerated by light and by heat, so that the tissue should be kept in the dark and in a cool place. Fixation may also be effected by simple immersion of the tissue in the fixative, instead of by injection, but this procedure is not recommended.

After this prolonged fixation the tissue is mordanted in a fresh 3 per cent solution of potassium bichromate, in which it remains for 8 or 9 days, changing every second day.

Wash in running water for 24 hours. The object of this careful washing is to remove most of the formalin and bichromate, for otherwise the tissue will be extremely brittle and hard to cut.

Dehydration and embedding:

50 per cent alcohol 12 hours; 70 per cent and 95 per cent alcohol 24 hours each; absolute alcohol 6 to 12 hours; half absolute and xylol 6 hours; xylol 3 hours; paraffin 60° C. 3 hours; cut in 4- μ serial sections.

Staining:

(1) Pass the sections, mounted on slides, down through toluol, absolute, 95, 70, and 50 per cent alcohol to distilled water.

(2) 1 per cent aqueous solution of potassium permanganate 30 seconds; but this time must be determined experimentally.

(3) 5 per cent aqueous solution of oxalic acid also about 30 seconds.

(4) Rinse in several changes of distilled water about a minute. Incomplete washing prevents the staining with fuchsin.

(5) Stain in Altmann's anilin fuchsin, which is to be made up as follows: Make a saturated solution of anilin oil in distilled water by shaking the two together (anilin oil goes into solution in water in about 1 per cent). Filter and add 20 grams of acid fuchsin to 100 c.c. of the filtrate. The stain should be ready to use in about 24 hours. It goes bad in about a month. To stain, dry the slide with a towel, except the small area to which the sections are attached. Cover the sections on the slide with a small amount of the stain and heat over a spirit lamp until fumes, smelling strongly of anilin oil,

come off. Allow to cool. Let the stain remain on the sections for about 6 minutes. Return the stain to the bottle.

(6) Dry off most of the stain with a towel and rinse in distilled water, so that the only stain remaining is in the sections. If a large amount of the free stain remains it will form a troublesome precipitate with the methyl green; on the other hand, if too much stain is removed the coloration of the mitochondria will be impaired.

(7) Again dry the slide with a towel, except for the area covered by sections. Allow a little 1 per cent methyl green, added with a pipette, to flow over the sections, holding the slide over a piece of white paper so that the colors may be seen. Apply the methyl green for about 5 seconds at first and then modify the time to suit the needs of the tissue.

(8) Drain off excess of stain and plunge the slide into 95 per cent alcohol for a second or two, then rinse in absolute for the same time, clear in toluol, and mount in balsam.

Several difficulties may be met with: (1) The methyl green may remove all the fuchsin, even when it is only applied for a short time. This is due to incomplete mordanting of the mitochondria by the chrome salts in the fixative. It may often be avoided, either by omitting the treatment with permanganate and oxalic acid, or by treating the sections with a 2 per cent solution of potassium bichromate for a few minutes immediately before staining (as advised by Benschley). The action of the permanganate and oxalic is to remove the excess of bichromate. (2) Or the fuchsin may stain so intensely that the methyl green removes it very slowly or not at all. This, on the other hand, is due to too much mordanting. It may be corrected by prolonging the action of the permanganate and oxalic. (3) Sometimes, after obtaining a good differentiation, the methyl green is washed out before the slide is placed in toluol. This may be avoided by omitting the 95 per cent alcohol, by passing from the methyl green to the absolute direct. (4) Unfortunately the stain is not very permanent. Under favorable conditions it will last for 3 or 4 years. The fading in color is hastened by light and by heat, and it proceeds very rapidly in a damp atmosphere.

Cajal's (1912, p. 211) uranium-nitrate method was employed for the canalicular apparatus in its original form, except for the substitution of methyl green in the place of carmalum as a counterstain.

Control preparations were fixed in a variety of fluids and were stained in many ways, as will appear later.

The figures have been made from specimens prepared by the above-mentioned fuchsin-methyl green method, by which the mitochondria are stained red, the Nissl substance green, while the canalicular apparatus remains uncolored; and also from specimens made by the uranium-nitrate method, which blackens the canalicular apparatus and colors the Nissl substance green.

OBSERVATIONS.

Chromophile cells, as the name implies, possess an unusual affinity for stains, which may be either acid or basic. Their structure is variable. A glance at the figures is sufficient to show this. The variations may represent stages in a process, which, when pushed to an extreme, results in a cell in an advanced stage of chromophilia, but of this we have no conclusive proof. Neither can we assert that the process proceeds in this direction, for the changes observed may equally well be interpreted as taking place in the reverse order. We do not yet know whether the series is homogeneous: that is to say, whether we are not arbitrarily grouping several processes of different nature under the same heading. For instance, a mitochondrial increase (figures 1 and 2) may not precede a diffuse staining of the whole cell with mitochondrial dyes (figure 6), which may be brought about in an entirely different way. Nevertheless, the cells are all chromophile in the sense already defined, that they stain deeply.

Some chromophile cells differ only from other cells by a slight increase in the amount and in the intensity of the staining of the mitochondria (figure 1). There is apparently no corresponding change in the Nissl substance and the morphology of the mitochondria is unaltered.

Other cells show a remarkable increase in the number of mitochondria. For example, a cell (figure 2) frequently contains three or four times as many mitochondria as its neighbor; this increase in mitochondria is associated with a slight but perceptible increase in the amount of the diffuse Nissl substance in the cytoplasm and with a darker staining of the acidophilic and basophilic nucleoli and the ground-substance of the nucleus. Cells in this condition show no evidence of shrinkage. They may be recognized in Cajal preparations (figure 7) by the changes in the nucleus and the Nissl substance. The Cajal preparations show that the canalicular apparatus is unaltered.

There may be a great increase in the Nissl substance, which is present as a diffuse deposit (figure 3). At the same time some of the mitochondria often lose their discrete outlines and seem to merge into the surrounding cytoplasm. Mitochondria may not be very numerous in cells of this kind. The nucleus stains intensely and a few clear canals may be seen in its vicinity. The cell has apparently shrinkage spaces on either side of it. Preparations, made by fixing in alcohol and staining with toluidin blue, contain cells in which the Nissl substance is in this condition and Cajal preparations show that the canals are unaltered.

Figure 4 illustrates a cell in a rather more advanced stage of chromophilia. In this cell there is an unusually large amount of Nissl substance and there are further evidences of the disappearance of formed mitochondria, especially in the cell process. The outlines of the nucleus can barely be made out. The canalicular apparatus shows no modifications either by this method or by the Cajal technique.

A very interesting condition is shown in figure 5. Here, with this degree of differentiation, only a few typical mitochondria persist near the origin of the cell process. The Nissl substance is overshadowed by a cloud of material staining the same way as the formed mitochondria do in adjacent cells. Figure 8 illustrates a similar cell in a Cajal preparation. The Nissl substance in it is increased and there is no modification in the blackened canalicular apparatus. Cells in this condition are often shrunken. It is difficult to determine whether the shrinkage is the expression of an actual diminution in the size of the cells during life, or whether it is simply the result of a difference in the reaction of chromophile cells to the fixation and subsequent treatment. The presence of what appear to be shrinkage spaces around the cells seems to indicate that it is in reality due to the technique employed, because if, on the other hand, it was due to a decrease in the size of the cell during life, one would expect the space to be filled up by a shifting of neighboring structures. It may be emphasized that the fact that other cells, in actual contact with chromophile cells, show no signs whatever of shrinkage must be regarded as one of the distinctive properties of cells in the chromophilic condition. There is, of course, still another interpretation, namely, that the spaces in question are unusually large perineuronal spaces, the enlargement being in some way connected with the difference in the physiological condition of chromophile cells as contrasted with other cells.

The mitochondria may apparently disappear more or less completely in certain cells, and their place be taken by a mass of amorphous material with the same staining properties (figure 6). The nucleus may or may not be visible. Cajal preparations of cells in the same condition (figure 9) show that the canals are unaltered. The nucleus is obscured by the cloud of Nissl substance. The appearance of these cells, in advanced stages of chromophilia, would perhaps lead one to suppose that they are degenerating and that their nuclei have disappeared. That this is not the case may be seen if one of the mitochondrial preparations is stained with hematoxylin and eosin. The hematoxylin and eosin does not color either the amorphous deposit or the Nissl substance, which, in the mitochondrial and in the Cajal preparations, hides the nuclei. The nuclei have in reality distinct and definite outlines and appear to be quite unaltered, except that they contain rather more than the usual amount of chromatin. In fact, the change in the mitochondria and the increase in the amount of the Nissl substance would never have been suspected if hematoxylin and eosin had alone been used.

The distribution of chromophile cells is important. They often occur singly. They may be surrounded on all sides by cells which show no tendency toward an assumption of the chromophilic condition. They may, on the other hand, occur in clumps. The clumps vary greatly in size. They contain cells in all stages of chromophilia in addition to a variable number of unaltered cells, which are always present, scattered among them.

The neuropil in which the chromophile cells are embedded differs in no way from the neuropil elsewhere. It seems, by all the mitochondrial methods, to be studded with mitochondria. But it must not be thought that the mitochondria occur in anything like equal numbers in the neuropil of different regions, because there is a remarkable variation in this respect. The mitochondria appear to be intercellular, but unhappily a source of error is introduced by the fact that the unmyelinated, and to a lesser extent the myelinated, processes stain in much the same way as the mitochondria, so that in some cases it is impossible to distinguish between them. Undoubtedly a large number of the mitochondria in the neuropil are contained in nerve-cell processes cut in section, but there is no *a priori* reason why they should not occur free from the cells as an intercellular deposit. This important question can only be solved by a detailed study of staining reactions, possibly by the elaboration of new methods, or by taking advantage of the differential solubilities of mitochondria. It has a direct bearing upon the rôle of intercellular material in the metabolism of the central nervous system.

Cells in the chromophilic condition are comparatively rare in the olfactory bulb as compared with the cerebral cortex. In fact, they are more abundant in the cerebral cortex than in any other part of the brain. Clumps of them are more common here than in other regions. The clumps vary in size, in shape, and in position in the brains of animals from the same litter, apparently treated in exactly the same way. Chromophile cells are also numerous in the hippocampus. They are, on the contrary, comparatively rare in the corpus striatum and in the thalamus, in both of which they are more frequently met with singly than in groups. In the midbrain they are found in about the same number. It is interesting to note that they are

quite numerous in the cerebellar cortex. The Purkinje cells are particularly liable to show this condition. They are infrequent in the medulla and they scarcely ever occur in the spinal cord, in the spinal ganglia, or in the sensory ganglia of the cranial nerves, as, for example, the Gasserian ganglion. In other words, this remarkable condition of the nerve-cell is more prevalent in the higher centers than in the lower ones. This is particularly true of chromophile cells in advanced stages of chromophilia.

The question at once arises as to whether these changes in the appearance of the cells are indicative of real alterations in the cells themselves or whether they are merely the result of the treatment to which they have been subjected.

Unfortunately it was found impossible to confirm these observations by the study of unstained, living cells by reason of the difficulties met with in attempts to isolate the cells without injuring them. Attempts to stain the mitochondria in living cells by injecting a solution of janus green into the brain through the blood-vessels did not yield satisfactory results because the janus green was almost immediately reduced, first to the leucobase, and then to the red diethylsafranin, by the reducing action of the brain-substance and the absence of an adequate supply of atmospheric oxygen, so that observations could not be made. Pure oxygen was bubbled through the janus-green solution while it was being injected, in the hope that the reduction of the janus green might thus be retarded, but without success. Attempts to tease out individual cells in the nervous system and to stain them by simple immersion in the janus-green solution resulted, of course, in a coloration of the mitochondria, but it was on the whole unsatisfactory on account of the unavoidable injury to the cells. Consequently I have had to rely solely upon the study of fixed material.

The results obtained with the fuchsin-methyl green method and with the Cajal technique have been confirmed by the detailed examination of material stained by the Benda method, the Altmann method, and with iron hematoxylin. Chromophile cells are, I think, not artefacts due to alcohol fixation, as Barker (1899, p. 124) supposes, because I have observed them in tissues fixed in a great variety of fluids not containing alcohol. Moreover, Flesch (1887, p. 197) found years ago that they could be identified in the fresh, unstained condition as well as in tissues stained vitally with methylene blue.

The fact that the chromophile cells are very abundant in the superficial layers of the cortex would at first seem to indicate, as some investigators believe, that they are artefacts due to mechanical manipulation. The clusters of chromophile cells are sometimes cone-shaped, with the base on the surface of the cortex and the apex of the cone extending inwards, which looks as if they might have been produced by pressure from without which radiated inwards. But isolated clumps of chromophile cells occur in deeper parts of the brain, which can not be explained in this way. Moreover, a number of other facts seem to be incompatible with this view. In the first place, since all the brains were fixed, before removal, by the injection of the fixative through the blood-vessels, it follows that there could be no mechanical injury until after fixation. The invariable occurrence of unaltered cells, side by side with the chromophile cells, is hard to explain on the basis of mechanical injury, because whatever pressure had been brought to bear upon the tissue must necessarily

have acted upon both; but one shows the condition and the other does not (as is shown in all the figures). Furthermore, if mechanical injury is the cause of the condition, it is difficult to understand why chromophile cells are so rare in the spinal cord and in the ganglia of the cranial nerves, which are bound down by membranes and which in removal are consequently subjected to greater mechanical injury than the cortex of the brain.

In order to settle the question the results of intentional mechanical injury brought about by bruising the cerebrum and the spinal ganglia with a blunt instrument were studied. It was found that the lesion produced was characterized by the flattening or compression of many cells in the same direction, at right angles to the direction in which the pressure had been exerted. All the cells in the area were uniformly affected. Normal cells were not scattered among them. The injured cells stained intensely, but they did not simulate the chromophile cells. The neuropil between them showed marked changes and could readily be distinguished from the neuropil elsewhere in the same section.

Chromophile cells are not the result of differences in the time or in the degree of fixation. The whole brain is uniformly fixed by the methods of technique employed. The distribution of chromophile cells is not related to the arrangement of the blood-vessels, which are the avenues of approach of the fixative. Neither do the mitochondria vary in number with the vascularity of the region.

The condition is not due to irregular mordanting with the potassium bichromate, because complete extraction of the bichromate by prolonged treatment with permanganate and oxalic acid does not essentially modify the appearance of the chromophile cells when the sections are stained.

Another possibility is that the intense staining of the chromophile cells results from incomplete differentiation. Even if this were the case the differences in the rate of decolorization must be the visible expression of real differences in the cells themselves. I have found, however, that the same differences obtain in undifferentiated specimens stained lightly with fuchsin, crystal violet, and iron hematoxylin. I have made a number of experiments to determine whether more complete differentiation would bring to light formed mitochondria in cells in which they appear to have been replaced by the amorphous deposit which stains in the same way.

Specimens were stained in the usual fashion with fuchsin and methyl green and were mounted in balsam. Drawings were then made of chromophile cells which had been stained intensely with the fuchsin and in which no formed mitochondria could be seen. The cover-glass was then dissolved off and the slide was passed down through toluol and graded alcohols to water. It was then restained with fuchsin, differentiated more strongly with the methyl green, mounted in balsam, and examined. The same condition was apparent, except that the homogeneous deposit had a distinctly greenish color. The same process was repeated as many as five times with the same cell, increasing each time the extent of differentiation, until the cell stained intensely with methyl green and very little trace of the fuchsin was left: still no formed mitochondria were observed; this was repeated with other cells with the result that in some of them formed mitochondria were brought to light, while in others they were not.

Similar experiments were performed with individual cells stained a homogeneous black color with iron hematoxylin. The results obtained are easier to interpret because the differentiator, iron alum, does not itself color the tissue like the methyl green. This advantage is counterbalanced by the fact that both the mitochondria and the Nissl substance stain in the same way and it is often difficult to distinguish between them. In many cases, particularly in slightly undifferentiated specimens, the extraction of the stain from chromophile cells by further differentiation brought to light a variable number of formed mitochondria. Moreover, it is worthy of note that the chromophile cells in the cerebral cortex are the last to become decolorized and that the differentiation occurs with unequal rapidity in different parts of the cell, thus indicating that the homogeneous deposit is not present in the same concentration in all parts of the cell.

The end-result of this experimentation is that chromophile cells, particularly those in advanced stages of the condition, contain a diffuse deposit, which stains in a typical way with all mitochondrial dyes, and which is probably formed by the solution of some of the mitochondria in the cell.

The condition is not due to technique and it is not associated with a visible pathological change on the part of the animal.

All the mice employed were apparently normal. They ate well and showed no signs of sickness. They were killed with chloroform, and it may at once be said that the changes are not due to acute chloroform poisoning, because animals killed in other ways, by decapitation, for example, showed the same condition. The mice were not excited, or disturbed or exercised in any unusual way before they were killed. A careful autopsy of each mouse was made to make sure that it was quite normal. Some were found to contain a parasite, present in the cysticercus stage in the liver; these were invariably discarded. The chromophile cells were found in mice of both sexes in almost all seasons of the year. They were found in mice varying in age from 25 days to adults, so that they can not be regarded as an expression of senility. It was thought that they might occur in consequence of abnormal conditions due to captivity. In order to settle this point a wild field-mouse was captured alive and in good condition and its brain was prepared in the usual way. It, also, showed chromophile cells.

An apparently analogous partial solution of mitochondria was observed in liver-cells poisoned with phosphorus by Mayer, Rathery, and Schaeffer (1914, p. 609). Accordingly, W. J. M. Scott tried the effect of experimental phosphorus poisoning on the nervous system of white mice. The chromophile cells were apparently entirely unaffected and a solution of mitochondria was not brought about. Dr. Bensley made the interesting suggestion to me that this partial solution of mitochondria in chromophile cells might be due to a swing of the reaction in them toward the acid side, with the liberation of free fatty acids. I therefore made some preliminary experiments on acidosis in mice produced by the subcutaneous injection of dilute hydrochloric acid, all of which yielded negative results as far as the chromophile cells were concerned. I have, further, found that slight exercise does not alter the appearance of the chromophile cells in the brains of white mice to any noticeable extent.

It seems highly probable, therefore, that chromophile cells occur normally in the brain of the white mouse and that we have to reckon with a partial solution of mitochondria just as we have for many years recognized a chromatolysis, or solution of the Nissl substance.

DISCUSSION.

This work on chromophile cells has, I believe, an important bearing upon (1) the question of differential nerve-cell activity; (2) the phenomena of chondriolysis and hyperchromatism; (3) the functional independence of the mitochondria and the canalicular apparatus; and (4) our conception of the structure of living nerve-cells.

(1) The distribution of chromophile cells in the different parts of the brain is interesting. The fact that they occur most abundantly in the cerebral cortex and in the cerebellum, and that they are rarely found in the lower centers like the spinal cord, would seem to indicate that the central neurones differ in some way from the more peripheral ones. The difference may be one of lability, for Dolley (1914, p. 56) has found that more highly specialized cells are more prone than less specialized ones to respond with structural changes to physiological experimentation. Moreover, the occurrence of these cells in groups, which vary in size and in position in different brains, is in accordance with our conception of the alternation of rest and activity in the higher centers and may well have some bearing upon the vexed problem of cortical localization, for as yet neither the mitochondria nor the canalicular apparatus have been considered in this connection.

(2) We must recognize a "chondriolysis," or a partial solution of mitochondria, in nerve-cells as well as a "chromatolysis." The word "chondriolysis" was first employed by Romeis (1912, p. 139) to describe the disintegration of certain mitochondria which escaped from the cells into the uterine fluid of *Ascaris*. It is, to my mind, more appropriate than the term "chromatolysis," which is frequently applied to the so-called solution of Nissl bodies, for the simple reason that I am of the opinion (1914, p. 20) that the Nissl substance is usually in solution in the living nerve-cell, whereas the mitochondria are assuredly present as definite formed bodies (except of course in the chromophilic condition).

Chemical changes are undoubtedly involved in the phenomena of conduction (Tashiro and Adams, 1914, p. 329) and, in view of the distinct differences in the chemical constitution of the mitochondria and of the Nissl substance, the one being of a lipoid albumin nature (Fauré-Fremiet, Mayer and Schaeffer 1910, p. 95) and the other being apparently a complex nucleoprotein containing iron (Scott, 1905, p. 507), it seems probable that the study of mitochondria and the changes which they undergo may bring to light variations in the activity of the nerve-cell which could never be detected by the study of the Nissl substance alone. Quite apart from the rôle of the nucleus in the elaboration of the Nissl substance and the purely cytoplasmic nature of mitochondria, there is further evidence of a functional diversity between the two structures. I have found that in the nerve-cells of the mouse the mitochondria vary directly with the volume of the cytoplasm and that the Nissl substance varies inversely with the nucleus cytoplasmic ratio; also that the mitochondria are of more general occurrence in nerve-cells than the Nissl substance.

They are present in the granule-cells of the cerebellum, as is also evident from the earlier work of Altmann (1890, plate XIII, figure 1) and Nageotte (1909, p. 826), and in the granule-cells of the olfactory bulb of mice and rats, which are well known to be devoid of Nissl substance. Moreover, in certain cell-groups, under normal conditions, there is often a variation in the mitochondria, as between different cells, without any corresponding change in the Nissl substance. Mitochondria occur abundantly throughout the length of the axone, where no Nissl substance has ever been seen. They also occur in certain dendritic processes which do not contain any Nissl substance. Evidence of this sort may be multiplied.

Just how the mitochondria are concerned with the activity of the nervous system is unknown. I have presented evidence elsewhere (1914, p. 18) that they play a part in the basic processes of metabolism which are common to all cells, but this is unfortunately a very broad statement and we naturally desire to learn something rather more specific about them. Coghill's (1915, p. 350) belief that the mitochondria are concerned in the constructive (anabolic) side of metabolism is of interest in this connection, particularly since it falls so well in line with the well-known "eclectosome" theory of Regaud (1911, p. 699), which, in turn, is an extension of the "side chain" theory of Ehrlich. M. R. and W. H. Lewis (1915, p. 393) make the interesting suggestion that the mitochondria take part in cellular respiration, which is also a fundamental process common to all cells.

We may confidently expect that this new avenue of approach to the study of the activity of the nervous system will yield results of importance, not only because our histological methods of technique are now sufficiently accurate to permit of the actual enumeration of the mitochondria, a thing which can not be accomplished in the case of the Nissl substance, but also because Waldemar and Mathilde Koch (1913, p. 427) have recently succeeded in devising chemical methods for the qualitative and quantitative estimation of substances, very closely related, perhaps identical with mitochondria, in the nervous system. These substances are phospholipins. Hoppe-Seyler long ago pointed out that lecithin (a typical phospholipin) and cholesterol are to be found almost everywhere that life phenomena exist. In fact, a great wave of revived interest is manifested in recent chemical and pathological literature in these complex compounds of fatty acid, phosphorus, and nitrogen. Mathews (1915, p. 88) very aptly remarks that the phospholipins are the most important substances in living matter:

"For they are found in all cells, and it is undoubtedly their function to produce, with cholesterol, the peculiar semifluid, semisolid state of protoplasm. The latter holds much water in it, but it does not dissolve. Indeed it may be said that the phospholipins with cholesterol make the essential substratum of living matter. This physical substratum of phospholipin differs in different cells and probably in the same type of cell in different animals, but everywhere, from the lowest plants to the highly differentiated brain cells of mammals and of man himself, it possesses certain fundamental chemical and physical properties. In all cases the phospholipin substratum is soluble in alcohol containing some water," etc.

In view of these considerations it is interesting to inquire whether the distribution of mitochondria in cells corresponds with that of the phospholipins. It is certainly true that mitochondria are more widely distributed than any other kind

of cell granulation now known to us. They occur in almost all cells. Yet certain cells, like the fully differentiated non-nucleated red blood-cell, unquestionably contain a large amount of phospholipin, though no formed mitochondria can be seen. The mitochondrial substance is probably present in solution, just as it appears to be in chromophile cells, for it would obviously be absurd to state that it must always occur in that state of condensation which makes it visible with the aid of certain powers of the microscope. The recent investigations of Levene (1915, p. 41) on cephalin are of interest. A new field of investigation is evidently opened up. It may thus be possible to pursue this line of work with chemical as well as with histological and physiological methods, a combination which has been but rarely effected.

Work along these lines seems the more desirable since, as will be seen, it may throw new light upon certain problems in the pathological anatomy of the nervous system as well. Wells (1907, p. 460), in his discussion of mental fatigue, writes:

"Since the lecithin forms so important a part of the nervous system, it is tempting to imagine that in fatigue excessive quantities of its toxic decomposition product, *cholin*, and the still more toxic derivative of cholin, *neurin*, are formed in considerable amounts and cause part, at least, of the intoxication."

Now we have seen that, in the opinion of certain investigators, mitochondria are largely composed of lecithin. It is possible, therefore, if Wells's reasoning is correct, that the symptoms of mental fatigue are the result of their decomposition. Moreover, Halliburton (1907, p. 74) and others are convinced that organic diseases of the nervous system may be distinguished from functional neuroses on account of the formation of cholin in the one and not in the other. This opens up the possibility of a differentiation between these two great groups of diseases on the basis of cell structure, as to whether or not there is a change in the mitochondria.

(3) The persistence of the canalicular apparatus in chromophile cells is of interest in general cytology. In chromophile cells, in which there are marked structural changes, the canalicular apparatus remains without any great modification. This is rather surprising, since investigators have gradually come to regard the canalicular apparatus as the most labile cell organ; but it is in conformity with Key's as yet unpublished observations on degenerative changes in spinal ganglion cells. Key finds that the canalicular apparatus persists without much modification for from 12 to 24 hours after death in spinal-ganglion cells left in the animal.

I have shown (1912, p. 494) that a canalicular apparatus, in the form of a system of clear, uncolored canals, occurs in the same cell with typical mitochondria and that consequently the canalicular apparatus and the mitochondria are structurally distinct. This conclusion is strongly supported by my observation that they may likewise be seen together in chromophile cells, the difference being that while the mitochondria are greatly changed, the canalicular apparatus remains with little or no modification, so that they are functionally as well as structurally different. My positive impregnations of the canalicular apparatus by the uranium-nitrate method of Cajal confirm this observation.

Now, Cajal (1908, p. 123) is so certain of the identity of the clear canals (described originally by Holmgren) and the "Apparato reticolare interno" of Golgi

that he refers to them as "conduits de Golgi-Holmgren." But Rina Monti (1915, p. 40) has made the statement that the large internal reticular apparatus corresponds to the chondriome (*i. e.*, to mitochondria) in the nerve-cells of mammals; to quote her own words: "Il grande apparato reticolare interno dal Golgi nelle cellule nervose di mammiferi corrisponde adunque al condrioma, comme il grande apparato descritto dal Pensa nelle cellule cartilaginea." If Cajal is correct in his identification, it would appear that the canalicular apparatus and the mitochondria are identical. I have already discussed (1912, p. 490) the older statements of Popoff (1906, p. 258), Smirnow (1906, p. 153), Van Durme (1907, p. 84), Meves (1908, p. 846), and Hoven (1910, p. 479), who are inclined to believe this to be the case.

It is hard to see how these two views can be reconciled. I am inclined to think that the well-known lack of specificity of the methods of silver impregnation which Pensa (1913, p. 560) and Rina Monti (1915, p. 45) have employed are the cause of the confusion. I do not believe that the Golgi method can be trusted invariably to demonstrate a certain structure within the cell, like the canalicular apparatus; and, for this reason, I can not accept unreservedly Cajal's identification of the canalicular apparatus with the Golgi-apparatus. I agree with Duesberg that a more precise definition of the "Apparato reticolare interno" is highly desirable, but I do not agree with him in his attempt (1914, p. 37) to define it in terms of its relation to the centrosome, because our knowledge of the centrosome itself is so deplorably inadequate. We require, above all else, more accurate methods before the matter can be cleared up.

(4) This discussion of the structure of chromophile cells may be profitably concluded by a statement of our present knowledge of the cytoplasmic structure of living nerve-cells of vertebrates not in the chromophilic condition. Mitochondria unquestionably occur and may be seen as such in living nerve-cells even without any vital stain. The Nissl substance is usually present in solution, not in the form of discrete masses as seen in fixed preparations. I believe that there is also an amorphous argentophilic material which (when treated by appropriate but very capricious methods) assumes the form of fibrils. The canalicular apparatus, like the neurofibrils, is an unknown quantity in living nerve-cells, although it may be demonstrated in fixed tissues with considerable regularity. These structures, or more correctly speaking substances, are distinct and should not be confused with one another. Although the mitochondria alone have a definite morphology and can usually be seen in living nerve-cells, under ordinary conditions, with the present means at our disposal, it would be arbitrary in the extreme to say that the others can never be seen. Pigment, fat, lipoid, etc., may of course be seen in variable amount in living nerve-cells. It is the more fundamental constituents with which we are concerned.

The recent work in bio-chemistry, summarized by F. Gowland Hopkins (1913, p. 663) in his presidential address before the Physiological Section of the British Association, has, I believe, an important bearing here. The cell is regarded as a dynamic system of co-existing phases in more or less stable equilibrium, the condition of which is altered, from moment to moment, by processes of oxidation, reduction, desaturation, condensation, etc., which naturally result in physical changes in the cell, with the building-up and breaking-down of molecular aggregates which may or

may not be visible with the microscope or the ultra-microscope. The Nissl substance, argentophilous material, etc., doubtless undergo changes of this sort from liquid to fluid and semi-solid phases. It seems right and proper, therefore, to steer an intermediate course, as I have done, between those, on the one hand, who assert that the Nissl substance and the neuro-fibrils occur in living cells in approximately the same form as they appear in fixed and stained preparations, and those, on the other hand, who claim that they are artefacts pure and simple and that they can never be seen in the living cell. Our problem is more one of material than it is of form.

In this connection the solution of mitochondria in chromophile cells is a phenomenon of considerable significance. In addition to variations in the chemical constitution of mitochondria, there is also evidence to the effect that there may be variations in the condensation or density of the mitochondrial substance (*vide* Duesberg, 1915, p. 40). This is a factor which has been too often ignored. We are inclined to look for mitochondria in all cells which are functionally active and in which metabolic changes are taking place. The fluidity of the mitochondrial substance varies and I am prepared to believe that further investigation will bring to light cells which are active functionally, but in which no trace of formed mitochondria may be seen.

CONCLUSIONS.

- (1) Chromophile cells occur under normal conditions in the brains of white mice.
- (2) They are distributed unequally in the different parts of the nervous system. They are most abundant in the cerebral cortex. They are progressively less abundant in the cerebellum, corpus striatum, thalamus, midbrain, and medulla. They are of very rare occurrence in the spinal cord, spinal ganglia, and sensory ganglia of the cranial nerves.
- (3) This restriction of the chromophile cells to the higher centers is in full accord with the well-known lability of the central, more highly specialized cells as contrasted with the more primitive, peripheral neurones.
- (4) Chromophile cells, as seen in fixed and stained preparations, vary greatly in structure. There is usually more or less shrinkage of the cell-body. The nucleus may also be shrunken. The acidophilic and basophilic nucleoli are particularly prominent and the ground-substance of the nucleus stains intensely with both acid and basic dyes. There is an increase in the amount of Nissl substance. The Nissl bodies become confluent and form a homogeneous mass. The cell is hyperchromatic. The canalicular apparatus is unaltered. The mitochondria either increase in number and stain more intensely, or else some of them lose their discrete outlines and form a diffuse deposit which stains intensely by the mitochondrial methods of technique. This change in the mitochondria occurs in the cell processes in the neighborhood of the cell, as well as in the cell-body. Although the nucleus may be completely obscured by this cloud of mitochondrial substance, it still remains and stains in the usual way with hematoxylin and eosin.
- (5) The lability of the mitochondria and the constancy of the canalicular apparatus in chromophile cells confirms my earlier contention by showing that the two structures are physiologically as well as anatomically distinct.

EXPLANATION OF FIGURES.

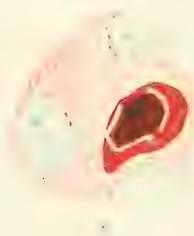
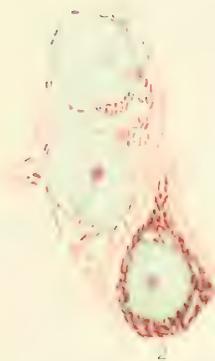
All the figures have been drawn with Zeiss apochromatic objective 1.5 mm. compensating ocular 6 and camera lucida giving a magnification of 1,500 diameters. The figures have not been reduced in reproduction. In all of them unaltered cells are represented side by side with chromophile cells just as they occur in the preparations.

Figures 1 to 6 represent cells in the cerebral cortex of a male white mouse, 26 days old and weighing 5 grams. The brain was cut into serial sections 4μ in thickness and stained with fuchsin and methyl green. All the figures were drawn from cells in the same section to insure uniformity in the action of the stain and of the differentiator. The mitochondria are stained red, the Nissl substance green, and the canalicular apparatus persists, in some of the cells, in the form of clear, uncolored spaces.

Figures 7 to 9 represent cells from the cerebral cortex of a male white mouse, 29 days old and weighing 10 grams. Portions of the brain were prepared by the uranium-nitrate method of Cajal and were cut into serial sections 4μ thick. These figures were also drawn from a single section to insure uniformity in the action of the counterstain, methyl green. The canalicular apparatus is in the form of a blackened network and the Nissl substance is colored green.

FIG. 1. Two cells, having a distinct increase in amount and intensity of the staining of the mitochondria. This change may mark the first stages in the assumption of the chromophilic condition.

2. A much greater increase in amount of mitochondria and a slight increase in intensity of the staining of the Nissl substance and the nucleus.
3. The Nissl substance is more abundant. It is diffuse and stains more brightly. The outlines of the mitochondria are indistinct. The nucleus stains darkly. A few clear canals are visible near it. There is what appears to be a shrinkage space on either side of the cell.
4. Still greater changes. The mitochondria appear to be going into solution; outlines of nucleus barely distinguishable.
5. The mitochondria have almost all gone into solution. The Nissl substance is almost entirely obscured by the cloud of mitochondrial material which stains with the most energetic of the two dyes, acid fuchsin. The nucleus is invisible.
6. A complete "chondriolysis" or solution of the mitochondria. The canalicular apparatus is present in the vicinity of the nucleus.
7. The increase in amount of the Nissl substance indicates a slight degree of chromophilia. The canalicular apparatus is blackened and shows no changes.
8. Greater increase in the Nissl substance. It is diffuse, with marked hyperchromatism. The nucleus stains diffusely with methyl green. Its outlines are obscure. The canalicular apparatus, in black, is unaltered and the cell as a whole is shrunken.
9. Cell so intensely stained with the methyl green that the nucleus can not be seen. Canalicular apparatus slightly condensed, otherwise unchanged. There is a considerable shrinkage of the cell.



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CONTRIBUTIONS TO EMBRYOLOGY, No. 12.

ON THE DEVELOPMENT OF THE LYMPHATICS OF THE LUNGS
IN THE EMBRYO PIG.

By R. S. CUNNINGHAM.

With five plates.

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ON THE DEVELOPMENT OF THE LYMPHATICS OF THE LUNGS IN THE EMBRYO PIG.

By R. S. CUNNINGHAM.

From an analysis of the literature on the development of the lymphatic system, it is clear that there is a general agreement among recent workers that the mammalian lymph-sacs precede the lymph-vessels in the time of their appearance, and hence constitute what may be called a primary lymphatic system. This system consists, in mammals, of 8 sacs: 3 paired, the jugular, the subclavian, and the posterior iliac lymph-sacs; and 2 unpaired, the retroperitoneal sac and the cysterna chyli.

The further development of the lymphatic system—that is, the formation of the thoracic ducts and the peripheral vessels—has been discussed at length by numerous workers during the past decade. These workers have been grouped into two general schools: the one holding that the lymphatics grow by a centrifugal sprouting of pre-existing endothelium, the other believing that these vessels are formed by a coalescence of numerous isolated spaces developing in the mesenchyme.

According to the centrifugal theory, briefly stated, the sacs arise from the veins and are joined together by vessels that sprout out from their endothelial walls. Thus the thoracic duct arises from both the retroperitoneal sac and the left jugular sac, and the two elements unite somewhere between the two points of origin. Supporters of the centrifugal theory claim that the secondary lymphatic system (the capillary bed) arises by the sprouting of the endothelial walls of the sacs and of the right and left thoracic ducts. These sprouts invade the organs and, becoming progressively more complex, assume the adult form of the lymphatic system. The supporters of the multiple-anlagen theories (whether they believe in coalescing tissue-spaces, multiple venous origins, or degenerating veno-lymphatics) agree in claiming that lymphatics do not grow by the centrifugal sprouting of the pre-existing endothelial walls.

It is not my intention to review here all the various theories that have been advanced, but only to call attention to the two general views, in order to correlate my findings with them. A very thorough discussion of these two views, as well as a comprehensive review of the literature, may be found in the *Ergebnisse der Anatomie und Entwicklungsgeschichte*, 1913. (Dr. F. R. Sabin, *Der Ursprung und die Entwicklung des Lymphgefäßsystems*.)

Though primarily concerned with the problems of origin and the method of growth of the lymphatic vessels, the supporters of both theories have aided in establishing the morphology of the primary system and have laid the foundation for the further study of the development of the system as a whole. If the centrifugal

theory is correct, it is clear that it should be possible to follow the growth of lymphatics from the sacs into any organ or group of organs. It should also be possible to demonstrate in progressively older stages constantly increasing lymphatic zones and decreasing non-lymphatic zones. The development of the lymphatics of the skin, of the intestine, and of the lung has now been studied in this manner.

In 1904, Dr. F. R. Sabin demonstrated that the skin received its lymphatic supply from the two jugular sacs and the two iliac sacs. From each of these sacs a group of radiating vessels invade the skin and form there a close-meshed plexus. These four plexuses gradually increase in size and finally unite, so that the entire skin is supplied with lymphatics. The differentiation which takes place varies with the location and depends upon the adaptation which the vessels must make to the other structures. Continuing the work of Baetjer (1908) on the retroperitoneal sac, Heuer (1909) studied the development of the intestinal lymphatics by the injection of this sac. He observed and described progressive changes in the intestinal supply, finding more complex injections possible in each older stage. He interpreted these results to mean that the lymphatics had not extended beyond the point which his injections reached and that the region beyond this point constituted a non-lymphatic zone.

There is, therefore, a primary and a secondary lymphatic system. The former consists of a series of sacs formed from the veins and connected by the right and left thoracic ducts. The secondary system consists of the peripheral vessels, which are held by some to be outgrowths from the sacs and by others to be formed *in situ*. With regard to the development of these peripheral vessels, only those of the skin and the intestine had been studied. There was need, therefore, for the study of the other abdominal and the thoracic lymphatics. This work was begun to establish a clearer conception of the development of the secondary system.

In presenting this study, I do not claim to have found any new evidence as to the mode of growth of lymphatics. This work supports the centrifugal theory in the same manner as does that of Heuer (1909); and it is certain that the theory is sufficiently well established to serve as a basis for this work. It is the object of the present paper to follow the gross morphological changes in the development of the lymphatic vessels of the lung from the primary stage to the adult form. It is desired to indicate the general lines of growth and the various stages which the system passes through in the course of its development. No attempt has been made to study the finer structure of the vessels or the mode of growth.

It is important to note that complete injections are very difficult to make, and that it is also difficult to be certain whether the injection in a particular specimen is complete or not. Therefore it is not claimed that any of the injections are complete; and the limits of the lymphatic and the non-lymphatic zones at any stage are defined in a general manner, depending on the comparison of a number of specimens.

The lymphatic supply of the lungs develops from three sources: the thoracic duct, the right thoracic duct, and the cephalad portion of the retroperitoneal sac. In 1913, Sabin remarked: "The right lymphatic duct curves ventralward and grows to the heart and lungs." This is the only statement which I have been able to find

in the literature regarding the development of the cardiac and pulmonary lymphatics from the right duct, or the morphological fate of the right duct in mammals. In the same report attention was called to the fact that the lung-vessels can be injected from the retroperitoneal sac, but this was not studied further at that time. The right duct grows primarily to the heart, just as the left grows to the aorta, this asymmetry depending upon that of the cardio-vascular system, according to the general rule that the principal lymphatic trunks follow the large blood-vessels, and grow with the greatest rapidity where the blood-supply is most abundant.

In the beginning I wish to lay emphasis upon the fact that the lung lymphatics develop partly from the retroperitoneal sac, and to call attention to the fact that these vessels persist in the adult as part of the permanent drainage of the lung, and hence may be of importance in pathology. On account of the complexity of the development of the lung lymphatics, it has seemed best to present this work, not by describing and figuring a series of progressively more complex specimens, but by describing the development as a consecutive growth and illustrating with those preparations that may seem best to clarify the text. However, as a matter of reference, the following table of periods has been arranged, in order to offer a brief outline of the complexity at varying stages. These stages are selected with regard to the more important principles of growth and are as follows:

(1) The downgrowth of the two ducts, completion of the primitive system, and the first vessels to the trachea and lungs. Embryos 2.3 to 3.5 cm.

(2) The migration of the heart; the coalescence of the cardiac drainage with that of the lungs, by the formation of the tracheal plexus and the plexus on the arch of the aorta; the growth of the vessels in the lung from the earliest sprouts along the bronchi to the primitive pleural plexus, and the early marking-off of the connective-tissue septa; and the growth up from the retroperitoneal sac through the ligamentum latum and the anastomosis in the primitive septa into which the vessels grow. Embryos 3.5 to 4.5 cm.

(3) The completion of the primary lymphatic system; that is, when the entire organ is supplied, and the further development is in an increasing complexity of the plexuses already present, incident to the increase in the size of the organ and its assumption of mature activities. During this period the formation of the valves and nodes begins. Embryos 4.5 to 7 cm.

(4) The remainder of the development is considered a period, as it is, in reality, an adaptation of the system already present to the increasing needs of the organ. This includes the differentiation of the drainage-lines and the final development of the nodes.

In describing the development of the lymphatics of the lung, the growth of the left duct down to the aorta, of the right duct to the heart, and the formation of the primitive tracheal plexus and the early vessels to the lungs from both ducts will be considered first; the further development of the tracheal plexus, together with the changes incident to the descent of the heart, will follow; then the origin of the vessels from the retroperitoneal sac and their growth up through the ligamentum pulmonale into the lungs will be considered. After the anastomoses of the two sets of lymphatics, the lung will be considered as a whole, inasmuch as the further development is symmetrical for the entire organ, with the exception of the final lines of drainage and the development of the nodes.

I wish to express here my indebtedness to Professor F. R. Sabin for her constant advice and assistance throughout this work. Also I wish to thank Mr. James F. Didusch and Miss Flora Schaeffer for the illustrations.

METHODS.

The injection method has been principally used, but it has been supplemented and supported by evidence from both single and serial sections. The collection of pig embryo of the Anatomical Laboratory has been at my disposal, and I have also studied a number of especially prepared series. Many of the series have been of embryos in which the blood-vessels have been injected, and this has materially aided in their interpretation: in fact, in all the especially prepared series the blood-vessels were injected. All these embryos were fixed in Carnoy's fixing fluid, consisting of 6 parts of absolute alcohol, 3 parts of chloroform, and 1 part of glacial acetic acid.

The method of fixation is as follows: Place the embryo immediately in the fluid and allow it to remain there 6 to 8 hours; then transfer directly to 70 per cent alcohol; dehydrate by ascending grades of alcohol with 2 per cent difference until 95 per cent is reached; then change to absolute. This gives excellent fixation with very little shrinkage. The stains used were Ehrlich's hematoxylin and a mixture of eosin, aurantia, and orange G.

The injection masses used were india ink, a saturated solution of prussian blue, a 5 per cent aqueous solution of silver nitrate, and an aqueous suspension of lamp-black. The india ink and prussian blue give about the same results, except that the ink renders them more opaque. The india ink, however, flows more easily and hence the injections are more nearly complete. The silver-nitrate injections are easiest to analyze and give beautiful preparations, but its caustic action prevents the finer vessels from filling, so that only the larger trunks are injected; however, it furnishes an extremely valuable method of following the principal drainage-lines at different stages. The lampblack is the mass which gives the most nearly complete injections, but unfortunately it precipitates in fine flakes and gives a feathery appearance to the specimen, thus rendering it difficult to use for illustrating.

It will be necessary to review the methods used in injecting the various stages, as they differ considerably and are of especial importance in interpreting the results. The earliest injections were made by filling the jugular sacs from the superficial plexuses and then gently moving the embryo. I have succeeded in injecting the early vessels to the trachea and the lungs in only a few pigs less than 3 cm. long, because the injection mass usually follows the path of least resistance, which is into the jugular vein.

In injecting embryos between 3 and 6 cm. in length, three general methods have been employed:

- (1) The best and by far the easiest method of obtaining good preparations of the left part of the tracheal plexus is to inject through the retroperitoneal sac in the manner described by Heuer (1909); but this seldom gives good preparations of any of the vessels of the lung except those of the lower lobe. However, this method has been of particular importance in following the lymphatics up from the retroperitoneal sac to the posterior poles of the lower lobes.

- (2) One may inject the tracheal plexus, especially the left part, by plunging the needle deep behind the aorta and injecting cerebralwards; the right plexus is sometimes filled also, and often the vessels of the left lobe of the lung.

(3) Finally, the vessels of the lung are best injected by a puncture just ventral to the trachea (the tracheal plexus) and behind the arch of the aorta. Here the tracheal plexus is always extravasated, but the lung-vessels fill up nicely.

The embryos older than these mentioned, that is, longer than 7 cm. (or after the valves are formed), are much more difficult to inject, and this difficulty increases with further development. The method employed has been to inject directly into the connective-tissue septa of the lung and to continue the injection slowly until there is some extravasation at the point of puncture, when a part of the lung surrounding the area of extravasation is well injected. This method has been very satisfactory in all specimens that were obtained very soon after the removal of the uterus; most of the injections were made while the heart was still beating.

In order to study the relations between the blood-vessels, bronchi, and lymphatics, multiple injections had to be made. Various combinations were employed. In some, the lymphatics were injected together with veins and arteries; in others with either veins or arteries alone. Again, the lymphatics and the bronchi were injected; and in still others the lymphatics were combined with either veins or arteries. In these multiple injections prussian blue, india ink, and carmine were used, the lymphatics being injected with either the blue or the ink.

The specimens in which three systems were injected were difficult to clear, unless only the large bronchi and blood-vessels were filled.

In order to trace the vessels more accurately, many of the injected lungs were embedded in paraffin and cut in thick serial sections (100 to 500 μ); these were mounted in balsam but not stained. Other lungs were cut at 10 to 20 μ and stained similarly to the series already referred to.

All measurements of embryos refer to crown-rump diameter and were taken before fixation, as is customary in this laboratory. The illustrations are labeled "C. R. —"; this refers to the crown-rump measurement.

In 1906, Flint published his study on the development of the lungs in the pig, and his work has been taken as a basis of the general structure of the lungs, especially with reference to the development of the bronchi and blood-vessels. He reviewed all the important literature on the embryology of the mammalian lung, studied the lymphatics in sections, and briefly summarized their structure and distribution at various stages, but he did not attempt to inject them. I have been able to confirm most of his observations. However, he labored under the difficulty of having neither reconstructions nor injections. He gives a short summary of each stage, and of these summaries I quote the more important parts:

Stage 3 cm.: At the root of the lung a few dilated lymphatics may be noted near the bronchi and pulmonary vessels; however, they have not grown beyond this point into the substance of the lung wings.

Stage 5 cm.: From the root of the lung the lymphatics have gone some distance into its substance. They have thin walls composed of young fibrils lined with endothelium with occasional valves. They are confined, however, to the immediate neighborhood of the main bronchi and their chief subdivisions.

Stage 7 cm.: The most interesting change, however, lies in the further growth of the lymphatics, which in the earlier stages are found in the root of the lung in the neighborhood of the pulmonary vessels and the large bronchi. As they grow in, they accompany these structures for a distance;

then approaching the end branches they leave them and run in a plexiform manner midway between the bronchial tubes until they reach the pleura. This gives the lung now an indefinitely lobulated appearance in which the periphery of the simple lobule is indicated by the lymphatic vessels and the center by the bronchi. The lymphatics are lined with flattened endothelium; their walls are formed by the young connective-tissue fibrils, and here and there valves are beautifully shown which, in general, point away from the pleura.

Stage 13 cm.: The lymphatics, forming a plexus around the bronchial veins and arteries at the root of the lung, accompany them towards the periphery, giving off branches to the interlobular spaces en route. * * * On reaching the periphery of the lung they leave these structures and pass out, as in the preceding stages, to the pleura. They have a plexiform arrangement and may be traced at times into the substance of the lobules. This course may be observed in the deeper lobules of the lung as well as in those on the surface under the pleura.

Stage 19 cm.: In general the relations of the lymphatic system have not changed.

Stage 23 cm.: At 23 cm. the first evidence of the submucous lymphatic system is seen in the stem bronchi. It may, however, be found earlier, but the vessels are difficult to follow. It would seem thus that we have in the pig's lung, besides the lymphatic plexuses accompanying the bronchi, arteries, and veins, an interlobular system which Miller has been unable to find in the human lung. Injections pointing to such a relationship he has interpreted as artefacts. If Miller's conclusions prove correct, then the lymphatics of the human lung must develop, so far as the interlobular system is concerned, in some other way.

I quote at length from Flint because he alone, of the many workers on lung lymphatics, has approached the subject from the embryological side. As I have said, Flint was seriously handicapped by having only sections from which to draw his conclusions. He was especially struck by the prominence of the vessels lying in the interlobular septa, and attempted to explain their apparent change of course (*i. e.*, from the bronchi to the septa) by the theory that the density of the tissue was greater around the bronchi and vessels and that the lymphatics chose the path of least resistance. He did not call attention to the relation of the veins to this point in the development of the lymphatics, which will be discussed later, but emphasized the fact, so amply shown by injections, that these interlobular vessels grow much more rapidly than the vessels around the bronchi and arteries.

It will be necessary hereafter to discuss the work of Miller on the adult lymphatic system, in connection with the later stages; therefore it will suffice to refer here to the statement which Flint discussed in the quotation given above. Miller has called attention to the fact that the terminal vein lies in the periphery of the lobule and that the lymphatics accompanying the vein communicate with those of the pleura. He cites Councilman's (1900) description of the interlobular vessels, but does not claim to have found the same vessels. I think that these different views will be reconcilable when we have followed the development of the lymphatics through the various stages that lead to the adult form. The literature on the lymphatics of the adult mammalian lung is very large, and for a comprehensive review of it the reader is referred to the papers of Miller (1893, 1896, 1900, 1902, 1911). It seems needless to discuss it more at length here.

THE VESSELS ARISING FROM THE LEFT DUCT.

As has been said, the lymphatics of the lungs arise partly from the two thoracic ducts by sprouts. These vessels grow to the mesenchymal wall of the trachea and form there a plexus which sends vessels down into the lungs. Other vessels grow directly into the lungs.

The thoracic duct, as has been shown by Sabin (1913), Baetjer (1908), and Kampmeier (1912), is complete—that is, it connects the jugular sac with the retro-peritoneal sac—in a pig embryo 2.5 cm. long. Very soon after this the first evidence of the pulmonary supply may be found. I have obtained partial injections at 2.8 cm., and have found some small vessels in serial sections at 2.6 cm.; so it is evident that these sprouts are either formed from the thoracic duct as it grows down or very soon after the primary system is completed.

About midway between the jugular anastomosis and the arch of the aorta the thoracic duct leaves its position lateral to the trachea and bends dorsalward to lie near the dorso-lateral border of the esophagus. In this position it comes down behind the arch of the aorta. This transition is shown by Sabin (1913, figures 12 and 13). Just at the point where the duct begins to bend dorsally the earliest sprout to the lung is formed. At this point a single large vessel buds off from the thoracic duct and passes down over the arch of the aorta to reach the hilum of the lung. This vessel unites with the vessels that grow up from the thoracic duct just caudal to the arch of the aorta and forms the lower part of the tracheal plexus. This vessel usually persists in the adult as one of the drainage trunks from the hilar nodes to the thoracic duct. It is shown in figure 5, plate 1, and figure 2, plate 4, marked with an asterisk. From the region of the thoracic duct, where this vessel buds off to a point about the level of the aortic arch, a number of other vessels are formed very soon afterwards. These vessels arise very close together and grow across to the lateral wall of the trachea, where they anastomose and form the primitive left tracheal plexus; they lie in the undifferentiated mesenchymal tissue that surrounds the tracheal lumen. These lymphatics have formed a plexus by the time the embryo has reached a length of 3 cm. From this plexus vessels grow across the trachea to anastomose with other vessels from the similar plexus on the opposite side; other lymphatics grow up the trachea and form a coarse-meshed plexus around it. This is the anlage of the adult supply of that structure. But the most important of the branches of this plexus, as far as the present work is concerned, are those from the lower part. These pass down the trachea and, being joined by other vessels that leave the duct near the arch, pass up over the bifurcation and into the lung. The left tracheal plexus is shown in figure 5, plate 1, and figures 1 and 3, plate 2. Here must be noted the fact that the plexus of the left side supplies the greater portion of the ventral surface of the trachea and forms the largest part of the great sheet of lymphatics around the primary bronchi. Later these vessels anastomose freely with those from the right side. It is important to call especial attention to the difference in the richness of the supply of the dorsal and the ventral surfaces of the trachea. There are vessels that grow to each from the left plexus, but a much greater number pass to the ventral surface than to the dorsal. Thus the plexus formed from the two lateral groups is much more closely meshed on the ventral surface, and from it is derived the greater part of the lung supply. Over the bifurcation there is a very complex group of vessels, and these form tubes around the principal bronchi as they grow on into the lung.

Below the level of the hilum several vessels, three or four in number, grow up from the thoracic duct and its plexus surrounding the aorta, to join with the large

vessel which has been described as the first to the lung and which comes over the arch to reach the hilum. These vessels from the duct below the hilum form a plexus with the vessel from above, as has been described. It is well known that the thoracic duct is double below the level of the arch of the aorta and that the two divisions are connected by numerous anastomotic vessels (figure 1, plate 2). This system is the anlage of the vessels that surround the aorta in the adult. This relation has been figured by Heuer (1909). One of the lymphatics that pass up from below to join the first vessel from the thoracic duct above leaves the duct near the diaphragm and is consequently very conspicuous in injections of this region. Heuer has figured this lymphatic as one that goes to the heart, a conclusion entirely justifiable from the general appearance of the injected specimen. Figure 1, plate 2, is from a dissected embryo 4 cm. long, in which the lymphatics were injected from the retroperitoneal sac. The thoracic duct and part of the left tracheal plexus are injected, and the extension of the plexus down on the bronchus is also shown. Below the arch may be seen some of the vessels that grow up to meet the branch from above. These vessels have been cut off, with the arch, to expose the tracheal plexus. The double duct is also shown, the more ventral element being the one figured by Heuer.

The pulmonary vessels reach the hilum when the embryo is about 2.8 cm. long, and can be seen in sections at 3 cm. (see figure 1, plate 1). The lung-tissue is at this time very slightly differentiated mesenchyme, containing the early bronchi and blood-vessels. For a further description of the structure of the lung at this stage see Flint (1906). These early lymphatics are grouped in an irregular manner in the hilum of the lung and may be found at 2.9 and 3 cm. in sections. But I have not been able to inject them earlier than 3.3 and 3.5 cm. Figure 1, plate 1, is of a section from an embryo 3 cm. long, in which the blood-vessels were injected while the embryo was still living. The lymphatics are shown as a few dilated spaces (blue) in the hilum. These vessels are beginning their invasion of the lung-tissue while the tracheal plexus is forming. It is necessary, however, to complete the description of this plexus before considering the portion of this study which relates to the lung proper. The development of the vessels within the lung-substance will be considered after the formation of the right lymphatic plexus has been described. It is important, however, to note here that all the vessels to the left lung come from the closely united group of vessels on the trachea and around the aortic arch, as has been described. This will be studied in relation to the first vessels to the lung on the right side, which will next be considered.

On the right side the development is, in general, similar to that on the left, but differs in a few particulars, chiefly relating to and in consequence of the asymmetry of the vascular system. The right duct is primarily to the heart, or perhaps to the vena cava, since it follows that vessel to reach the cardiac base. But while the heart supply is at first only from the right side, the vessels to the lung and the trachea develop at about the same time. The right duct grows caudalward parallel to the thoracic duct to the point where the vena cava arches ventralward to reach the heart. There it divides, and one branch follows the posterior wall of the vena cava to reach the cardiac base, while the other passes into the hilum of the lung. The

cardiac division, after reaching the base of the heart, along the posterior wall of the vena cava, passes around the bulbous arteriosus to reach the anterior surface of the heart, where it divides to form the primitive pericardial plexus. By introducing a canula dorsal to the vena cava and injecting towards the heart, I was able to fill this plexus in a pig 3 cm. long. At this stage it extends about one-fourth of the distance from the base to the apex of the heart. Figure 13 in Volume V of the Johns Hopkins Hospital Reports, Monograph Series (Sabín on "The Origin and Development of the Lymphatic System"), shows the right duct near the heart in an embryo pig 2.5 cm. long. In that paper attention was called to the fact that the duct grows towards the heart and that it probably represents the cardiac supply.

The second of the two terminal branches of the right duct passes down parallel to the dorsal wall of the trachea in about the same general position as that occupied by the duct above the point of division. Thus it might seem proper to consider the lung division as the more fundamental of the two, as it appears to be the continuation of the undivided duct. However, the heart branch is probably the more fundamental and the earlier of the two, since it is a general principle in the growth of lymphatic trunks for the principal vessels to follow the larger blood vascular channels. Hence we consider the left duct as primarily aortic and the right as primarily cardiac in distribution.

This vessel enters the hilum of the lung and breaks up into a few branches that are grouped around the bronchi and blood-vessels as on the left. The nature of the grouping and the further development are similar on the two sides, and hence both will be considered together. There is, however, an interesting difference between the two upper lobes, which is dependent upon the relation of the aortic arch to the hilum on the left. On the right the lung is distinctly higher (*i. e.*, nearer the neck) than on the left, because on the latter side the aortic arch lies in the groove made at the juncture of the upper lobe with the trachea. Thus the vein to the upper lobe on the left passes close to the bronchus under the aortic arch, while on the right it is well above the bronchus. This allows more freedom in the lymphatic growth on the right, so that the vessels to the upper lobe come down directly into it instead of growing back from a single group, as they do on the left. It must be understood that the stage referred to is between 2.5 and 3 cm., when the heart is still higher than the bifurcation. Later the heart passes still farther down into the thoracic cavity, and these differences disappear as the cardiac and aortic relations to the lung begin to assume their adult form. There is, however, one very important effect of this asymmetry; the lymphatics of the right duct pass directly into the lung, while those of the left must course up over the arch of the aorta and the bifurcation of the trachea to reach the lung-tissue. This has been mentioned briefly before. It is clear that the principal supply of the bronchi, and therefore, ultimately, of the lungs, comes from the left duct. This is in large measure the result of the asymmetric relations of the heart and aorta.

The development of the first vessels to the trachea and lungs on the right side will next be described in detail. From the heart limb of the right duct a few vessels arise and grow down over the vein to the upper lobe on the right side; after crossing the vein they enter the lung near the hilum and divide into several branches, some

of which anastomose with those mentioned above as growing down into the hilum of the lung from the pulmonary limb of the right duct. Other vessels turn outwards along the bronchi and veins and grow into the lung-tissue of the upper lobe. This process will be described later.

Along the right duct, cephalad to the division into the two branches, other vessels are given off; some grow down to anastomose with ascending branches lying along the tracheal wall and coming from the vessels described above, while others grow to the tracheal wall at varying positions along the section lying between the jugular anastomosis and the bifurcation, corresponding somewhat to the vessels on the other side, with which their branches anastomose, forming the tracheal supply. The earliest injection of the lymphatics of the right side were at 2.8 and 2.9 cm.

Figure 2, plate 3, shows an embryo of 3 cm., where the injection was made into the right sac, which illustrates the relative position of the vessels to the upper right lobe and the limb that follows the vena cava to the heart. This drawing is diagrammatic and does not show the different vessels to the lobes on the right side, though some of them were injected. The left duct is shown without any branches.

In figure 1, plate 2, the right tracheal plexus is represented. Though it is very incomplete, it shows the general form of the plexus and its relation to the similar plexus on the other side. The right tracheal plexus, in its simplest form, consists of a few vessels which are beginning to anastomose along the lateral wall. These anastomoses become more and more complex and numerous until, along the right side of the trachea, a plexus somewhat similar to that of the other side is formed. They differ, however, in that on the right there is no aortic arch to complicate the form. Therefore the plexus is a simple sheet-like group of vessels which lie along the lateral wall of the trachea, but do not extend up over the ventral surface of the bifurcation, except by a few anastomosing vessels. It anastomoses freely with the larger plexus from the other side on the ventral surface of the trachea, and later the combined plexuses lose their individuality and appear continuous. In the meantime the two tracheal plexuses have begun to anastomose. This will next be described.

Between 3.3 and 4.5 cm. the two tracheal plexuses anastomose by means of numerous vessels which grow around the trachea, both dorsally and ventrally. Above the level of the aortic arch these connecting vessels are far less numerous than below, where the two are merged into a sheet-like plexus that surrounds the trachea and passes down into the lungs as tubes of vessels surrounding the bronchi. Above the bifurcation the dorsal surface of the trachea has fewer vessels than the ventral, while the two original lateral plexuses are much more closely meshed, representing the anlagen of the two lateral groups of lymph nodes of the adult.

From the close-meshed plexus on the left side of the trachea just at the bifurcation a group of lymphatics pass up over the left stem bronchus and sweep across to the right bronchus, forming the upper group of vessels lying on the bronchial wall. These grow down on the side and anastomose with the vessels coming down from the plexus on the right side. Thus it will be seen that the left supply is a more important part of the general origin than the right, supplying, as it does, all of the left lung and part of the right.

It is of importance to note here that the heart is migrating downwards (*i. e.*, caudalwards) during this period, and, by the time the embryo has reached 4.5 cm. in length it has come to lie almost directly over the hilum of the lung. Hence the vessels that formerly ran in a long course from their point of origin in the heart limb of the right thoracic duct to reach the upper lobe and the hilum of the lung have become a part of the common tracheal plexus, and the formerly distinct duct to the heart has also been absorbed by the plexus over the bifurcation.

The cardiac vessels then (at 4.5 cm.) drain directly into the plexus over the hilum of the lung (figures 1 and 3, plate 2). This relation remains in the adult in the drainage of the cardiac vessels into the mediastinal nodes and the union of the efferent trunks of these nodes with those from the hilum of the lungs.

Here must be mentioned, though not bearing particularly on the lymphatics of the lungs, the connection between the right and the left ducts. In specimens of about 3.5 to 4 cm. in length, I have regularly found a vessel arising from the dorsal part of the right tracheal plexus and joining the thoracic duct behind the aorta. As has been said, it seems best to consider the vessel to the heart as the continuation of the right thoracic duct; hence this vessel must be considered, as was the one to the lung, as a part of the collateral supply.

The lung, as has been stated, also derives lymphatics from another source—the cephalad portion of the retroperitoneal sac. These vessels are growing into the lung during the period when those already described are differentiating, but it seems best to postpone the discussion of this portion of the pulmonary supply until we have studied the early changes that take place in the lung itself, following the invasion by the vessels already described. The desirability of this is evident when it is remembered that the vessels from below must follow a similar course in the lung, with the exception that this course is reversed, due to the fact that these vessels invade the lung through the pleura instead of the hilum, and must reach the other supply through the interlobular septa, to be described later.

At 3 cm. there are two primary bronchi and two veins on either side, one of each to each upper lobe and one to each lower lobe. From these the secondary branches are beginning to form. From 3 cm. to 5 cm., these secondary branches are developing rapidly and are very large in comparison to the size of the lung. The arteries are very much smaller, and the veins are somewhat larger than the arteries, but much smaller than the bronchi. It is of great importance to note the relations of these structures to each other during this period. Flint has studied their development very thoroughly, but he does not call attention to the fact, so important with reference to the lymphatics, that the developing vein is separated as widely as possible from the bronchus with which it is morphologically associated. The artery, on the other hand, follows the bronchus very closely and is distributed with it to the center of the developing lobule. The two primary branches of the pulmonary vein lie close to the corresponding bronchi. This is, indeed, as far separate as is possible, since there is almost no lung-tissue at this period, while the secondary vessels which may be considered the terminal branches lie about equidistant from the two adjacent bronchi. The arteries follow the bronchi more closely. This fact is of the greatest importance in the development of the lymphatics and also in the relation of the veins to the periphery of the lobule in the adult, as has been shown by Miller (1900).

As the lung increases in size and the veins and bronchi which we have termed secondary give off other branches, these in turn become the terminal ones and assume the relations that have been described. The others are, by the increasing amount of lung-tissue, forced closer together. Thus it is seen that it is only the terminal veins that occupy the position described; that is, pass along the periphery of the lobule. In the pig there is considerable connective tissue forming definite lobules in the adult lung; and these septa, bounding as they do the area supplied by terminal bronchi, divide the lung into a large number of irregular cones or pyramids, which have the bronchus and artery in the center and the veins passing along the periphery until close to the apex, where they enter veins of the next larger size. For further discussion of this arrangement see Miller's article (1900).

As we have seen, a few dilated lymphatics are found in the hilum of the lung at 2.9 and 3 cm. These are the first branches from the vessels that are forming the plexus on the trachei and bronchi already described. The bronchi, as has been said, are surrounded by lymphatics which follow them into the lung-tissue; and, as secondary bronchi are formed, lymphatics from these plexuses branch off to follow them.

The primary veins lie very close to the corresponding bronchi at this stage, and are accompanied by a few lymphatic trunks which arise from the same general plexus that covers the bifurcation. These vessels anastomose very richly with those of the bronchi, and, close to the point where the trachea divides, they merge together. We have seen that the secondary veins lie midway between the adjacent bronchi, and represent the outer border of the primitive lobule of the developing lung. Along these veins the lymphatics grow towards the pleura: they are derived both from the plexus that follows the primary vein and from the vessels that surround the primary bronchi. The lymphatics from the bronchial supply join those from the vein, and the combined group passes along the vein, spreading out on either side to form a sheet, until the vessels reach the pleura. Flint observed these sheets of lymphatics, but thought that there must be some difference in the density of the tissues to account for their leaving the bronchi to run midway between. He did not recognize the relation between the veins and the lymphatics. It will be clear, when it is remembered that the smaller branches of one vein spread out fan-like to meet those of the other vein, that the sheets of lymphatics lying between the bronchi are directed by the veins as well as the separate lymph-vessels directly associated with them.

In this manner the true primitive lobules are formed by the interpolation of a sheet of rapidly growing lymphatics between the bronchial tubes. It is along the distal margin of these plexuses that the pleural marking begins. When these vessels reach the pleura there is a marking-out of the characteristic coarsely-meshed plexus, each interspace corresponding to the sheet beneath (figure 3, plate 1). It must be remembered that these vessels, growing as they do very rapidly, reach the pleura very early, and hence the pleural plexus is developing while the above-mentioned interlobular plexuses are forming. We have so far described only the formation of the large parallel plexuses shown in figure 1, plate 4, figure 2, plate 5, and figure 1, plate 3. But the formation of veins in other planes directs the growth of the lym-

phatics, so that with each bronchus there are several veins and several sheets of lymphatics developing. Thus the series of cone-shaped or pyramid-shaped lobules are surrounded by plexuses of lymphatics. Along these plexuses the differentiation of the connective-tissue layers takes place, for, when the lymphatics invade these areas, there is only an undifferentiated tissue, which is characteristic of the lung. Flint suggested that the lymphatics followed the bronchi for a certain distance and then turned away midway between them, because of some relative difference in the density of the tissues. It is quite impossible to observe the relation to the veins in uninjected sections, and consequently this point was not discussed in relation to the problem of the question of tissue density. Notwithstanding this phase of the development which Flint was unable to follow, there still remains considerable probability in his suggestion. The fundamental reason for the direction of growth is as yet entirely a mystery, but there seems to be little doubt that the principal lines of lymphatic development are along the larger blood-channels; and, in general, the veins are chosen, though the left duct may be considered as following the aorta.

The much slower-growing lymph-vessels on the bronchi follow each branch out towards the periphery. The primary bronchus is surrounded by a very close-meshed plexus, which consists of a large number of vessels; in cross-section one can count from 50 to 75. However, this number is very greatly reduced on the secondary bronchi, each of which has four or five trunks following it. These are closely bound together by anastomosing collaterals.

With reference to the secondary bronchi, almost the same series of events occur as given above for the primary ones. These secondary bronchi are likewise marked off by interlobular septa in which the lymphatics develop more rapidly than along the bronchus whose lobule they mark off. The lymphatics around the bronchus give off small vessels near each branch of the bronchus, and these pass across to join the plexuses that surround the area of the lobule (figure 1, plate 3). As the new-formed bronchi grow larger they are, in turn, followed by two or three lymphatics, which end, as did those around the secondary bronchi, by passing over to join the septa or, if close to the pleura, the vessels there. These lymphatics that pass from the bronchial system to join those in the septa follow the branches of the veins which bend in from the septa to reach the capillary bed of the arterial tree. These persist in the adult as the vessels that pass from the bronchus to the vein and thence to the pleura (figure 2, plate 1).

We will consider now the lymphatics that grow up from the retroperitoneal sac into the caudal pole of the lower lobe.

In 1906 F. T. Lewis described, in rabbit embryos, a lymphatic sac just median to the mesonephritic vein. Baetjer (1908) showed that it arises from the ventral surface of the large vein which connects the two Wolffian bodies (embryos 17 to 23 mm.); Heuer, following Baetjer, found that numerous lymphatic sprouts arise from this sac and invade the intestine through the mesentery. This sac supplies lymph-vessels to the stomach, the liver capsule, the Wolffian bodies, and the reproductive glands.

The lower pole of the lower lobe of the lung is continuous with the mesentery in the early stages. As the embryo develops, this connection becomes a thin band

of tissue that passes down behind the diaphragm to end in the tissue around the aorta; it corresponds to the ligamentum pulmonale in the human. It is through this prolongation of the lower lobe that the lymphatics from the retroperitoneal sac grow up to reach the lung. These vessels arise from the cephalad portion of the sac and pass up behind the dorsal wall of the stomach to enter this long posterior or lower pole of the lung (figure 2, plate 4). There are three or four vessels that grow out from the sac and up into the lung; these are closely associated with those that pass to the diaphragm and, in adult life, join with them just before reaching the nodes into which they drain. They pass upward and divide, on reaching the lung, into two groups, one of which passes up over the diaphragmatic surface and the other over the outer or lateral surface of the lower lobe.

The anlage of the ligamentum pulmonale is connected not only with the lower pole of the lung, but also with the median border of the lower lobe. Thus the lymphatics grow directly up about one-third of the way to the hilum in this medial extension of the ligament, and from there sweep out in a fork-like division which produces the two plexuses on the two borders of the lung (figure 3, plate 5). I have injected these vessels at 3.4 cm.; but I think that they reach the lung border a little earlier.

From the two plexuses described above vessels grow into the lung in exactly the reverse order to that followed by those developing from the hilum. They grow in just where they will meet the veins, and along these form the septal plexuses, exactly similar to those described above. These rapidly anastomose with the other lymphatics, and, by the time the embryo has reached 4 cm. in length, the entire lung is uniformly supplied.

It is very pertinent to inquire why the lymphatics that reach the lung from below select these points for the invasion of the deeper tissue of the lung. However, when it is recalled that the lymphatic vessels which lie in the mesenchymal tissue (the pleural anlage) are very large in proportion to the other structures and that the budding vessel would be in direct relation to the outgoing veins, it is easily understood that exactly the same causes must be acting here as those which direct the growth from above. So here, as above, the position of the veins controls the direction of growth. Of course, the plexuses on the two surfaces become more complex as the lung is invaded and follow the same steps as the pleural supply in general. As has been said, there are branches along the pleura, and these anastomose with the other pleural vessels, so that the supply becomes general. The drainage in the early stages—that is, before the formation of the valves—is probably divided; the flow of lymph might be to the retroperitoneal sac via the vessels that grow up from that structure, or to the thoracic ducts through the tracheal plexuses and the vessels accompanying the veins and the bronchi.

We have seen how the lymphatics grow into the lung-tissue and there form two distinct groups, and how one of these rapidly reaches the pleura and there forms the characteristic plexus-pattern marking off the boundaries of the lobules; also how the vessels grow into the posterior poles of the lower lobes and anastomose with the system from above, which follows the veins in the connective-tissue septa.

Now, it will be well to review briefly the state of the development of the lung lymphatics at the time that the primary system is complete—that is, in 6 cm. embryos. At 6 cm. the lymphatics around the trachea form a close-meshed plexus near the bifurcation, extending down into the lung around the bronchi. Above the bifurcation there are only a few connecting vessels on the ventral and dorsal surfaces of the trachea, but the two plexuses on the lateral surface are very close-meshed. From the left plexus the principal supply of both lungs is derived, but there are numerous vessels passing down into the right lung from the right plexus, and the two are closely bound together, especially near the bifurcation, where they have fused into one plexus. The vessels surrounding the bronchi follow them towards the periphery, giving off branches to the venous tree at every division of the bronchial tree. Each smaller bronchus derives its lymphatic supply from the plexus that accompanies the parent bronchus. These vessels are very difficult to inject.

Accompanying the primary divisions of the pulmonary vein there is another group of vessels that is closely bound, by anastomoses, to the lymphatics around the principal bronchi (figure 4, plate 1). Along each of the tributary veins vessels pass to the pleura and spread out in the region that has been described as the septa between the lobules. Each of these dividing sheets anastomose with other sheets and with the pleural vessels. The vessels derived from the retroperitoneal sac are continuous with those derived from the two duets; there can be determined no line of differentiation either within the lung-tissue or on the pleural surface. The posterior pole is connected with the retroperitoneal sac by three or four vessels that pass down in the fold of tissue that precedes the *ligamentum pulmonale* (figure 3, plate 5). The pleural plexus has begun to form within the gross markings that we have described as corresponding to the connective-tissue septa. These vessels are very superficial and are not connected, at this time, with the deeper vessels.

The further development is chiefly due to the multiplication of the lung units and the increase in volume of the interbronchial tissue. As new bronchi are formed, new groups of lymphatics bud off from the plexus that accompanied the parent bronchus and follow the new-formed structure towards the periphery. These lymphatics leave the bronchus and pass to the venous group when they reach the region where the air-sacs are developing.

As the lung-tissue differentiates further and further, the larger veins become more closely associated with the bronchi and only the terminal vessels are peripheral with reference to the lobule. This brings about the relations that are found in the adult, where the principal veins and bronchi are closely associated, while the terminal ones have the same relative positions that have been described for the developing structures.

The arteries in early stages lie very close to the bronchi and are associated with the plexuses that follow that structure. As these blood-vessels increase in size the bronchial plexus differentiates into two parts, following the arteries and the bronchi. This is accomplished by the growth of vessels around the arteries, and, as the artery increases in size, the two plexuses become entirely distinct, but are still connected by numerous anastomotic vessels.

In the meantime, the vessels of the pleura, which at from 5 to 6 cm. we have seen beginning to form the true pleural plexus, continue to proliferate, and thus form a fine-meshed plexus in the pleura between the blocking-off of the lobules.

The completion of the primary plexus is shown in figure 3, plate 1. This is the surface of the lung in a pig embryo of 6 cm. with the pleural vessels injected. Each of these uninjected areas represents a primary lobule, and the surrounding lymphatics mark out the connective-tissue plexuses. Figure 1, plate 5, illustrates one of the primary lobules, and the close-meshed plexus is the true pleural supply. It is still seen to be connected with the deep vessels of the septum.

Here and there one finds vessels passing from the terminal bronchi to the surface, in the lobule proper, to join with the fine-meshed plexus of the pleura. These pass around the air-cells, but are never found on their walls, and, uniting with the terminal vessels of the end veins, pass to join those in the pleura. These are the vessels described by Flint (1906) as seeming to dip down into the lobules from the pleura; these, he said, he could follow only a little way into the lobule. This is easily understood from the information gained from injections, for the vessels around the bronchi can not be seen in uninjected specimens, and consequently those which remain patent in sections seem to end abruptly in the midst of a lobule, whereas they in reality connect with those following the bronchi and terminal veins. The lymphatics that follow the terminal bronchi leave them just before the atria are reached and cross over to join the lymphatics which follow the veins. The lymphatics which accompany the veins pass to the pleura just where the veins bend to reach the center of the lobule.

Flint first observed the submucous plexus of the bronchi and trachea in embryos 23 cm. long. It was surprising that injections did not reveal this plexus very much earlier. I have not been able to demonstrate any lymphatics in the submucosa before the embryo reached a length of 19 cm. This plexus develops, as do all the secondary plexuses, by the outgrowth of vessels from the primary one and their coalescence to form the new group. This process has been carefully studied by Heuer in the formation of the mucosal plexus in the intestine. The submucosal plexus is complete just before birth and consists of numerous fine vessels that lie just beneath the bronchial epithelium. From this plexus numerous vessels pass down between the cartilaginous rings and join the lymphatic trunks which follow the bronchi, as has been described. In those bronchi having no cartilaginous rings there is only the one group of lymph-vessels to be found, and these have already been described.

The lymphatics of the adult lung were first described by Olaf Rudbeck in 1651-1654 (quoted from Miller, 1900). Since that time numerous workers have studied these vessels. In 1900 W. S. Miller reviewed the literature very thoroughly, and it will, therefore, be unnecessary to repeat that here. Miller studied the lymphatics in the lungs of adult cats and dogs by injecting them from one of the pleural vessels. He divided the lymphatics into four groups, as follows:

- | | |
|-----------------------------------|------------------------------------|
| A. The lymphatics of the bronchi. | C. The lymphatics of the arteries. |
| B. The lymphatics of the veins. | D. The lymphatics of the pleura. |

The lymphatics of the bronchi.—Miller describes two sets of lymph-vessels associated with those bronchi which have cartilaginous rings and only one with those which have no rings. In the former the two sets are connected by vessels that pass between the rings and join the trunks situated on the outer side of these structures. These trunks drain the lymphatics that accompany the smaller bronchi and empty into the nodes which are situated at the hilum of the lung. While there are several lymphatics accompanying the larger bronchi, only three are to be found with those nearer the air-sacs. These end by leaving the terminal bronchus just before it ends in the atria; one of them passes to the artery, while the other two join the lymphatics of the vein.

The lymphatics of the veins.—There is a single group of vessels that extends from the terminal vein to the hilae nodes. Along the larger veins there are several vessels, but the terminal ones are accompanied by only one or two lymphatics. Anastomatic vessels pass from the bronchial lymphatics to join those of the vein at each branching of the bronchial tree. The lymphatics that accompany those veins which go to the pleura join the pleural lymphatics.

The lymphatics of the arteries.—The lymphatics which accompany the arteries are very similar to those of the veins, with the exception that none of them pass to the pleura.

The lymphatics of the pleura.—There is only one plexus in the pleura, and this drains through several large trunks to the nodes at the hilum. There are anastomoses with the lymphatics of the veins, as has been said, but the drainage probably does not pass through these. Miller put his canula into a large pleural vessel and injected towards the hilum. After some time the deep lymphatics, as well as those of the pleura, were filled. He thought that the injection mass backed up into the deep vessels from the nodes at the hilum, since both the sets of vessels drain into the same nodes.

Miller does not confirm the findings of Sappey (1874) and of Councilman (1900) with regard to the interlobular lymphatics. Sappy thought that it was wrong to divide the lung lymphatics into superficial and deep groups on account of the rich anastomosis of these vessels. He thought that the lobules were surrounded by lymphatics which formed networks between the adjacent lobules in much the same manner as the blood capillaries do around the air-sacs. Councilman divided the deep lymphatics of the lung into two sets, the bronchial and the interlobular; the latter he interpreted as very important in infections.

While Miller does not agree with these observers in regard to the interlobular lymphatics, he does describe anastomoses between the lymph-vessels of the venous radicles and those of the pleura, and he emphasizes the peripheral location of the veins. It might well be that the vessels which Sappey and Councilman found in the interlobular septa were the lymphatics of the veins, since they did not have very accurate methods for the differentiation of these structures. It becomes more difficult to reconcile Miller's findings with those of Flint and the results of this study. Both Flint and I have found distinct groups of vessels in the interlobular connective tissue in embryo pigs. These groups of vessels are directed in their growth and location by the position of the veins, but are not limited in their distri-

bution to the venous trunks. The fact that so careful an observer as Miller does not find these lymphatics in the septa suggests the possibility that the assumption of mature activities in some way brings about the atrophy of all of the interlobular lymphatics except those that accompany the veins. Again, this plexus may be peculiar to the pig. It seems necessary that this question must remain unsettled until studied by some method other than simple injection.

The question of the drainage of the lung lymphatics is of exceptional interest and importance; and while we must depend, for the final settlement, upon physiological methods, there is much evidence available from morphological observations. In the larger vessels on the bronchi, the veins, and the arteries there are valves which point towards the hilum. This is assumed to be very good evidence that the flow is in that direction. No valves have been described in the lymph-vessels which accompany the smaller bronchi, veins, and arteries. Hence it can not be stated whether the lymph flow, in the lymph-vessels of the veins, is towards the pleura of the hilum; and, in like manner, the flow in the bronchial vessels might be either towards the hilum or towards the veins and arteries. With regard to the vessels on the pleura, all of the lymphatics above a certain regional level of the lower lobe drain either towards the mid-line and then course up in the pulmonary ligament to end in the nodes at the hilum, or pass by direct paths to these nodes. Those below this level drain to the nodes lying in the mesentery of the lesser curvature of the stomach. Some of these drain as do those above—towards the median and pass down in the ligamentum pulmonale—while others pass directly down from the posterior pole. This group of vessels which pass to the preaortic nodes drains about one-third of the lower lobe of the lung. This varies considerably; in some specimens as much as half of the lung has been found to drain in this direction.

This peculiar drainage of the lower lobes seems especially important from the bearing that it may have on the pathology of the lungs. It has long been known that the diaphragmatic vessels drain to these nodes, but there is no connection between these vessels and those of the lung proper. The lymphatics that pass through the pulmonary ligament apparently drain only the pleura; but, as has been shown, the deep lymphatics anastomose with those of the pleura, and therefore it seems possible for substances to pass from the lung-tissue to the preaortic nodes. What bearing this may have upon the pathology of the lungs or of the abdomen remains to be settled.

SUMMARY.

The lymphatics of the lungs are derived from three sources—the right and the left thoracic ducts and the retroperitoneal sac.

In embryos 2.6 to 3 cm. long, vessels bud off from the thoracic duct and grow across to the trachea, forming there a plexus that gradually extends over the ventral surface of the trachea, and especially down over the bifurcation. From this plexus vessels pass into both lungs and into the pleura.

The right thoracic duct divides, in embryos about 2.5 cm. long, into two vessels. One passes to the heart, while the other breaks up to form a plexus on the right lateral wall of the trachea. Some vessels from this plexus pass down into the hilum

of the right lung, while others anastomose with the plexus from the left side, which extends up over the trachea. The development of the lymphatics within the lung depends upon the division of the vessels into two groups—those associated with the veins and connective-tissue septa, and those associated with the arteries and the bronchi.

The former grow very rapidly, and following each of the branches of the pulmonary vein, pass to the pleura. There are at first only two or three lymphatics with each vein. In the early stages the terminal veins lie about midway between the adjacent bronchi, and in this plane a sheet of lymphatics develops from the vessels surrounding the veins and passes to the pleura, where they mark out the boundaries of the distribution of each bronchus. These vessels anastomose with those that grow direct to the pleura from the plexus on the trachea.

The bronchial vessels develop more slowly and at first are to be found only around the larger bronchi. As these structures increase in size and number, the lymphatics surrounding the main bronchi send vessels to the smaller ones and these form a plexus around each of the bronchi, so that the bronchial tree is surrounded by a continual series of branching tubes made up of lymphatic vessels. From every point of division of the bronchi, lymphatic vessels pass to the lymphatics of the veins; those around the terminal bronchus leave it near its ending in the atria, and pass to join the lymphatics of the veins or septa, or, more rarely, those of the pleura.

Lymphatics also arise from the retroperitoneal sac and grow up posterior to the diaphragm to enter the lower pole of the lower lobe of the lung. These vessels form a plexus on the median surface of the lower lobe, and send branches both to the pleura of the other surfaces and into the lung along the veins. Plexuses develop here as with those that come from above and the two groups soon anastomose.

The further development consists in the multiplication of the plexuses on the bronchi and blood-vessels, following their continued differentiation. As the lung increases in size, the larger veins become approximated to the bronchi and only the terminal ones are separated from them; these lie in the periphery of the lobule. Connective tissue is formed along the sheets of lymphatic vessels, and these become the septa of the lung, containing a definite set of vessels which develop from the early vessels following the veins. The lymphatics accompanying the veins remain connected with those of the bronchi and septa.

The common plexus surrounding the artery and bronchus is separated into two individual plexuses, incident to the increase in size of the artery; however, these continue to have anastomosing branches.

The vessels of the pleura mark out the early connective-tissue septa, but later there develops a fine-meshed plexus between these larger vessels, which is not connected with the vessels of the lung-tissue. The valves begin to form at about 6 cm. and, in general, point away from the pleura. None, however, have been found in the smaller vessels which accompany the terminal bronchi.

In the adult there are lymphatic vessels accompanying the bronchi, the arteries and the veins; these anastomose freely. There are also vessels in the connective-tissue septa which drain chiefly into those around the veins, and, to some extent,

into those of the bronchi and arteries, near the point where the vein and the bronchus separate to take their relative positions with relation to the lobule. There are numerous anastomoses between the deep vessels and those of the pleura, but probably most of the flow is towards the hilum. All the deep vessels, together with the greater number of the pleural vessels, drain into the nodes at the hilum; but the vessels of the lower half of the pleura of the lower lobe drain through several vessels to the preaortic nodes. These vessels pass through the ligament of the lower lobe and behind the diaphragm.

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EXPLANATION OF PLATES.

PLATE 1.

- FIG. 1. Diagram of transverse section of left lung of an embryo pig 3 cm. long, in which the blood-vessels were injected through the umbilical artery with india ink. The lymphatics appear as dilated spaces (blue). The section is 20μ thick and is stained with hematoxylin and eosin, aurantia, and orange G. $\times 55$. *Ao*, aorta; *T*, trachea.
- FIG. 2. Diagram of section through lobule of lung of an embryo pig 7 cm. long, in which the lymphatics were injected with india ink through the left tracheal plexus. The veins were slightly injected by the rupture of a lymphatic vessel into a vein near the hilum. The section is 100μ thick and is unstained. $\times 47.5$. *A*, artery; *B*, bronchus; *V*, vein; *Pl*, pleura.
- FIG. 3. Surface of lung of an embryo pig 6 cm. long, in which the lymphatics were injected with india ink through the left tracheal plexus. The section was taken from the ventro-lateral surface of the left lower lobe and is about 200μ thick and is unstained. $\times 29.4$. *P L*, primary lobule.
- FIG. 4. Longitudinal section of lung of an embryo pig 6 cm. long, in which the lymphatics were injected with india ink through the left tracheal plexus. The veins contain some blood pigment. The section is 400μ thick and is unstained. $\times 33$. *V*, vein; *B*, bronchus.
- FIG. 5. Diagram of left tracheal plexus in an embryo pig 6 cm. long, in which the lymphatics were injected through the thoracic duct. Cleared by Spalteholz method. Note that part of the vessel marked with an asterisk (*) has been removed in dissecting the body-wall away. This vessel is the one described as the first to the lung. $\times 15$. *, first vessel to lung; *T*, trachea; *Th D*, thoracic duct; *L T P*, left tracheal plexus; *Ao*, aorta.

PLATE 2.

- FIG. 1. Dissection of an embryo pig 4 cm. long, in which the lymphatics were injected with prussian blue through the retroperitoneal sac. The heart, aortic arch, left lung, and the body-wall have been removed. Cleared by the Spalteholz method. $\times 19$. *Th D*, thoracic duct; *R Th D* right thoracic duct; *R T P*, right tracheal plexus; *L T P*, left tracheal plexus; *C L*, cardiac lymphatics; *Ao*, aorta; *B*, bronchus; *Oc*, esophagus.
- FIG. 2. Section of a small area of the lung of an embryo pig 7 cm. long, in which the lymphatics were injected with prussian blue through the retroperitoneal sac. Drawing to show the relation of the peri-bronchial lymphatics to the wall of the bronchus. Section is 20μ thick and is stained with hematoxylin and eosin, aurantia, and orange G. $\times 93$. *B*, bronchus.
- FIG. 3. Dissection of an embryo pig 4 cm. long, in which the lymphatics were injected with prussian blue through the retroperitoneal sac. The left lung, the arch of the aorta, the pulmonary artery, and the body-wall have been removed. Cleared by the Spalteholz method. The left tracheal plexus is shown as a solid blue mass because the meshes are so close that they could not be analyzed in the drawing. $\times 15$. *Th D*, thoracic duct; *R Th D*, right thoracic duct; *Ao*, aorta; *L T P*, left tracheal plexus; *B*, bronchus.
- FIG. 4. Longitudinal section of upper lobe of right lung of an embryo pig 6 cm. long, in which the lymphatics were injected with prussian blue through the retroperitoneal sac, and the veins were injected with india ink through the pulmonary vein. The section is 400μ thick and is unstained. Cleared by the Spalteholz method. $\times 39$.

PLATE 3.

- FIG. 1. Small block of an embryo pig 15 cm. long, in which the lymphatics were injected with prussian blue by puncture of an interlobular septum. The arteries were injected with india ink through the pulmonary artery. Cleared by the Spalteholz method and mounted in balsam. The specimen was mounted at a convenient angle to best show the interlobular septum; unfortunately, it was jarred out of position while being drawn and hence the group of lymphatics in the septum is shown bent to one side. $\times 40$. *Pl*, pleura; *A*, artery; *I L S*, interlobular septum.
- FIG. 2. Diagram of a dissection of an embryo pig 3 cm. long, in which both the right and left jugular sacs were injected and, from them, the right and the left thoracic ducts respectively. India ink was used. The body-wall, heart, and left lung have been removed. Cleared by Spalteholz method. $\times 30$. *Th D*, thoracic duct; *R Th D*, right thoracic duct; *V C S*, vena cava superior; *Ao*, aorta; *P A*, pulmonary artery; *C L*, cardiac branch of the right thoracic duct.
- FIG. 3. Diagram of a section of the right lung of an embryo pig 6 cm. long. This is the same specimen from which figure 4, plate 2, was made; the part of the section shown in that figure is indicated by an X. $\times 20$.

PLATE 4.

- FIG. 1. Longitudinal section of the left lung of an embryo pig 5 cm. long, in which the lymphatics were injected with prussian blue through the retroperitoneal sac. The veins have some blood pigment in them. The section is 400μ thick and is unstained. $\times 22$. *V*, vein.
- FIG. 2. Dissection of an embryo pig 4 cm. long, in which the lymphatics were injected with prussian blue from the retroperitoneal sac. The right lung, esophagus, and body-wall have been removed. The stomach was pulled to the left side of the embryo in order to expose the retroperitoneal sac. Cleared by the Spalteholz method. $\times 20$. *Ao*, aorta; *L L*, left lung; *Th D*, thoracic duct; *D*, diaphragm; *R P S*, retroperitoneal sac; *, first vessel to the lung.

PLATE 5.

- FIG. 1. Surface of lung of an embryo pig 23 cm. long, in which the lymphatics were injected with prussian blue, by puncture of an interlobular septum. Cleared by Spalteholz method. The interlobular septum is indicated by a very large lymphatic trunk. $\times 28$. *I L S*, interlobular septum.
- FIG. 2. Longitudinal section of the upper portion of the lower lobe of the left lung of an embryo pig 5 cm. long, in which the lymphatics were injected with prussian blue through the retroperitoneal sac, and the veins have retained a little blood pigment. The section is 400μ thick and is unstained. $\times 57$. *V*, vein; *A*, artery; *Pl*, pleura; *B*, bronchus.
- FIG. 3. Lower portion of left lung of an embryo pig 5 cm. long, in which the lymphatics were injected with india ink through the retroperitoneal sac. Cleared by the Spalteholz method and mounted in balsam. $\times 28$.



Fig. 100

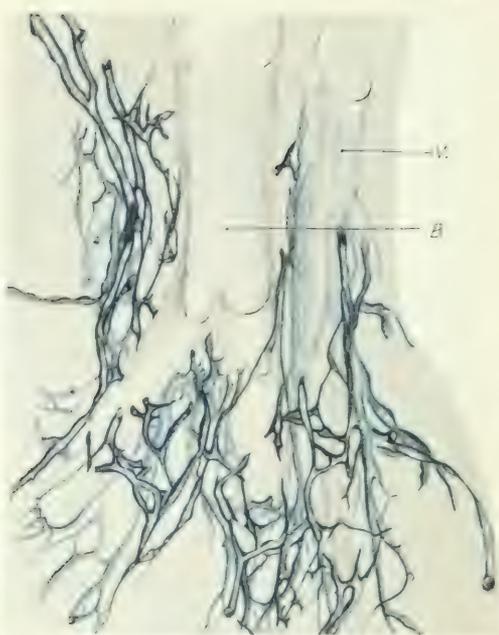


Fig. 101



Fig. 102

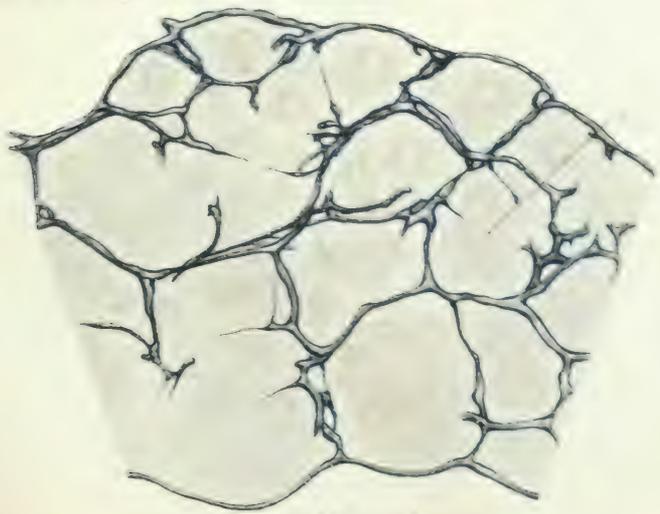


Fig. 103



Fig. 104

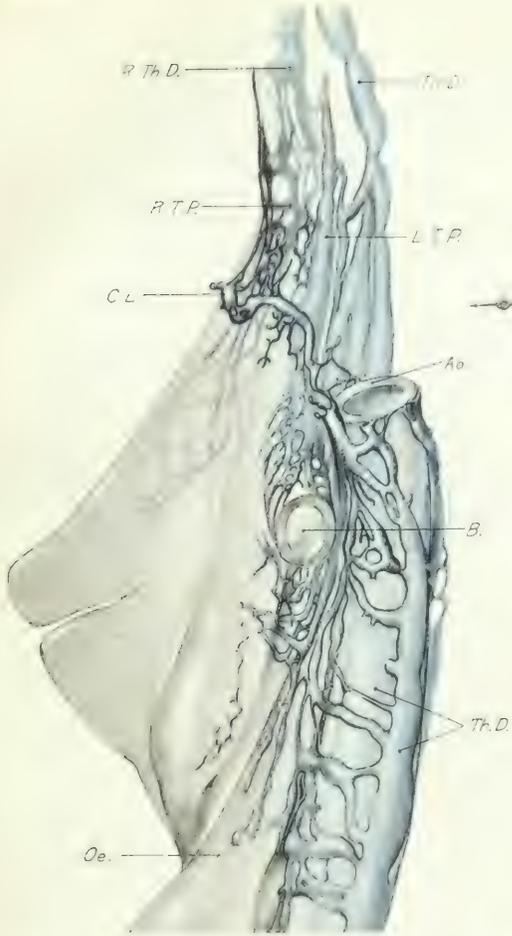


FIG. 1 (C. S. 1889)

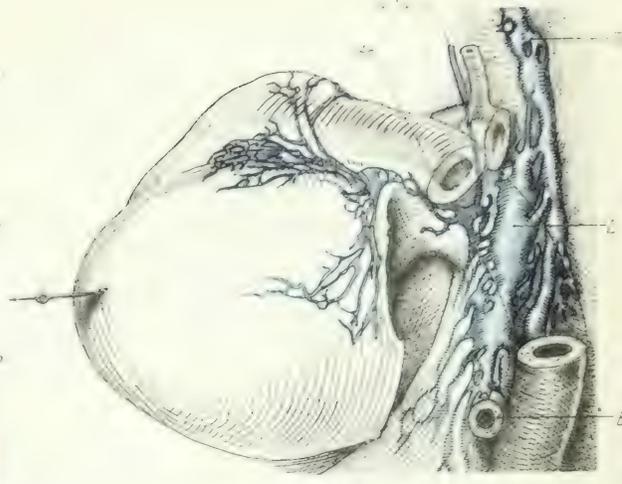


FIG. 3 (C. S. 1889)

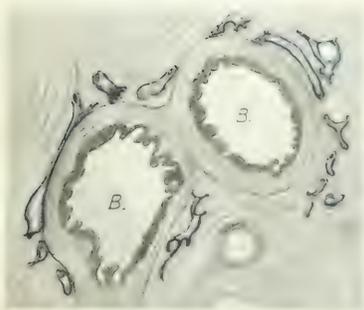


FIG. 2 (C. S. 1889)

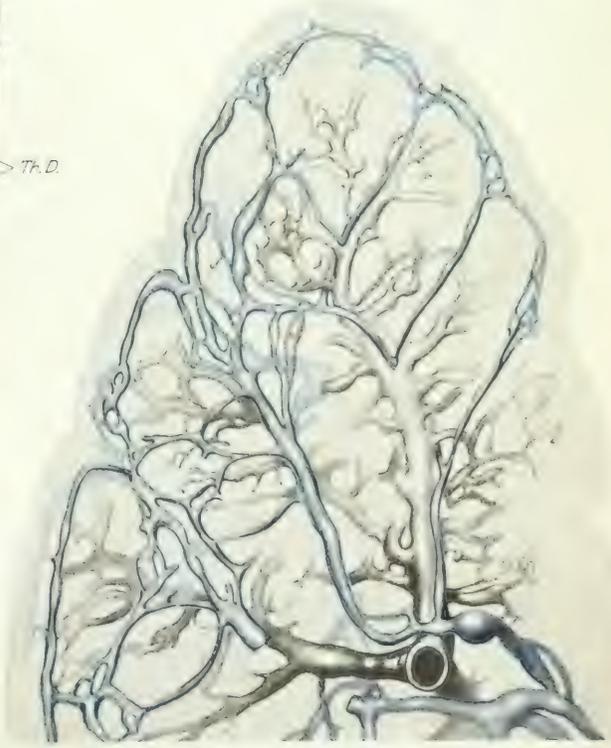


FIG. 4 (C. S. 1889)

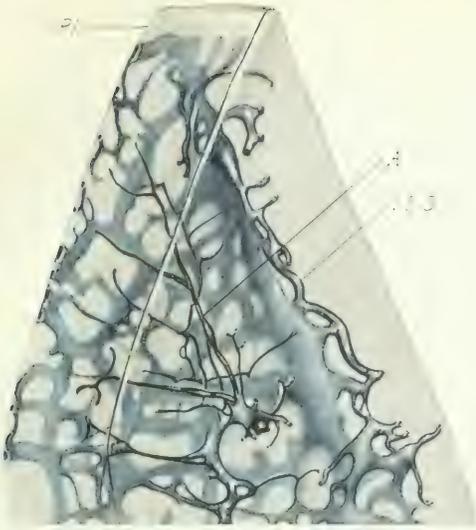


Fig. 1 (C. R. 17 m)

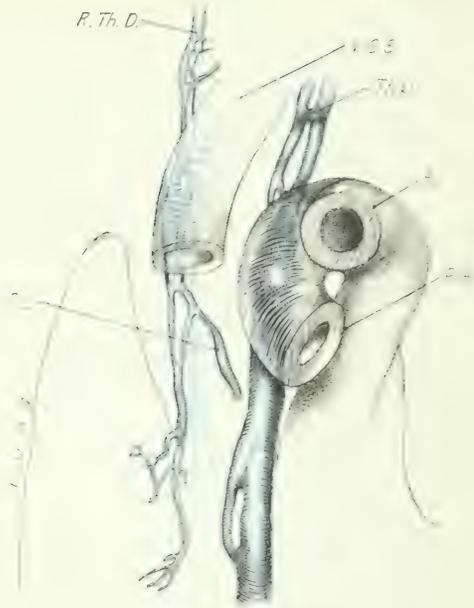


FIG. 2 (C. R. 20 m)

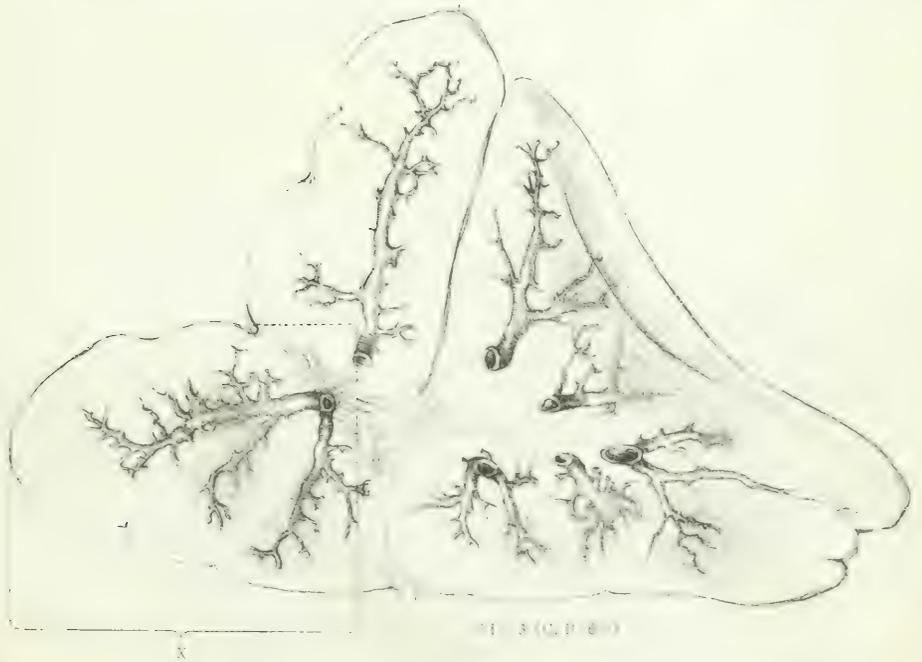


FIG. 3 (C. P. 8 m)



Fig. 2 (C. R. 4cm)

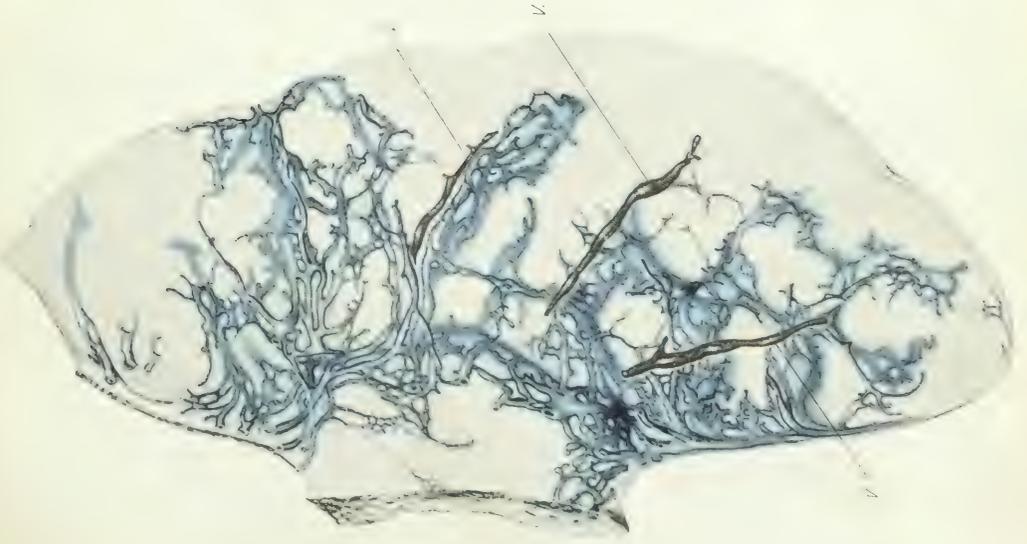


Fig. 1 (C. R. 5cm)

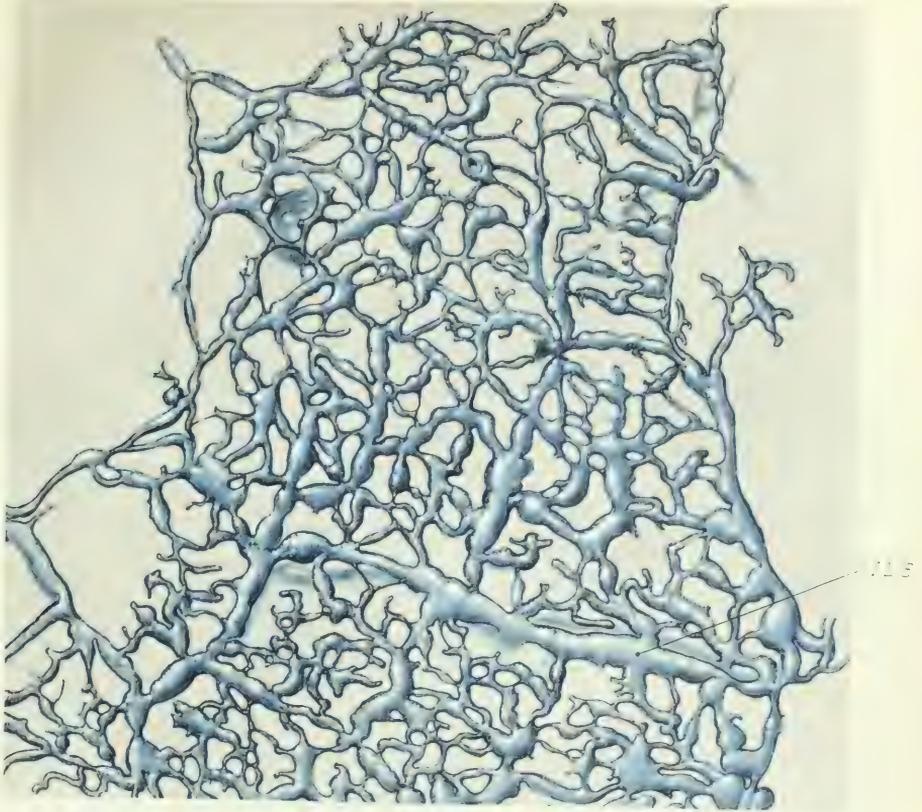


Fig. 1 (C. R. 20m)



Fig. 2 (C. R. 20m)

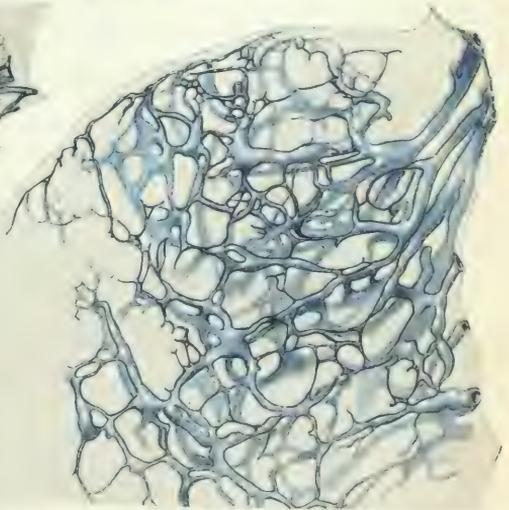


Fig. 3 (C. R. 20m)

CONTRIBUTIONS TO EMBRYOLOGY, No. 13.

BINUCLEATE CELLS IN TISSUE CULTURES.

BY CHARLES C. MACKLIN.

Four plates, containing seventy figures.

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BINUCLEATE CELLS IN TISSUE CULTURES.

By CHARLES C. MACKLIN.

INTRODUCTION.

In examining a living tissue culture, or a preparation from the same, one frequently finds a cell which contains two or more nuclei, of about equal size, slightly separated or in contact. In a communication of Lewis and Lewis (1912 *c*, fig. 12) a binucleate cell from a tissue culture is shown; more recently these authors (1915, p. 391) have referred to the occurrence of such cells in tissue cultures under the heading "Amitosis and giant cells." That they may be quite numerous in an area of new growth is seen by referring to figure 1, where one quadrinucleate and six binucleate cells appear in a small field.

The question of their origin and fate in cultures of embryonic tissue, involving as it does the idea of direct nuclear division, gathers interest from the fact that such cells are found in embryonic tissue developing *in vivo*, and from the further fact that they probably represent the first stage in the formation of certain giant cells. The problem of inquiring into their history by prolonged observation of the living cell was suggested by M. R. and W. H. Lewis.

METHOD.

Cultures were grown in ordinary hanging-drop preparations, the technique of W. H. and M. R. Lewis (1911, 1912*a*, 1912*b*, 1915) being employed. The tissue was obtained from embryo chicks of from three to ten days' incubation. Heart tissue was most frequently used, and gave very satisfactory results.

Locke solution as a culture medium was used. A stock saline solution was first made up as follows: NaCl, 18 grams, 0.9 per cent; KCl, 0.84 gram, 0.042 per cent; CaCl₂, 0.5 gram, 0.025 per cent; NaHCO₃, 0.4 gram, 0.02 per cent; H₂O, 2,000 c.c. Freshly distilled water and absolutely clean bottles are indispensable. The solution will keep apparently good for months.

Culture media was made up, from time to time, as required, from this stock solution, in 100 c.c. lots, by dissolving from 0.25 to 1 gram of dextrose in 100 c.c. of saline, thus making a solution of 0.25 to 1 per cent of dextrose. The media was then placed in clean plugged test-tubes, 10 c.c. in each, and sterilized in the Arnold sterilizer for 30 minutes, after which it was stored for use as required. It should be made up fresh every two weeks.

The best results were obtained by diluting this media, when it was being used, by the addition of 20 to 25 per cent of freshly distilled, sterile water, since some evaporation went on during the planting, and further concentration of the media often occurred in the preparation from evaporation of the hanging drop and conden-

sation of the vapor about the walls of the moist chamber on the depressed slide. By using a slide with a deep depression, containing a little distilled water, this evaporation was lessened.

Cultures planted in a hanging drop of this media, upon a sterile, clean cover-slip, inverted over a depressed slide, which was sealed with vaseline, grew very well at a temperature of 39° to 40°. However, it was found that if a small quantity of extract of chick embryo (Carrel, 1913) were added to the media better growths were obtained, *i. e.*, cells migrated out from the original piece earlier, growth was more rapid and vigorous, mitoses were more frequent, and a larger percentage of growths was obtained. Hence this addition to the media was generally made. Ordinary bouillon had a similar activating effect.

The embryonic extract was prepared as follows: After the embryo had been removed from the egg, under sterile conditions, and with as little contamination with yolk as possible, it was placed in a Petri dish containing 10 c.c. of sterile Locke's media and washed. The tissue to be planted having been dissected out and removed to another dish of media, the remainder of the embryo was cut up and placed in a small, sterile test tube with a little media, and carefully ground up with a glass rod. This mixture was next centrifugalized, and the supernatant fluid added to the culture, generally in the proportion of equal parts of this fluid and Locke. Too high a proportion of embryonic extract was undesirable on account of its richness in food material, in that it produced a cell overloaded with fat globules, which interfered with observation.

The advantages of glycosaline over plasma have been noted by Lewis and Lewis (1912*a*, p. 10). It is more transparent and practically all of the growth is upon the lower surface of the cover-slip—not scattered throughout the hanging drop, as in the case of the plasma clot. The cells, unimpeded by the fibrin network, migrate freely along the cover-slip, upon which they spread themselves flat and thin, thus facilitating observation. The quantity of fat being much less than in plasma-grown cells, the cytoplasmic constituents, such as the centrosphere and mitochondria, are much more easily observed and studied. There are, too, the additional very considerable advantages that the media is more convenient to handle, fixed preparations are more easily made and are not marred by stained fibrin and coagulated albumen, and experimental operations, such as staining with vital dyes, are more satisfactorily carried out.

Immediately after planting, the cultures were transferred to a warm box, kept at a constant temperature of 39 to 40 degrees by means of an electric thermostat. With the microscope inside this box it was not necessary to remove the cultures from their warm environment for purposes of observation, and it was owing to this manifest advantage that the earlier method of observation of the living cultures upon a warm stage outside the incubator was discontinued. In addition, the warm-stage method of heating, from one side only, was found to be inferior to that of the warm box, in which the culture was completely surrounded by an environment of uniform temperature.

Illumination was furnished by daylight, Tungsten globe, or Welsbach burner. A ray filter, consisting of a glass vessel filled with a solution of copper sulphate or

copper acetate, placed between the source of illumination and the condenser, when artificial light was used, was found to be an advantage (Kite 1913b, p. 149).

In studying the grosser changes, such as the variation in shape of the nucleus, the 4 mm. Leitz apochromatic objective was used; for the finer details the Leitz $1\frac{1}{2}$ oil-immersion objective was found to be satisfactory. Oculars were Leitz Compensating Nos. 4 and 6.

For observation of the living cells the cultures of the second day were generally the most favorable; the growth was then usually abundant and the cells in a healthy condition, with a fair proportion of mitoses.

It is quite evident that continuous observation of the living cell, provided it can be carried out successfully, is the ideal method of studying the sequence of changes occurring therein. Indeed, for the study of amitosis it has been regarded as indispensable, as witness the statement of Richards (1911, p. 125): "For amitosis there is but one absolutely certain criterion, the observation of living material and subsequent study of material fixed under observation;" he adds, "this is, of course, impossible in most cases."

The method has already been used in the study of living multinucleate cells of tissue cultures, Lambert (1912a) having attempted to settle the question of the origin of giant cells growing from explanted tissue by its aid, and the character of the results attained through its use was sufficiently encouraging to warrant its application to the problem in hand, though not altogether satisfactory in view of the obvious difficulties. It was hoped, too, that these difficulties would be minimized by the use of glycosaline media, which produced films of tissue sufficiently thin for study in the living condition.

It was first planned to ascertain the full history of the binucleate cell by selecting a cell with a single nucleus and observing it continuously on the stage in the warm box till either the nucleus divided and formed a double-nucleated cell or the cytoplasm became merged with that of another mononucleate cell to form a single cell containing two separate nuclei. Observations upon this binucleate cell were then to be continued until the ultimate fate of the double nucleus was disclosed. Drawings were to be made from time to time with the camera lucida.

This ideal was found to be impossible of realization, on account of the technical difficulties. Cultures under continuous observation, exposed, as they were, to strong light, often showed evidences of degeneration; even daylight seemed to cause this and the use of ray filters did not altogether eliminate it. Degeneration was noticed at times even when the plan was followed of making short observations and immediately turning the light off, the culture remaining continuously on the stage.

Living cells show a marked tendency to migrate; hence the cell under observation had to be closely watched to prevent its escape from the field of vision. Other cells often wandered over the cell under inspection, and so interfered with the work. Added to these difficulties is the length of time involved in the process, which necessitates many hours—even days—of continuous observation. Then, too, the minuter cell changes are very difficult to follow, even for short periods, the only

optical picture presented being very delicate shades of difference in refractivity. The obscure and peculiar optical properties of living matter, as Kite (1913, p. 148) points out, give rise to an important source of error.

The ideal procedure having to be abandoned, the alternative practice was adopted of following shorter periods of change and piecing the records of these together. A start was made with the formation of the double nucleus, and here another difficulty was encountered; it was manifestly impossible to tell which of the thousands of nuclei in the culture was about to divide, and by selecting nuclei at random, months might be spent without getting one which ultimately divided. It was thus necessary to select a nucleus which gave some indication of being on the way to division, *i. e.*, by elongation, or equatorial constriction; such a cell was observed continuously until it divided or became degenerate. The subsequent history was studied by selecting a double-nucleated cell and observing it continuously.

In the observations the shape of the nucleus was particularly noted, and with this was considered the behavior of the centrosphere, mitochondria, fat globules, nucleoli, shape of the cell generally, and whether or not the cell itself ultimately divided following nuclear division. Cells on or near the outer border of the new growth were found most favorable, since they were larger and more free from surrounding cells. They appeared to be usually quite healthy during the first 48 hours at least.

As has here been noted, the morphology of the cell is difficult to make out in the living and unstained condition, and it was thought that inspection would be much easier and more accurate if the details could be rendered visible by the use of stains which would not impair the vitality of the cell.

Churchman and Russell (1914) and Russell (1914) have recorded satisfactory results with gentian violet in staining embryonic and adult tissues of the frog growing *in vitro*. They stated that endothelium from adult frog pericardium in frog's plasma to which gentian violet had been added grew definitely when the strength of gentian violet was 1 in 2,000, and actively in a dilution of 1 in 20,000. Furthermore, their records show that they were able to follow cell division in their stained preparations. Clear karyokinetic figures were not seen in growing adult frog tissue, but in embryonic frog tissue these figures were found in the dividing stained nuclei. They believe that the nucleus is stained intravitaly and that growth continues in the presence of the dye. Toxic action was shown when this stain was used upon paramecium, even in dilutions of 1 in 1,000,000. They believe that "the use of stains in the plasma in which tissue is grown will probably facilitate the study of nuclear growth."

My results with gentian violet in chick tissue growing in Locke do not bear out those of Churchman and Russell, for the staining could not be considered as in any sense *intra vitam*, under the conditions existing in my experiments.

A solution of Grüber's gentian violet was made up in a strength of 1 in 100,000 with slightly diluted saline. Without removing the culture from the warm box, the cover-slip was lifted off and a small drop of the stain (warmed to the same tem-

perature as the culture and of about the size of the hanging drop) was added to the latter, the excess fluid being withdrawn. The dilution of the stain was thus approximately 1 in 200,000. The culture was immediately examined under the microscope. The dye rapidly diffused through the cytoplasm into the nucleus, the nucleoplasm taking on a finely granular appearance; this latter was, apparently, the result of the coagulative action of the dye. The nucleoli were distinctly marked out, and stained much more darkly than the nucleoplasm or cytoplasm. The nuclear membrane, too, was sharply outlined as a dark violet ring. This staining was very valuable in delineating indistinct nuclear boundaries, since these, in the living unstained culture, are often obscure. Irregularities in outline, such as indentations, were rendered very plain, and the method was of assistance in studying the relationship to one another of double nuclei.

The cytoplasm, after this treatment, consisted of coarse violet granules in a very faintly stained matrix, showing at times a slightly fibrous structure. Cell borders were well marked, especially the pseudopodia, which, however, lost their power of movement upon being stained. Intercellular bridges could be studied. Mitochondria were not specifically stained, and degenerated in a short time. In a culture so stained evidence of life, such as pseudopodial and mitochondrial movement, cell migration, and mitosis, ceased almost at once, and in a few minutes vacuoles formed in the cells and the entire culture became degenerate.

Though gentian violet staining is of great assistance in obtaining a conception of the morphology of the cells rapidly, under the conditions of the experiments its toxic action precludes the possibility of the stained cell undergoing vital changes. Owing to its coagulative action the appearance of the living protoplasm is not accurately reproduced in the stained preparation. In spite, however, of these disadvantages, the use of gentian violet enables one to inspect portions of the cells which, in the living condition, are almost, or quite, invisible, and also to examine more accurately and easily some of the visible parts.

M. R. and W. H. Lewis (1914 and 1915, p. 376) used janus green as a vital stain in tissue cultures growing in Loeke solution, and found that, although the mitochondria were specifically stained, the dye was toxic in as low dilutions as 1 in 200,000, and caused speedy death of the cells, as well as distortion of the mitochondria.

Janus green (Hoechst), di-ethyl saffranin azo di-methylanilin, in Loeke's solution, in a strength of 1 in 40,000, was applied to living cultures in the same manner as the gentian violet, and was found to stain the mitochondria specifically in about 5 minutes, but no movements of these bodies could then be noted, and the threads broke up into a row of granules. The cells soon died, as evidenced by their vacuolated and degenerated appearance.

While janus green staining provided a rapid and convenient method of observing mitochondria, its toxic action rendered it valueless as a means of studying vital changes; moreover, the stained mitochondria soon lost their normal optical characters, thus prohibiting extended observation.

Some of these living cultures were fixed and stained. Osmic-acid vapor was used for fixation, and Heidenhain's iron hematoxylin was found to give the best staining.

The culture, growing in the hanging drop, was removed from the vaselined slide and exposed to the fumes from a 2 per cent aqueous solution of the tetroxid of osmium. This may be done by placing the cover-slip, drop down, over the mouth of a bottle containing the fixative (the vaseline adhering to the cover-slip and preventing the escape of the vapor) or, as suggested by M. R. Lewis, by floating the cover-slip, drop up, upon the osmic solution. Fixation is complete in 5 or 6 minutes, and the preparation is then dark brown or black. It is now rinsed off with distilled water, and passed rapidly through ethyl alcohol solutions of 35, 50, and 70 per cent. To the latter a few drops of hydrogen peroxid are added, which bleaches the preparation. It is then passed back rapidly through the same alcohols, rinsed in distilled water, and washed in running tap-water for 5 minutes. Too long immersion in alcohol will cause the mitochondria to become dissolved out.

The cover-slip, culture downward, is now floated upon a solution of 4 per cent iron alum and allowed to remain for 12 to 24 hours; next it is washed in running tap-water for 5 minutes and then immersed in 0.5 per cent aqueous hematoxylin for 24 to 48 hours, after which it is washed in running tap-water for 1 minute, differentiated in 2 per cent iron alum and again washed in tap-water for 10 minutes, dehydrated through the alcohol series, cleared in xylol, and mounted in balsam. The hematoxylin solution is prepared as follows: Hematoxylin (10 per cent in absolute alcohol), 0.5 c.c.; distilled water, 10.0 c.c.

These fixed preparations were used to make clear the morphology of the living cells, especially such details as nuclear membranes, nucleoli, mitochondria, and centrosomes. An attempt was made to pick out the successive stages in the process of direct division of the nucleus for comparison with the observations upon living material, and thus to build up a series exhibiting the various changes. The phases of mitosis were also studied, and drawings were made of interesting cells. For statistical purposes cell counts were made of some of these preparations by placing a glass disk, upon which squares had been ruled, in the ocular, and using the mechanical stage.

THE BINUCLEATE CELL.

INCIDENCE.

The frequency of occurrence of binucleate cells varies within wide limits in cultures from different tissues. They were found to be most numerous in membranes growing from the heart, and were not uncommon in cells of the connective-tissue type from this and other tissues, but in the endodermal membranes from stomach and intestine they were exceedingly rare. They may be even altogether absent from the new growth. Lewis (1915, p. 156) notes that in growths from the leg of chicks no amitotic forms were noted.

To get an idea of the relative number of these cells as compared with the total number of cells in the new growth, careful counts were made of 20 fixed cultures from chick heart. Imperfect cells and those situated so close to the original piece as to be indefinitely outlined were omitted. In these 20 preparations there was a total of 41,725 cells, of which 375 were binucleate, or an average of 1 binucleate to

each 111 cells; thus the binucleate cells made up 0.9 per cent of the total cells appearing in the new growth.

Even in different preparations from the same tissue binucleate forms were found with varying frequency. Among the 20 cultures of heart mentioned above, one preparation showed 1 double nucleus to each 28 cells, while in another the ratio was 1 to 1,180.

Age of tissue, too, in these 20 heart preparations, had a bearing upon the incidence of binucleate forms, new growths from the younger hearts showing a somewhat greater proportion of double nuclei than those from older cardiac tissue. In hearts from chicks of 5 days' incubation there was, on the average, one binucleate to each 105 cells; in 7-day hearts the ratio was 1 to 123, and in 8-day hearts it was 1 to 233.

Finally, duration of growth seemed to be related to the relative frequency of occurrence of these cells. In the same 20 preparations it was found that cultures of the first 24 hours showed one double nucleus to each 183 cells; in cultures of the second 24 hours the ratio was 1 to 86 cells. This seems to point to a considerable amount of nuclear splitting in the second 24 hours, of which some at least probably occurred within the new growth. Cultures of older duration were, in the slides counted, not sufficiently numerous and typical to base accurate conclusions upon.

MORPHOLOGY.

The average binucleate cell (figs. 1*a*, 7, and 9) is somewhat larger than the average mononucleate, the area occupied by the nucleus being approximately twice as great. Each nuclear part is, in size, shape, and general appearance, very similar to the nucleus of the mononucleate cell. The nuclear parts are often pressed close together (figs. 1*a*, 60) and their adjacent surfaces are consequently flattened, the intranuclear pressure in each being evidently equal. When thus related, the appearance of the double nucleus in the living preparation—and indeed in some of the fixed preparations—simulates a single nucleus which looks as though it were separated by an equatorial membrane. Such an appearance has been interpreted as a nuclear plate, or intranuclear membrane, and so described by Child (1904, 1907*b*, and 1911, p. 283), and others; but for reasons which will appear later on, I believe that such appearances in tissue cultures are due to the apposition of nuclear surfaces, as above described.

In an elongated nucleus which has become bent upon itself the folded free edge of nuclear membrane, projecting into the karyoplasm, may simulate a partition which seems to be growing across the nucleus from one side to the other. A nuclear configuration of this character is presented in Child's (1911) figure 16, page 293, and in other of his figures. It is not to be wondered at that the approximated areas of nuclear wall at the folded edge are somewhat attenuated and appear thin, as Child (1911) has observed (p. 283). Such reduplications of nuclear membrane are not to be looked upon as intranuclear membranes which cleave the nucleus by growing across its equator. I have seen no evidence of a type of amitosis of this kind.

Sometimes an equatorial membrane is simulated by an elongated nucleolus lying across the nucleus. Again, as Richards (1911, p. 124) suggests: "A strand of

linin stretched across a nucleus with chromatin granules upon it often gives the appearance of a membrane dividing the nucleus amitotically (endogenous division?). He also states that he has found no evidence of the "endogenous" division of Child (1907*a*, p. 95); nor have I seen anything of this kind in tissue cultures. Optical appearances similar to Child's (1911) figure 6 have been seen in living cells and interpreted as indentations and infoldings of the nuclear membrane. All these conditions can be made clear by the use of a dye like gentian violet upon the living culture, or by proper fixation and staining. In no case has a *bona fide* intranuclear membranous partition been found in any kind of preparation.

I may also state here that my observations upon fixed and stained cells in tissue cultures have not disclosed cases where one nuclear half was more darkly stained than the other, such as those mentioned by Child (1904, p. 549; 1906, p. 595; 1907*c*, p. 171; and other places) and which he believes to indicate "a certain degree of physiological independence before separation of the parts." In the living condition, too, the nuclear portions present no evident difference in cytoplasm. The contents of the nuclear parts are in every way similar to those of the single nuclei. The nucleoplasm appears homogeneous during life and when fixed with osmic-acid vapor is finely granular. This method of fixation preserves most accurately the details of the living cell (Lewis and Lewis, 1915).

There is usually at least one nucleolus or karyosome in each nuclear portion, and more often two (figs. 7 and 9) or even more. The nucleoli of the connective-tissue type of cell are irregular in shape, often elongated, and vary greatly in size (fig. 8). In the living cell they are highly refractive. They continuously undergo changes in shape, size, and number during the life of the cell (figs. 24 to 35, and plate IV), as can be seen by watching the living nucleus. It is then apparent that their outline is "ragged," as Lewis and Lewis (1915) describe it. The bodies even appear to break up from time to time, and afterward to recombine (figs. 24 to 35). At times the nucleolus comes to lie very close to the nuclear membrane (fig. 29) and it may even appear to be attached to it. These bodies take the gentian violet dye very well and stain darkly with hematoxylin. If overdifferentiated with iron alum the nucleolus appears as an agglomeration of small granules of about equal size (fig. 10); it is probably to be regarded as a gel of varying density, the densest portions being represented by these darkly staining granules.

During mitosis the nucleolus disappears with the formation of the spireme, and the daughter nucleoli reappear in the reorganizing daughter nuclei. The nuclear portions may be separated by an interval (fig. 9), or simply touching one another (fig. 7), or may be pressed so close together that their adjacent surfaces are flattened, similar to the condition in the early cleavages of *Moniezia*, as mentioned by Harman (1913, p. 221). They tend to remain close to one another, and do not migrate far apart, as nuclei in a syncytium. When separated, the nuclear portions show mitochondria between them (fig. 9) and usually the centrosphere is situated either in the interval between the nuclear portions, or opposite this interval, as in figures 7 and 59.

In the living condition the centrosphere or "central body" of Lewis and Lewis (1915) appears as an area of slightly greater refractivity situated at one side of the nucleus in mononucleate cells; this side is frequently concave, with the centro-

sphere situated in the concavity (fig. 24c). This concave side then appears indistinctly marked out in the living culture, the close proximity of the centrosphere and mitochondria obscuring the nuclear outline. Its relation to the parts of the double nucleus has been noted.

I have not observed the centrosome (centriole) in the living cell, but when stained with iron hematoxylin this body appears usually as two minute dark granules, lying close together (fig. 7). The centrosphere takes a slightly darker stain than the area surrounding it, and thus appears to be a somewhat more concentrated area of the protoplasm. From this area mitochondria radiate, as seen in figure 8. In the living condition the centrosphere shows an indefinite, irregular, apparently serrated edge, the toothlike processes of which undergo a curious constant, slow, almost imperceptible indrawing and outpushing. The mitochondria seem to be intimately connected with this body, as observed by Lewis and Lewis (1915, p. 349), but they differ from it in their reaction to janus green and to certain methods of staining in the fixed condition, such as iron hematoxylin.

Mitochondria in tissue cultures have been described at length by Lewis and Lewis (1914, 1915). Their curious movement, mentioned by these authors, is plainly evident. The special relation of these bodies to the binucleate cell is their position between the nuclear portions, as in figures 8 and 9, unless, as in figure 7, the parts of the nucleus are too close together to permit of this. The relationship of the mitochondria and adjacent centrosphere to the portions of the double nucleus is similar to that of the Netzapparat of Deineka (1912, figs. 2 and 12) under similar conditions.

Fat, though not so abundant as in plasma-grown cultures, nevertheless occurs as fine globules which tend to crowd together at the nuclear poles (fig. 32) and often become arranged in rows between the mitochondria.

The other details of the binucleate cell are very similar to those found in the mononucleate.

Occasionally cells are found which contain three or more distinct nuclei (fig. 1b) and the evidence seems to indicate that the binucleate cell is the first stage in the formation of the giant cell: this stage, however, is seldom passed, for giant cells are comparatively rare. Such multinucleate cells are quite different from the foreign-body giant cells of Lambert (1912 *a* and *b*), which have been shown by him to arise by fusion of previously separate wandering cells.

Binucleate cells, and the intermediate stages leading up to them, have long been known in embryonic tissue. Child (1907c) shows several such from chick embryos in his figure 12. Maximow (1908) describes and figures double nucleated cells, similar to those found in tissue cultures, in mesenchyme of embryo rabbits of 11½ to 13½ days, and he has found amitosis also in the guinea pig in the same region and stage. Patterson (1908) shows illustrations of cells of the same type in developing pigeon's eggs, and such cells have been described by many others. Thus it is certain that, since the paired nucleus occurring in the tissue-culture cell is similar to that found in the cells of embryonic tissue, it can not be considered as an abnormality due to its artificial mode of life.

Harrison (1913, p. 67) has shown that the behavior of cells growing in culture media is comparable to that of cells growing in the embryonic body, and it is reasonable to assume that the behavior of these binucleate cells in tissue cultures approximates the behavior of similar cells in the developing embryo. Hence the vital phenomena manifested by such binucleate cells in tissue cultures afford reliable evidence as to the changes which take place in similar cells living under normal conditions in the corresponding embryo.

ORIGIN.

If we inquire as to the origin of these binucleate cells of the new growth we are confronted with four possibilities, viz:

- (a) Migration as a binucleate cell from the explanted tissue.
- (b) Fusion of the cytoplasm of two previously separate cells without fusion of the nuclei.
- (c) Division of the nucleus by mitosis without division of the cytoplasm.
- (d) Division of the nucleus by amitosis without division of the cytoplasm.

This list does not include the theoretical origin of nuclei *de novo* from the cytoplasm, or their development from extruded chromidial substance (Young, 1913). These hypotheses do not appear to have been substantiated, and no evidence in favor of either appears in tissue-culture preparations.

First, considering (a), we find that twin nuclei occur in the area of new growth immediately surrounding the original tissue, and such forms are well known in embryonic tissue. Thus it is probable that many of the binucleate cells in the new growth have migrated as such from the explanted tissue. The great increase in proportion of double-nucleated cells in the second 24 hours, however, as has been noted, suggests that not all of these cells are of migratory origin, but that some have probably arisen in the new growth itself. This view is borne out by observations upon the living cell, as will be shown, where a single nucleus has been seen to become divided directly into two parts, and also by the finding of nuclei in the act of direct division in the fixed preparations.

The binucleate cells which have migrated as such from the original piece have probably originated therein in the same manner as those arising in the new growth. Giant cells can hardly be considered to have migrated as such from the original piece, for in the zone immediately surrounding the latter they are not found.

Regarding (b), it may be said that no appearances which could be interpreted as transitional forms have been found in fixed and stained preparations or in cultures vitally stained, neither has the process been observed in the living culture. I therefore regard it as an improbable hypothesis. This could hardly be considered as an explanation of the formation of giant cells, for that would postulate the fusion of a multitude of previously separate cells, of which there is no evidence in the material examined.

It may be noted that Lambert (1912*b*), who brought about the formation of giant cells by fusion of mononuclear cells in cultures from chick spleen, failed to get such cells in cultures from chick heart. Furthermore, Lambert (1912*a*) recognized three other types of giant cells in tissue cultures, besides this.

Considering next (*c*), we find that this also is improbable. It is easy to observe the process of mitosis *in vitro*, and to follow the various changes. Many such cases have been observed, and in none has there been seen a failure of the cytoplasm to divide following separation of the chromosomes. This process of cytoplasmic constriction is well shown in figures 68, 69, and 70, and in the living culture it is very evident and easy to watch. In no case has it been observed, in following these cells dividing by karyokinesis, that a binucleate cell was formed; always the end result was two distinct daughter cells, often widely separated, connected by a thin strand of protoplasm (fig. 1, *t*). If crowding of the cells occurs, separation of the daughter cells may be interfered with to some extent, but it is doubtful if this interference ever is so serious as to prevent cytoplasmic fission altogether and thus result in the formation of a single cell containing two nuclei. At least no evidence has been found from observation of the cells of tissue cultures that this is ever the case.

Upon this point my observations agree with those of Child (1911, p. 283). He says: "In *Moniezia nuelei* which arise by mitosis are separated by an appreciable distance when they form." Again (p. 292), in describing a "double" nucleus, represented in his figure 11, he says: "The two parts of the nucleus . . . are in immediate contact and flattened against each other. It is difficult to understand how they could attain such a position as the result of mitotic cleavage, like that of the earlier stages."

It must be said that my observations upon living cells have principally been made with cells of the connective-tissue type. In the case of membranes, however, there is always a well-marked dividing line between the cells, which is made evident by staining with iron hematoxylin or the use of silver; also this potential isolation of the cells is made apparent by the fact, when cells do separate, that the cleavage is along this line of partition, as is shown from the study of fixed preparations (Lewis and Lewis, 1912c, figs. 14, 13, and 12). No such partition is ever found between the nuclear parts of binucleate cells.

In fixed preparations of connective-tissue cells there is no indication of any failure of the cytoplasm to divide in the later stages of mitosis; that is to say, we find no telophases where separation of the cytoplasm is not evident (fig. 17).

Again, these double nuclei almost always have only a single centrosphere (fig. 7), whereas nuclei arising by karyokinesis have each a centrosphere. This finding as to the centrosphere agrees entirely with that of Deineka (1912) for the *Netzapparat* in the dividing epithelial cells of Descemet's membrane and connective-tissue cells of the cornea. This author is of the opinion that the *Netzapparat* surrounds the centrosome, and its changes appear to follow the variations of the latter body. In binucleate cells of these tissues, in which the nucleus divides by amitosis, the *Netzapparat* remains single, whereas if the nuclear division takes place by mitosis each of the daughter nuclei obtains a separate *Netzapparat*. By reference to this disposition of the *Netzapparat*, Deineka is even able to tell the manner of origin of such double nucleus, whether by amitosis or mitosis in which cleavage of the cytoplasm has been delayed. I have never observed this cell organ in living tissue-cultures.

The fact that the centrosphere in the binucleate cell is single seems to indicate that the twin nucleus is single so far as its reproductive capacity is concerned. This inference is borne out by observations, later to be referred to.

Considering finally (*d*), it seems probable that these twin nuclei arise through direct equal binary fission of the nucleus without division of the cytoplasm. The evidence upon which this assumption rests is, first, the inadequacy of other explanatory hypotheses; and, second, the observation in living cells of a process which is apparently direct nuclear division, and the occurrence in fixed preparations of tissue cultures of what must be regarded as transitional forms between single and double nuclei.

It is true, as Harman (1913) remarks (p. 219), that "the fact that two nuclei lie in contact is no evidence that they have arisen by amitotic division," and in the material which she studied, viz. early cleavage stages of *Tania teniaformis* and *Mougeotia*, she undoubtedly presents convincing evidence that nuclei which have arisen by mitosis may lie quite close to one another within the same cell. This, however, is a case of delayed cleavage, for she states (p. 215):

"In cleavage, nuclear division takes place very much in advance of cytoplasmic division. In the early divisions it is the exception and not the rule to find even a constriction in the cytoplasm. This gives rise to a syneuctial condition. This syneuctium persists until very late cleavage."

This is quite a different condition from that obtaining in the cells of tissue cultures. Then, too, many of her nuclei contain spiremes. Her contention in no way counts against the view that the double nuclei of tissue cultures are of amitotic origin.

Observations on direct nuclear fission will now be recorded, first to be described being the process as it was seen to occur in the living cell. As has been pointed out, it is impossible to tell from inspection of the living culture which of the thousands of mononucleate cells will divide directly, and so to follow the process of nuclear amitosis in the living cell it is necessary to select a cell which shows some indication of beginning direct division, *i. e.*, by elongation and constriction. Figures 24 and 25 appear to be typical of the early stages of direct division of the nucleus.

Many attempts to trace the changes in such a cell were made, with, however, only partial success, for in almost every case the nucleus lost its constriction and became rounded again, or the cell degenerated. However, one case was found where what appeared to be direct division of the nucleus occurred during observation. The various phases are shown in the series of figures 24 to 35, which were drawn at 15-minute intervals from a single cell growing in a culture from a 5-day chick heart in Locke solution with extract from chick embryo. The culture was of 57 hours' duration. A cell was first selected which contained an elongated nucleus with a marked notch in one side. In this notch the centrosphere was situated, and consequently this side was somewhat indistinctly outlined (24). Instead of dividing, the cell straightened out, almost losing the indentation (25). It contained two nucleoli, one situated in the uppermost pole, and the other, which was paired, about the equator. The nucleus next became rounded (26 and 27) and, after one hour's observation, its outline was almost circular (28). In the latter figure there appeared to be only a single paired nucleolus.

The nucleus now became elongated and a refractive mass appeared in the lowermost pole—apparently another nucleolus; at the same time the central nucleolus became a single mass, and was somewhat longer than before (29). Next, a shallow notch formed in one side, and the nucleus became shorter and thicker, its nucleoli undergoing minor changes (30 and 31). At the end of two hours the nucleus again elongated and a deep notch appeared, indistinctly marked out on one side (32). This seemed to become shallower in 33, but the presence of the centrosphere prevented this portion of the nuclear membrane from being well defined.

The next change was the formation of another notch on the opposite side, both notches forming what seemed like a zone of constriction about the nucleus. A refractive mass stretched across the equator of the nucleus between these notches (34). This is apparently a strand of mitochondria rather than a nucleolus, for, in the next drawing (35), $2\frac{3}{4}$ hours after the observation began, this strand is situated between two apparently separate nuclear portions, the nucleus having divided directly. In no fixed and stained cell has a nucleolus been seen to occupy this position; on the other hand, mitochondria have frequently been seen between these nuclear parts, as in figure 8. There was here no evidence of the formation of either a spireme or an amphaster, and thus Wilson's (1900) criterion for amitosis was fulfilled. It may also be noted that the centrosphere did not divide and the nuclear membrane remained intact.

The final division apparently took place very rapidly, since the actual separation was completed in the 15-minute interval between 34 and 35. This rapidity of the end process of nuclear cleavage accounts for the infrequency of such terminal constricting forms as figures 6 and 8, and makes the relatively small number of these later transitional forms adequate to account for the number of binucleate cells which originate therefrom. The cell was allowed to remain on the microscope stage all night, but unfortunately wandered away and was lost, so the subsequent changes could not be followed. The drawings were made from direct observation, but not with the aid of the camera lucida. Mitochondria and centrospheres are partially diagrammatic. This process, though traced with difficulty, and though somewhat obscure, seems to follow the classic descriptions of amitotic division of the nucleus, viz. elongation with equatorial constriction, forming a somewhat dumb-bell shaped figure, and final separation of the two nuclear portions.

A similar elongated nucleus in a connective-tissue cell was followed for 6½ hours, and did not divide, but finally degenerated; in the meantime it underwent various changes in shape and was rounded when last observed. The changes in nucleoli were similar to those in figures 24 to 35.

Thus it appears that a nucleus in a condition of elongation and constriction may remain undivided for a long time and may even return to the rounded form without dividing at all. In cases, however, where the constriction has passed a critical point, as apparently was the case in the nucleus represented in figure 34, the process of division proceeds rapidly.

The study of fixed preparations, too, throws some light on nuclear amitosis, for in these one frequently finds nuclei evidently undergoing direct division. Such

forms are to be regarded as transitional stages between the mononucleate and binucleate cell. Figure 2 shows a nucleus which has undergone elongation and equatorial constriction, so that there is an indentation on either side. The nucleolus appears to be dividing also; this condition of the nucleolus is, however, not constant. Figure 3 shows a cell in which constriction is somewhat farther advanced; here the nucleoli have apparently divided, two being seen in each nuclear portion. In these cells the method of preparation does not show cytoplasmic details.

Figures 6 and 8 show nuclei in which direct division is almost complete, the nuclear parts being held together only by the finest filament. Similar nuclear figures were found by Maximow (1908) in embryonic rabbit tissue, as shown in his figure 4, and the upper two nuclei in his figure 10. In figure 8 the nucleus has divided unequally, and the larger portion contains two nucleoli, while the smaller has but one. In figure 6 only one nuclear portion contains a nucleolus, and this is single. In both cells the unchanged centrosphere is situated in its characteristic position between the two nuclear portions, while the mitochondria radiate out from this body, and a strand of mitochondria passes over the bridge connecting the nuclear parts.

There is nothing in the appearance of these nuclei to suggest the late telophase of an intranuclear mitosis, such as those shown by Cary (1909) and referred to by Richards (1911, p. 158). The clearness characteristic of the cells of tissue cultures prevents confusion of nuclear amitosis with the late telophase of mitosis, such as has been shown by Richards (1909) to be possible in the cells of *Tenia*.

In figure 9 nuclear separation has been completed, the two portions being quite free from one another. These are of about equal size and appearance, and each contains two nucleoli. Mitochondria and centrosomes occupy their typical positions; the former are short rods, this being a cell from heart membrane.

In figure 7 the separate nuclear parts have come together and their surfaces are just touching. Mitochondria have been forced out, but the centrosphere is characteristically opposite the area of contact of the nuclear portions. Figure 4 shows a somewhat similar binucleate cell, from a Zenker and Mallory preparation.

It would appear that the nucleus may sometimes divide by a gradually deepening cleavage from one side, which finally cuts it into two pieces. This may be regarded as an asymmetrical type of constriction. Figure 5 may be taken as representative of the beginning of this process and figure 6 the end. The centrosphere is found typically in the notch, as has many times been recorded in amitotically dividing nuclei, as by Maximow (1908). In the rare exceptions to this rule the centrosphere may have been originally situated in the notch and subsequently have left it. No evidence of separation of the centriole-pair during nuclear amitosis has been found.

Richards (1911, p. 156) finds constricted and indented nuclei in his material only in cases of imperfect fixation. Whatever may be said as to the nuclear distortion brought about by many fixatives, this is not an explanation of such figures as 6 and 8 seen in tissue cultures, for here osmic-acid vapor was used as a fixative and this does not change the nuclear outline, as may be proved by observing a living nucleus and the same nucleus after fixation (Lewis and Lewis, 1915). Then, too, only a small proportion of nuclei appear thus, whereas if the appearances were to

be interpreted as due to the fixative they should be abundant. Again, the actual observation of such nuclei in living cells is proof absolute that they are not artifacts.

The only type of nuclear fission which I have observed in tissue cultures is that which occurs, apparently, by constriction.

An estimate of the frequency of occurrence of such transitional amitotic nuclear forms as those shown in figures 2, 3, 6, etc., was made by making careful counts, the aforementioned series of 20 heart cultures being used. Out of a total of 41,725 cells in this series, 50 cells were found to contain constricted nuclei of such a character as would warrant their being considered as amitotic. This is a proportion of one amitotic nucleus to 835 ordinary nuclei, or 0.1198 per cent. In the same series there were 375 binucleate cells, which are regarded as end products of nuclear amitosis. The proportion of transitional forms to end products is thus 50 : 365, 1 : 7.5 or 13.33 per cent. So high a percentage of transitional forms seems to indicate that the nuclei remain a long time in this condition, and the observations upon living cells bear this out. The final stages of direct nuclear fission, as shown in figures 6 and 8, are, as has been noted, rarely found.

In this connection it is of interest to compare the incidence of amitotic with that of mitotic nuclei. In the same series there were found to be 170 cells undoubtedly in mitosis. The ratio of mitotic cells to total cells is thus 170 : 41,725, or 1 : 245, or 0.4 per cent. There were probably many more mitoses than this, for some are undoubtedly rubbed off in preparation, since their rounded and thickened form exposes them to friction in washing, etc.; also 62 doubtful mitotic forms were not included.

It is an easy matter to calculate the relative proportion of amitotic to mitotic forms. As has been stated, the ratio of amitotic nuclei to total cells is 1 : 835, while that of mitotic nuclei to total cells is 1 : 245. It is evident that the mitotic forms are 3.4 times as numerous as the amitotic, even when we leave out the doubtful forms and the cells in mitosis which have been rubbed off. Again, when we consider that the amitotic process is a slow one, as has been shown, and that mitosis is relatively rapid ($1\frac{1}{4}$ to $2\frac{1}{2}$ hours according to Lewis and Lewis, 1915, p. 371), it will be realized that the amitotic method of nuclear division is unimportant, so far as nuclear multiplication is concerned, as compared with mitosis.

Thus, examination of living and fixed preparations makes reasonable the view that direct division of the nucleus occurs where this structure is elongated, and sometimes bent upon itself, by a karyoplasmic streaming, away from the nuclear equator, and a gradually deepening constriction which encircles the nucleus more or less symmetrically and cuts it into two parts, the constricted area becoming a narrow tube and finally a thread, which ultimately disappears.

The behavior of the nuclear membrane during amitosis in the cells of tissue cultures seems to be essentially the same as that of the same structure in the cells of the trematode described by Cary (1909) during intranuclear mitosis. There is, however, no intranuclear spindle in the cells which I have examined.

The final separation of the constricted nucleus takes only a short time, as has been noted, but a nucleus may remain for a long time apparently about to divide without actually doing so.

In the process of direct division of the nucleus various factors may play a part. First, we may refer the different changes in form of the nucleus to changes in form of the cell as a whole. It is of frequent occurrence that a cell, by reason of the tension exerted by attached cells, or of its own amœboid movement, becomes elongated. In consequence of this stretching of the cell the nucleus also becomes drawn out, it being simply a sac of fluid, and it is possible that it may become broken into two parts much in the same manner that an oil globule, floating upon water, becomes broken up if stretched. It may be assumed that there is a streaming of protoplasm away from the equator, with a constriction in this region, which becomes deeper and deeper until the nucleus is divided into two more or less equal portions, these now tending to assume a more globular shape. This view of the cause of nuclear amitosis is somewhat similar to that of Maximow (1908), who believes that amitosis in the mesenchyme cells of developing rabbits may be brought about by the stretching of such cells consequent upon rapid growth of the adjacent liver.

The process of direct division of the nucleus as described is strikingly like the division of the cytoplasm of ova which had been replaced in normal sea-water after having been treated with hypertonic sea-water (J. Loeb, 1906, p. 66, figs. 10, 11, 12, and 13). It appears that here the cell first becomes ineat from one side; the protoplasm thereupon streams off in opposite directions, forming two globules connected by a narrow isthmus. This soon becomes reduced to a mere thread composed of the attenuated cell membrane, which finally disappears, so that there remain two sacs of protoplasm, quite without connection one with another. The physical changes involved in this process seem to be very much like those seen in direct division of the nucleus. Loeb's figures are very similar to those illustrating nuclear amitosis.

That the size of the nucleus is not a material factor in this process is seen by the variation in size of the twin nuclei, some of which are quite small. Although a twin nucleus is frequently found in a cell which is not elongated, it may be assumed that such a cell has subsequently changed its form, but that it was extended when the separation of the nucleus occurred. This hypothesis would not, however, explain the formation of giant cells, multinucleated muscle-cells, etc., and it does not provide an explanation for the evident activity of the centrosphere and mitochondria in direct division.

A second hypothesis to account for the separation of the nucleus directly postulates the active participation of the centrosphere, or mitochondria, or both, and here we may assume a purely mechanical and a purely physico-chemical activity. It has been noted that the centrosphere is found commonly in the invagination of the nucleus; moreover, its edge shows evidence of a curious type of movement—a slow, indefinite retraction and elongation of the marginal processes—which seems to be associated with movements of the mitochondria. It is possible that, through the mechanical influence of the centrosphere upon the adjacent nuclear membrane, the constriction of the latter is favored and the nucleus ultimately divided, and it is easy to conceive how the mitochondria may assist in this nuclear separation

through their own movements (as they have been described by Lewis and Lewis, 1915), since they are typically found between the nuclear parts when these are separated to any extent (fig. 9), and a strand of mitochondria may even be seen lying across the constricted isthmus of the nucleus, when this has not become completely divided (figs. 6 and 81). This position of the centrosphere and mitochondria undoubtedly seems to have some significance in separation of the nucleus, and is seen even where the nucleus is dividing irregularly, as in figures 48 to 58.

The relation of the Netzapparat of Deimeka (1912, fig. 3) to the nucleus is similar to that of the centrosphere as just described, viz. it is found in the cleft separating the nuclear portions. This author, however, does not ascribe to it any function in nuclear separation. He believes that it surrounds the centrosome.

The position of the centrosphere and mitochondria may, of course, be without significance, so far as the actual division of the nucleus is concerned, and it is possible that the relationship of these cytoplasmic bodies to the amitotic nucleus is purely fortuitous, or, at most, occasioned through their adjustment to conditions of intracellular pressure. The occasional absence of the centrosphere from the cleft (once in each 50 cases as determined by counts) and the presence of a cleft opposite the one in which the centrosphere is found are points which count against this second hypothesis. Again, not all nuclei, in which the centrosphere appears in a concavity on one side, divide directly; indeed, this relationship of centrosphere and nucleus has frequently been noted and illustrated in cells developing, without nuclear amitosis, in their normal environment. It would seem, therefore, that this relationship, of itself, can not bring about nuclear amitosis.

In no case has there been noted a ring-shaped centrosphere, like that described by Meves (1891), which encircles the constricted zone in the dumb-bell-shaped, amitotically dividing nucleus.

The centrosphere and mitochondria may be assumed to act in another way in accomplishing direct division of the nucleus, viz. by bringing about a change in the surface tension of the area of the nucleus to which they are opposed, through the elaboration of a chemical substance, and it may be possible to explain direct division of the nucleus upon some such hypothesis as that used by Robertson (1909, 1911, and 1913) to account for division of the cell in mitosis, viz. that there is produced in the region of cleavage some chemical substance which lowers the surface tension, such as soap, and that there results, in consequence, a streaming of protoplasm away from the equator, leading to separation of the cell. Robertson postulates a cholin-fatty acid soap, the cholin being derived from the splitting-up of lecithin. Since it has been shown that mitochondria are lecithinoid bodies (Cowdry, 1914, p. 18) it is not beyond the range of possibility to assume that they may act in the formation of a cholin soap. Indeed, the relation of mitochondria to the production of cholin in nerve-cells has recently been discussed by Cowdry (1915). The position of the mitochondria, lying across the zone of nuclear constriction (fig. 8) is eminently favorable for the action of such a soap, should it be formed there.

A third theory to account for direct division of the nucleus is based upon the assumption that some intranuclear change inaugurates the process. As long ago

as 1855 and 1858, Remak set forth a theory to account for the division of the cell, which may be stated in the words of Wilson (1900, p. 63) as follows:

"Cell-division proceeds from the center toward the periphery. It begins with the division of the nucleolus, is continued by simple constriction and division of the nucleus, and is completed by division of the cell-body and membrane."

A type of division which bears a close resemblance to this has recently been described by Howard and Schultz (1911) in the cells of a giant-celled sarcoma from the human oesophagus. To this type of division Schultz (1915) has proposed the name "promitosis," and these investigators believe it to be intermediate between amitosis and mitosis. This form of cell division seems to have an interesting parallel in certain protozoa, and they regard it as a reversion to a primitive biological condition in which the division sphere is permanently intranuclear—an idea analogous to that of Wieman (1910, p. 175) for a similar form of nuclear division.

The first step in the division of the nucleus here is taken to be a separation of the karyosome into two or more parts, of equal or unequal size, followed by a breaking-up of the nucleus into portions corresponding in number and size with the fragments of the karyosome, each nuclear part coming to contain a portion of the latter. This function of the karyosome in initiating division of the nucleus is analogous to that of the centrosome in mitosis.

This form of nuclear division is essentially the same as that described by Conklin (1903) in the follicular epithelium of the common cricket, and that it is by no means infrequent is gathered from the numerous references to it which this author has found in the literature. Conklin, however, has never seen actual cell division following nuclear amitosis, and from the fact that the cells in which direct division of the nucleus is found speedily degenerate after the egg is laid he believes that it is, in the material examined, "one of the last functions of these cells and that it is therefore an accompaniment of cellular senescence and decay." Conklin, however, believes that in most cases of amitosis the nucleolus does not divide.

The evidence from tissue-culture cells does not lend much support to a hypothesis ascribing to the fission of the nucleolus the initiation of nuclear division; true, we have in figure 2 a nucleus which shows lateral constrictions at the equator, and within it, lying with its long axis parallel to that of the nucleus, is an elongated karyosome, which also appears to be undergoing division in the same plane as the nucleus. This somewhat resembles the nuclei described by Howard and Schultz; the karyosomes of tissue-culture cells, however, are decidedly simpler in structure than those of the cells of the giant-cell sarcoma. Again, the fact that in the binucleate cell each nuclear portion is usually supplied with one or more karyosomes seems to point to this body having been divided before or during the division of the nucleus; but against this circumstance, weighing in favor of the view that the division of the karyosome acts to excite direct nuclear division, is the occurrence of such division where the karyosome has evidently not divided (fig. 6), since it is present in only one of the nuclear parts. Such nuclei are not uncommon in tissue cultures. The peculiar condition of the nucleolus in figure 2 may thus be purely accidental, since it is not at all constant.

The division of the nucleolus thus seems to have nothing to do with the separation of the nucleus; indeed, after the nucleolus has divided, the nucleus may not divide at all. It may, however, have to do with the size of the nuclear portions; where these latter are equal they each contain one or two nucleoli, of about equal size, whereas where they are unequal one portion—usually the smaller—may not contain a nucleolus.

That direct division of the nucleus may take place without preliminary fission of the karyosome in tissues developing normally is evident from the statement of Wilson (1900, p. 115): "In many cases, however, no preliminary fission of the nucleolus occurs; and Remak's scheme must, therefore, be regarded as one of the rarest forms of cell division." It is interesting to note that Schultz finds evidences of such a simple form of direct division in the nuclei of cells of the same tumor in which he finds "promitosis."

Summing up, then, the process of direct nuclear fission, it is probable that various factors are involved. Elongation of the nucleus is undoubtedly sometimes followed by its cleavage, and, since it is always present in nuclear amitosis, it may be regarded probably as an essential in this. The activity of centrosphere and mitochondria must also be considered as a factor in equal, as well as unequal, nuclear fission, and this activity is apparently made effective by nuclear elongation.

Fission of the nucleolus, while possibly concerned with the relative size of the nuclear parts, is not necessarily associated with the initiation or carrying out of nuclear cleavage.

Inasmuch as binucleate cells, and constricted nuclei which must be regarded as their precursors, are found in apparently normal embryonic tissue, they can hardly be considered as abnormal or as evidence of a reversion to a more primitive type of cell division; furthermore, their healthy condition is manifest from their capacity to divide by mitosis, as will be shown hereafter. Thus it is reasonable to suppose that the factors operative in nuclear division in tissue cultures are those which function in embryonic cells *in vivo*.

Since these binucleate cells seem to represent the first step on the road to certain giant cells it may be concluded that the latter are the result of a repetition of the same processes which bring about the formation of the former. This view is in accord with that of Lewis and Lewis (1915), p. 391, who state: "These giant cells appear to be formed by an amitotic division of the nucleus without a coincident division of the cytoplasm."

FATE.

The nucleus having divided directly, what becomes of it? Obviously the most certain method of settling this question is to select a living binucleate cell and watch it constantly as it passes through its various changes. This course has been followed with several cells, and the evidence at hand does not show that the cell as a whole divides otherwise than by the regular process of mitosis; in the early stages of this process there is a combination of the two nuclear portions to form a single mitotic figure.

Plate IV is a series of camera-lucida drawings representing successive stages in the history of one of these twin nuclei, in a living connective-tissue cell, grown from

a 7-day chick heart in glyeosaline with autogenous embryonic extract, the culture being 19 hours old when the observation commenced.

At 11^h 55^m a. m., when the observation began, the nucleus (fig. 60) was seen to be composed of two portions, approximately equal, separated by what appeared to be a single membrane, but what really represents, as has been shown, the apposed areas of nuclear membrane of the two portions. This double partition was seen, by focusing at different levels, to be a plane surface. The first three drawings show roughly the appearance of such a double nucleus during life. The parts are of about the same size and each at first contains a single nucleolus. These latter undergo obvious changes in size, shape, and number. There is a single centrosphere *c*. Fat globules are numerous, and the mitochondria are thread-like and plainly visible, and show their characteristic movement.

The nucleus remained in much the same condition, undergoing minor changes in outline, for about 2 hours, when, at 1^h 50^m p. m. (63) the division between the nuclear parts was seen to become less clearly defined at one side and, gradually, refractive material from the nucleus accumulated in this equatorial plane until, at 5^h 05^m p. m. (65), there was a distinct refractive mass in this region, which was evidently chromatin. Soon the entire cell began to contract, to become rounded, and to draw in its processes; the nuclear outline became indistinct, the position of the nucleus being represented by a clear space surrounded by a ring of fat globules and mitochondria (66). By focusing up and down it is seen that the cell is much thicker than before—in fact, it is almost spherical, the mitochondria and particles of fat forming a hollow globe which incloses the nuclear space. The portions of the twin nucleus have quite evidently fused and (from our knowledge of mitosis) it is plain that the cell is now in the prophase. A spireme, however, could not be made out. The refractive material which had been seen between the nuclear portions has become indistinct. This stage was seen at 6 p. m.

If we could see the cell represented in 65 in the fixed and stained condition we would doubtless find something like figure 22; here the spireme is forming in a binucleate cell and the nucleoli are becoming smaller and are breaking up. It is evidently composed of two such nuclei as are seen in figure 14, an early prophase in a mononucleate cell. The accumulated chromatin in the plane of contact of the two nuclear portions is clearly evident; this is obviously not the equatorial plate of mitosis. The nuclear membrane has almost disappeared, but the chromatic material is somewhat more concentrated about the periphery.

Figure 23 evidently represents a somewhat later stage of spireme formation in a double nucleus. Here the skein is well marked and the nuclear membrane has completely disappeared. These figures bear a striking resemblance to figure 6 of Rubaschkin (1905), in which he shows a spireme in a double nucleus.

The stage represented in 66, if fixed and stained, would probably resemble figure 19, drawn from a mononucleate cell in the late prophase. From this point on the behavior of the combined double nucleus is identical with that of an ordinary single nucleus.

As the cell was watched it was seen that a line, refractive in character, formed across its equator; this line, represented in 67, was somewhat irregular in outline, its borders being serrated. It did not remain unchanged, but on the contrary

showed almost constant minor variations in contour; it seemed to be composed of a row of small refractive bodies—chromosomes—undergoing constant, slow, and very slight movements. From this characteristic formation, situated as it was in a diamond-shaped field, surrounded, as before, by a granular ring of refractive globules and mitochondria, the metaphase of mitosis was easily recognized. This stage was drawn at 6^h 50^m p. m. (67) and would appear like figure 15 if fixed and stained. The cell is somewhat smaller and more condensed than that seen in 66, and the appearance plainly indicates that the centrosome has divided and that each part is performing its usual function at a pole of the spindle. The actual division of the centrosome was not observed.

After a short time the plate was seen to split, and the two halves, retaining their parallel relationship to one another, moved to opposite poles of the cell, and there remained, thus marking the anaphase. Figure 16, from a fixed preparation of a mononucleate cell, represents this stage. Almost immediately thereafter the granules and fat globules midway from the poles of the cell were seen to move inward as though a constriction were occurring about the nuclear area at this zone; the result was a dumb-bell-shaped mass within the elongated cell, formed of the nuclear area and surrounding protoplasm. Almost at once the cell membrane itself was seen to be undergoing constriction at this point, as shown in 68, at 7^h 05^m p. m. At the same time the nuclear areas at either end of the cell commenced to become free from granules of fat and other refractive material and the cell outline became larger, showing that the cell was flattening out and that the daughter nuclei were becoming reconstituted in the telophase.

That the intracellular pressure is considerably increased during this process is shown by the bulging outward of certain portions of the cell membrane, as illustrated in 68, to form bubble-like protuberances. Frequently the granules and fat globules may be seen to rush out into these evaginations, indicating the formation of cell currents, where pressure has been suddenly released, through giving way and stretching of localized areas of the cell wall. These protuberances soon flatten out, lie close to the cover-slip and expand, becoming armed with hyaline borders possessed of amœboid movement (Harrison, 1913, p. 67). The end of the cell opposite the connection with the daughter cell thus appears fimbriated, as shown by Lewis and Lewis (12c, figs. 8 and 10). These refractive borders act as pseudopodia to anchor the cell to the cover-slip and to drag the daughter cells apart.

The reforming nuclei, now more widely separated, and showing wider and clearer areas in the cell protoplasm, are seen in 69 at 7^h 25^m p. m., and at this time the cell was very much constricted, with the nuclei more widely separated. The constricted zone is somewhat more highly refractive than the surrounding tissue and resembles a short thread. Here also the cell processes are seen to be feeling their way outward and to be pulling the two daughter cells apart. The stage corresponding to this in the fixed preparations is shown in figure 17; here the chromatin is a closely clumped, darkly staining mass, and the individual chromosomes are becoming resolved into smaller granules. These subsequently become scattered, and appear in the later definitive, more lightly staining, nucleus as in figure 18. A marked expansion of cytoplasm is here to be noted.

There have thus been formed two separate and distinct daughter cells, in each of which the nucleus is becoming gradually reconstituted. As the cell was watched the nuclear areas became clear and the membranes distinct: nucleoli also appeared, two in each nucleus. Separation of the cells continued, their hyaline borders becoming very active, stretching away into the outlying media and writhing in a sluggish, eel-like manner. Soon the fat globules took up their characteristic arrangement in the cytoplasm, mitochondria appeared, and, in 70, at 8 p. m., 8 hours after the observation commenced, we have to recognize two cells, apparently normal, each with its own centrosome.

The process of mitosis was identical with that followed many times in mononucleate cells, except for the variation in the introductory stage, occasioned by the formation of the spireme from two nuclear parts instead of one. The various stages of mitosis, as it is found in the mononucleate cell, are well shown in the series, figures 14, 15, 16, 17, and 18, selected from a fixed preparation.

I have been unable to ascertain whether such spindle formations arising from the fusion of two nuclear portions are possessed of a double number of chromosomes, but the apparent identity of the mitotic process, after nuclear fusion has taken place, with that occurring in mononucleate cells, does not suggest any material variation in the chromatin arrangement. I am in agreement with Maximow (1908), when he says regarding similar spiremes (p. 95): "Aus diesen Spiremen entstehen immer regelmässige normale Mitosen."

These cells were not followed farther. The history for the period of 8 hours, however, shows conclusively that spiremes from these double nuclei may combine to form a single equatorial plate and division may occur by the ordinary mitotic process. That such mitosis occurs in all cases it is impossible to state from this isolated observation, but the presence of double nuclei (with spiremes like those shown in figures 22 and 23) here and there in the fixed preparations no doubt points to the occurrence of such nuclear fusion as a part of the process of mitotic division in the binucleate cell.

Cases have not been found where one portion only of a bipartite nucleus was in a condition of mitosis; hence it seems reasonable to conclude that both parts are always involved in the process. This much is demanded by our conception of the potential unity of the double nucleus, so far as its reproductive capacity is concerned.

In the case of the cells from which figures 22 and 23 were drawn, it may be argued that these represent telophases in which the daughter nuclei failed to separate. Many mononucleate cells have been followed entirely through the mitotic process, and failure of the daughter nuclei to separate has never been noted. Again, in figure 23, drawn from an iron-haematoxylin preparation, there is only a single centriole-pair, not two, as would be the case in a telophase.

It might even be suggested that such daughter nuclei have recombined, as observed by Kite and Chambers (1912); here, however, artificial conditions were existent, since the cells were being forcibly separated in the Barber moist chamber by mechanical means. Moreover, entire absence of constriction of the cytoplasm, as would occur in the telophase, points to the condition we are considering as representing the prophase. More than this, the fact that the process has been followed

in the living cell, from resting twin nucleus through mitosis to two separate and distinct daughter cells, would seem to be proof absolute that these figures 22 and 23 (which represent a phase of this process) are prophases of combining double nuclei.

The mere contact of two spireme-bearing nuclei (such as appear in figure 23), is of itself no evidence that they will combine, but when we bring to bear upon the interpretation of such a figure the evidence derived from a series such as that shown in plate IV, in which a nuclear formation, like that of figure 23, represents a stage, it seems obvious that these nuclear parts are undergoing fusion to form a single plate of chromosomes. Harman (1913) shows several figures of such nuclei in early cleavages of *Tenia tenuiformis* and *Moniezia* (her fig. c, plate 8), but here the separate nuclei have arisen by mitosis, according to her observations, and cleavage, which is delayed, will eventually separate the blastomeres. The nuclear membranes are here quite intact, and show no evidence of beginning dissolution.

It may be objected that the condition of spireme is no indication that mitosis is beginning. To this the reply may be made that in the cells of living tissue-cultures a nucleus showing a spireme of this kind, no companion cell in the same condition being present, always represented the prophase of mitosis.

Since mitosis occurs in binucleate cells *in vitro*, it might be assumed that it would also occur in such cells *in vivo*, and indeed this is the case, for Maximow (1908) has found figures in fixed preparations from the mesenchyme of embryo rabbits which strongly resemble those just described. In his figure 7 (p. 93) the spireme is forming in a dumb-bell-shaped nucleus, and in his figure 8 the nuclear fragments in which the spireme is found are quite separate. Maximow believes that his pictures represent the prophase of normal mitosis occurring in amitotic nuclei; this belief is supported by my observations upon the living cell shown in plate IV. In his figure 8 he finds the centriole-pair situated between the two coils of the spireme—a position corresponding to that characteristic for it in the amitotic nucleus, viz, in the cleft. In my figure 23, which is slightly later, the centrosome has shifted its position to the pole. He states that his results resemble the findings of Karpow (1904) for urodele amphibia; this latter author described a process of nuclear amitotic division, with subsequent formation of a spireme from the fragments (which may be two or more in number), with fusion to form one "mutterstern." It may therefore be concluded, from the finding of such double spiremes in embryonic tissue, that this process of mitosis in binucleate cells occurs in normal development. It is thus to be found in differentiating as well as non-differentiating cells.

I regret that I have seen no other living examples of combination of the parts of a double nucleus during mitosis, but the process is so rare that its observation thus is largely a matter of chance hitting upon a favorable cell. Mitosis occurs rather infrequently in the mononucleate cell, and when it is considered that the proportion of binucleate cells to total cells is very low (1 to 111) the remoteness of the possibility of finding a binucleate cell which will divide by mitosis may be realized. It is only in those cultures showing abundance of both binucleate cells and mitotic figures that there is any hope of finding such compound mitoses.

To ascertain the relative frequency with which mitosis occurred among the binucleate cells, as compared with the mononucleate, a study, by careful counting

and classification of cells, was made of the 20 preparations from chick heart mentioned before. In these estimates only the prophases were counted, since it is impossible to say of the cells in the later stages of mitosis whether they arose from a monopartite or bipartite nucleus. Degenerate cells were omitted, and also the area close to the original piece was not counted, since the cells here were usually too small and closely packed to be seen clearly. Nuclei with more than two parts of equal size were rare: such were grouped with the binucleate cells in this estimation.

It was found that there was a total of 41,106 mononucleate cells (excluding the later mitotic and amitotic forms); of these 47 were in the prophase of mitosis, or 0.114 per cent of the total. In the same series there was a total of 375 binucleate cells, 2 of which were in the prophase, or 0.53 per cent.

In spite of the rarity of occurrence of binucleate cells in prophase (there being only 2 in a total of 41,725 cells) it will be seen from this result that mitosis occurred even more frequently among the binucleate cells than among the mononucleate—in fact, 4.65 times as frequently. Thus, while it can not be stated definitely that mitosis with recombination of the nucleus always follows amitotic nuclear division, or, indeed, that it frequently does, it may nevertheless be affirmed with confidence, even allowing for the limited extent of the observation, that the incidence of mitosis in the binucleate cells is at least as high as that among the mononucleate.

If, in addition to this division by mitosis which these binucleate cells show, they be considered as also proliferating by direct division of the cytoplasm, it will be readily seen that their rate of proliferation would then be very much greater than that of the mononucleate cells. The improbability of this excessive multiplication strengthens the negative evidence to be put forward later that there is, in these binucleate cells, no division of the cytoplasm following direct division of the nucleus.

We have seen that a single mitotic figure can be formed from two nuclear portions, previously separate, but contained within the same cell. It has also been found that the spireme may form in a bent nucleus of a shape similar to those undergoing direct division. Figure 20 represents an early spireme in such a nucleus. There is apparently but a single centrosphere, situated in the cleft. Figure 21 shows a somewhat more advanced spireme. The nuclear membrane has disappeared and the chromosomes are more definite. One centrosphere is situated above, in the cleft, and there is an indistinct trace of a second in the clear area below.

We can thus construct a series, from cells taken from fixed specimens, illustrating prophases in single nuclei, in double nuclei, and in the intermediate forms connecting these. Figures 14 and 19 show spiremes in single nuclei. In the last are two well-marked centrospheres, indicating that a spindle is about to be formed. Figures 20 and 21 show the process in intermediate forms, and figures 22 and 23 show it in the double nucleus.

In figures 20 and 21 it is reasonable to suppose that the amitotic process has ceased, since the nuclear membrane has almost or quite disappeared, and for the same reason the process of karyokinesis, which is so obviously taking place in these cells and in those represented in figures 22 and 23, must in all of these cases be considered as starting up under circumstances where amitosis of the nucleus was under way, or was completed, rather than as having the process of amitosis superposed upon it.

Altogether the various forms of the nucleus in which spiremes are found in tissue-culture preparations resemble strikingly the findings of Karpow in the leucocytes of urodele amphibia. This similarity is brought out in the following paragraph from Maximow (1908, p. 95):

“Nun ist es aber nach Karpows Untersuchungen ziemlich sicher, dass hier die Kernamitose zwar zur Kernpolymorphie und sogar zu sicherer Kernteilung führt, dass sie aber doch keine richtige Zellvermehrung nach sich zieht. Wenn die Leukoeyten mit amitotisch zerschnürtem Kern sich teilen, so geschieht dies eben auf dem Wege der Karyokinese, und aus einem zerschnürten Kern oder sogar aus mehreren einzelnen, ganz getrennten, durch Amitose erzeugten Kernen entsteht dann eine einzige, gewöhnlich regelmässige, mitotische Figur. Man findet Spireme in ring-, hantel-, rosenkranzförmigen Kernen, oft auch zwei oder mehrere einzelne Kerne in einer Zelle, alle gleichzeitig im Zustande des Spirems, woraus dann immer ein gewöhnlicher Mutterstern resultiert.”

Maximow also shows a spireme in a dumb-bell-shaped nucleus found in his own material, and observes:

“Die tief eingeschnürten, oder auch schon ganz zerteilten Kerne können in Mitose treten und man bekommt dann hantelförmige Spireme (fig. 7) oder zwei kugelige Spireme nebeneinander in ein und derselben Zelle (fig. 8).”

Thus it would seem that the nucleus enters upon the process of mitosis whenever the stimulus initiating this process occurs, whether rounded, bent, undergoing constriction, or divided into two parts, and in all of these, after the single spireme has been formed, the process is apparently identical.

The question as to whether or not the cytoplasm of the cell divides following direct fission of the nucleus, to form two separate and distinct cells, has been much discussed by various authors, among them Maximow (1908), who found—besides the cases in which the amitotically divided nuclear portions formed a single combined mitotic figure and divided by karyokinesis—also instances where such portions simply became separated from one another and surrounded by protoplasm, to form ordinary mononucleate cells. In short, Maximow believes that, though amitosis of the nucleus may be followed by cell division arising through a process of mitosis involving the directly divided nuclear fragments, yet it can lead directly to cell proliferation without intervening mitosis. As such a method of actual cell multiplication, Maximow believes that amitosis functions in certain areas of the normal developing tissue of the rabbit, and he has found it also in the guinea pig. Furthermore, he expresses the view that cells arising by direct division can later divide by mitosis, but his reasons for the latter assumption are not given.

On the other hand there are those who oppose this view and believe that nuclear amitosis is never followed by cell amitosis. For instance, Karpow (1904), according to Maximow (1908, p. 89) came to the conclusion, based upon his observations upon the leucocytes of urodele amphibia: “dass in den Fällen, wo richtige Amitose wirklich vorliegt, man eigentlich doch nur Kernvermehrung annehmen kann, keine Zellvermehrung.” This view is in agreement with the findings of Conklin (1903, p. 671) for follicular epithelial cells of the common cricket.

No reliable evidence that fission of the cytoplasm follows that of the nucleus has been found in the tissue cultures examined by me. It is quite true that so-called “paired” cells (*i. e.*, cells closely resembling one another in form, staining, etc.,

lying side by side, but separated by cleavage of the cytoplasm) may be picked out in the fixed preparations, and it might be urged that such were of amitotic origin. This contention can not be proved, however, and it is more probable, in view of the lack of positive evidence of amitotic division of the cytoplasm, that these cells are either of mitotic origin or have migrated together.

The problem was attacked by the method of continuous observation of binucleate cells (in which the double nucleus has been shown to arise by direct fission), the object being to see if the cytoplasm would divide, and in this way give rise to two separate mononucleate cells. Several such cells containing twin nuclei were followed, but in every case the cell finally degenerated without dividing, after an observation of shorter or longer duration. As an example, the following may be recorded: In a connective-tissue binucleate cell from an 8-day chick heart of 24 hours' growth, the portions of the nucleus were at first pressed closely together, but after 30 minutes they separated slightly, as in figure 9, and remained apart for 2 hours, when they again became pressed together. The cell was observed for 11½ hours, and the process of separation and reapproximation of the nuclei occurred four times during this period. There was no trace of cytoplasmic division and the only changes noted were those mentioned—some shifting of position of the nuclei and a slight decrease in size of the nuclear parts; the latter is believed to be due to prolonged exposure to light. Continuous change in shape of the cell was followed by change in shape of the nucleus.

This observation shows conclusively that the binucleate cell may remain a very long time without direct division of the cytoplasm, and has been confirmed in the case of other binucleate cells. In living cultures the absence of evidence of direct division of the cytoplasm, combined with similar absence in the case of fixed preparations, leaves us with no ground for the assumption that such direct division ever occurs. Even granting that cytoplasmic division occurs at all, the process appears to be so long delayed that it can not be of much importance as a method of cell proliferation.

This view is in accord with that of Conklin (1903, p. 670), for follicular epithelial cells of the common cricket, but does not coincide with that of Child (1907, *c*, *d*, and *e*), who concluded from this examination of the cells of *Moniezia* and other animals that amitosis was a rapid method of division which occurred where the stimulus to divide was very great and the supply of nutrition was inadequate. Patterson (1908) and others hold similar views. From the evidence which tissue cultures afford, however, I am inclined to agree with Harman (1913, p. 219) that the assumption that amitosis is a more rapid method of cell proliferation than mitosis is hardly justified.

The observation just recorded also shows that the interpretation of "double" nuclei (such as those seen in my figures 4, 59, and 60 as separate nuclear sacs touching one another) is correct, for the sacs have been seen to move apart and afterward to return to their original contact with one another, and to repeat this process. As has been already mentioned, the apposed surfaces of such paired nuclei give rise to an appearance resembling an intranuclear plate; such a plate has, however, not been found by me in the cells of tissue cultures.

The twin nucleus is, then, to be regarded as potentially a single nucleus, in which the nuclear material is separated into two or more sacs. This nuclear material is not to be considered as in any way equally divided between the nuclear portions, which are by no means daughter nuclei. This view is strengthened by the fact that the centrosome, as has been observed, is single in binucleate cells. Before the cell containing such a single twin nucleus can divide, it seems to be essential, judging from the observations, that the nuclear material should recombine and a spireme be formed from the chromatin material in its entirety.

It may be asked whether nuclear fusion, in these binucleate cells, ever occurs without an accompanying mitosis. I have seen no evidence of such recombination, either in living or fixed preparations, and regard it as improbable, because (among other reasons) the parts increase in size following their division and the single nucleus, which would result from their reunion, would be unusually large.

Nothing was brought to light, in the material examined, which would in any way support the assumption that there are two distinct types of cell division, amitosis and mitosis, for the type of amitosis which I have described involves only the nucleus, and mitosis was the only process which resulted in the formation of two separate cells.

These observations upon nuclear amitosis do not point to its being an evidence of cell degeneration, for the cells in which it is found are not highly specialized and do not show any more tendency to degenerate than the other cells of the culture. It is generally assumed that mitosis takes place only in normal cells, so that the occurrence of mitosis in amitotically divided nuclei hardly allows them to be considered as degenerate. So, too, the occurrence of amitosis and mitosis in the same preparation (as in the culture from which figure 2 was drawn), where the conditions under which the cells are growing are apparently identical, militates against the view that the environment is not favorable, for the two processes are going on side by side, and mitosis demands suitable conditions. The statements of Wieman (1910, p. 174), "amitosis occurs usually under abnormal metabolic conditions which are unfavorable to normal metabolic processes" and "it can occur under circumstances that make mitosis impossible," are out of harmony with his finding of both direct and indirect division side by side in the same field, as shown in his figure 13. This coincident occurrence of mitosis and amitosis has been noted by other investigators.

The conception of amitosis which I have advanced thus differs radically from that of Flemming (1892 and 1893), vom Rath (1891 and 1895), Ziegler (1891), and Ziegler u. vom Rath (1891). They believed that amitosis occurred in cells which were of a transient character and in those which were very highly specialized or on the way to degeneration: and that in cells of amitotic origin the process of mitosis was not believed to take place. In their scheme the condition which I shall speak of as nuclear fragmentation seems to have a place.

According to this conception, then, amitosis constitutes simply a change in form of the nucleus without increase in its reproductive capacity, and not an actual cell division: and division of such an amitotic cell occurs only by karyokinesis in which there is a recombination of the nuclear material. If this view be correct,

and of universal application, it may be possible to reconcile amitosis with the chromosome hypothesis, for, since mitosis would be the only method of actual cell proliferation, an unequal distribution of chromatin material to the daughter cells would not be possible, according to our conception of the mitotic process.

NUCLEAR FRAGMENTATION.

A note may here be made regarding a curious form of nuclear division which bears some resemblance to the one just described, but which differs from it in many important particulars. It is known as nuclear fragmentation or unequal multiple nuclear fission, and was found to occur where the conditions for growth were not favorable—for instance, in old cultures, in which the food and oxygen supply had become depleted and katabolic products had accumulated (figs. 36 to 47) and in those to which a toxic constituent had been added (*e. g.*, ethyl alcohol, figs. 48 to 58). It thus seems to be a pathological condition and is characterized by marked malformation of the nucleus, manifesting itself in lobulation and by a breaking away of these lobules, so that what was formerly a single nucleus comes to consist of two, three, or as many as seven or eight apparently separate pieces.

The forms in which fragmentation presents itself are various, as may be seen by reference to figures 36 to 47, drawn from a 6-day growth from the stomach of a 5-day chick. The nucleus may be but moderately deformed, as in figure 49, where a small bud has become constricted off, or there may be two, three, or more lobes or appendages, as seen in figures 40 and 41. These small fragments are in all stages of constriction, ranging from a blunt, sessile protuberance to a small pedunculated mass, held sometimes by a mere thread, as in figure 46. Extremely irregular forms, as 37, are not infrequent, and completely separated portions, as in 36, 42, and 45, are quite often met with. Each fragment may or may not contain a nucleolus. In the smallest pieces it is absent. In some cases, as in figure 45, if the nucleolus happens to be caught in the constricting zone it may become separated, but this is a rare occurrence. Where the nucleus is lobulated the number of lobules usually exceeds the number of nucleolar portions. The culture shows other evidences of degeneration. The size of the nuclear portion seems here to bear no relationship to the size of the karyosome fragment, as it does in the multiple direct division of the nucleus described by Schultz (1915).

The extent to which this process of fragmentation may proceed is seen by reference to the fact that 66 per cent of the nuclei were malformed in some way, and 34 per cent were actually fragmented, in ten fields from the preparation from which figures 36 to 47 were drawn. There were no mitotic figures found in this preparation.

In no case was there found any evidence of division of the cell protoplasm following nuclear fragmentation; on the contrary, a sort of syncytium was formed, in which the cytoplasm was filled with nuclear fragments of varying size. The picture presented by such a nuclear complex is markedly different from that of the giant cell, among the points of differentiation being the widely varying size of the nuclei, their lobulation, and the presence of buds in process of separation from the main nuclear mass. Again, in fragmentation the cytoplasm does not increase, as in the case of the giant cell.

The entire absence of division of the cell protoplasm prevents this nuclear change from being regarded as a method of cell proliferation. Again, there is no evidence that such nuclear fragments ever reunite to form a spireme after the manner already described for the ordinary type of amitotic nucleus: indeed, mitotic figures are absent from such preparations—a fact which seems to indicate that the conditions which bring about fragmentation also prevent karyokinesis.

The differences which fragmentation presents as compared with the usual form of direct nuclear division may be briefly summarized as follows: The nucleus is of irregular contour, multilobulated, and breaks up into a number of small, unequal-sized parts, which frequently do not contain nucleoli; the nuclear parts remain small, indicating that they have little or no power of growth, for the total volume of the nuclear substance does not seem to be increased following division. There is no evidence of fusion of the fragments contained in a single mass of cytoplasm to form a single mitotic figure. Finally, the process is found in growths which are existing under abnormal conditions, such as the presence of toxins or a deficiency of oxygen, and such conditions act to prevent mitosis.

As contrasted with this we find, in the case of the ordinary binucleate or multinucleate cell, nuclear portions of regular contour, few in number (usually not more than two), of almost equal size, each containing as a rule one or more nucleoli. These parts apparently possess the power of growth, for in size they are comparable with the nuclei of the mononucleate cell. The fragments of the "double" nucleus are also able to combine and form a single mitotic figure. These cells are found in normal cultures, in which mitotic figures are frequently to be seen.

Fragmentation is similar to the division which produces the ordinary binucleate cell in that the position of the centrosphere and mitochondria with relation to the nucleus is the same. In figures 48 to 58 these structures will be seen occupying the cleft, as in 55 and 58, or situated between the fragments, as in 50 and 54.

Nuclear forms of this character are not infrequently found in the literature. Glaser (1907) describes an analogous form of nuclear fragmentation which occurs in the degenerating food ova of *Fasciolaria tulipa*. This he regards as "pathological amitosis" as distinguished from physiological amitosis. Child (1907c, p. 288) speaks of "degenerative amitosis" in starving planarians, stating that these forms "differ in appearance from the amitoses in regenerating tissues;" again (1907c, p. 173) he finds that "nuclear fragmentation is a frequent accompaniment of degeneration."

On the whole, therefore, judging from the prevailing views of authors, and from the conditions obtaining in the cultures in which it occurs, it seems reasonable to regard nuclear fragmentation as an evidence of degeneration. These final changes are, perhaps, to be looked upon as an active reaction of the nucleus to unfavorable conditions of its environment, as, for instance, the presence of toxins due to katabolism, or chemical change in the media, or injurious material added to the media, as alcohol, or to deficiency in food or oxygen.

In this connection it is interesting to note that Lewis (1911) and Miller and Reed (1912) demonstrated that the presence of toxins caused an increase in the number of lobes of the neutrophilic leucocyte in blood of the human subject and also in that of the guinea pig and rabbit. They looked upon this increase as a physiological reaction on the part of the leucocyte. Wherry (1913) found that amœbæ

grown in oxygen-poor media showed division of the nucleus without cleavage of the cell protoplasm, and Wieman (1910) expresses the view that lack of oxygen may be a cause of a similar nuclear fragmentation in the material which he examined. Again, Holmes (1914) noted such a fragmentation in tissue cultures kept a week or more without changing the medium; when, however, the medium was changed frequently there was no indication of such nuclear change. Fragmentation was accompanied by other evidences of degeneration. Here, too, lack of oxygen may be the underlying cause, and the increased nuclear surface due to the change in form and multiple division of the nucleus may represent the effort, on the part of the cell, to secure an increased respiratory area.

The mechanics of nuclear fragmentation is no less complicated than that of true nuclear amitosis; indeed, it is probable that new and obscure factors bring about a change in nuclear outline and division of its substance. The activity of the centrosphere and mitochondria may be regarded as similar to that found in the true form of nuclear amitosis, since their relation to the nucleus is the same.

Nuclear forms somewhat resembling those just described, but simpler in character, are occasionally seen in apparently normal tissue cultures; *e. g.*, those shown in figures 11, 12, and 13. Similar forms have been described in embryonic tissue developing normally, as figures 7*b*, 8*a* and *c* of Child (1904) and some of the figures of Maximow (1908). They appear to be examples of sporadic and simple fragmentation. The fate of these buds is obscure, but is probably degeneration.

SUMMARY.

The following general conclusions, based upon the results of the foregoing investigations, have been reached:

BINUCLEATE CELL.

Incidence: In 20 preparations the binucleate cells made up 0.9 per cent of the total cells appearing in the new growth. They were more abundant in membranes growing from the heart than in growths from any other tissue, and in cultures of hearts of 5 days of age than in those from older cardiac tissue. They were also more abundant in new growths from cultures of the second day than in those of the first; this suggests that some, at least, of these cells have arisen in the new growth rather than in the original piece, with subsequent migration into the new growth.

The proportion of cells containing amitotic (constricted) nuclei to the total number of cells was 1 to 835; that of amitotic nuclei to bipartite nuclei was 1 to 7.5, and that of amitotic to mitotic nuclei was 1 to 3.4.

Origin: The paired nuclei of binucleate cells in tissue cultures arise by direct division of the nucleus, or nuclear amitosis, without division of the cytoplasm. This occurred in perfectly normal cells.

Constriction of the nuclear membrane, from one or both sides, which seems to be associated with a karyoplasmic streaming away from the nuclear equator, was the only mechanism observed in direct nuclear fission, and in this process an activity of the centrosphere and mitochondria, combined with elongation of the nucleus, appeared to be the principal factors. The centrosphere does not divide, nor do the centrosomes separate.

The process of nuclear amitosis is slow, excepting the final stage, which is rapid. There seems to be a critical point in nuclear constriction: before this point is reached the nucleus may return to its original form, but after it has been passed the cleavage of the nucleus proceeds rapidly, and results in two separate nuclear parts.

Division of the nucleolus is not an essential of amitotic nuclear division; it may, however, be concerned with the size of the nuclear fragments.

There is no evidence of a form of nuclear amitosis that depends upon the formation of an intranuclear membrane which subsequently splits. Such a structure is simulated by the apposed surfaces of the nuclear membranes of the parts of the nucleus of a binucleate cell, when they are in close contact. Sometimes, also, nucleoli, mitochondria, and inbending of the nuclear wall may resemble such a membrane.

Fate: There is no evidence that direct division of the cytoplasm follows direct division of the nucleus: thus amitosis is not a method of complete cell division, but is to be looked upon as a change in form of a healthy nucleus.

The regular process of mitosis may occur in binucleate cells. During this process the chromatin material from both nuclear portions is merged into one equatorial plate of chromosomes, the spiremes, which begin to arise separately in the two nuclear parts, joining together to form the chromosomes. Furthermore, this is the only kind of cell division which was found to occur in binucleate cells; they either divide by mitosis or remain as they are, without fission of the protoplasm.

The separate parts of the double nucleus have no reproductive independence (though they may have metabolic independence), and act as a unit in mitosis. Hence the reproductive capacity of the bipartite and monopartite nucleus is the same.

Mitosis occurred as frequently in the binucleate as in the mononucleate cells.

Nuclear fusion, without mitosis, has not been found to occur.

GENERAL.

Mitosis occurs in a nucleus irrespective of its shape; thus the spireme was found in nuclei of rounded form, in those presenting equatorial constriction, and in those divided into two portions.

Chromosome hypothesis: Nuclear amitosis is not incompatible with theories of inheritance which assume that the chromosome is the bearer of hereditary characters.

Giant cells: The binucleate cell seems to be the first stage in the formation of the giant cell, which probably arises by a repetition of nuclear amitosis. This conception does not include the formation of the foreign-body giant cell.

Nuclear fragmentation was found to occur where conditions for life were not favorable, and was thus a form of degenerative change. Fission of a healthy nucleus (amitosis) must thus be distinguished from fission of an unhealthy nucleus (fragmentation).

Karyosomes of the cells examined were irregular in shape, underwent continuous change in morphology, size, number, and position, and were made up of numerous closely packed masses of gel, each with a core of greater density.

The centrosphere in the cells examined was a slightly concentrated gel containing a centrosome (usually paired). Its border is irregular, and this undergoes continu-

ous change in outline and appears to be intimately associated with adjacent mitochondria.

Vital dyes: Gentian violet did not prove to be a true vital dye. While it stained the intranuclear bodies and nuclear membrane, its action was toxic and coagulative, and the cells speedily degenerated.

Janus green, in low dilutions, was found to stain mitochondria specifically, but its action was destructive, causing speedy death of the cell, with dissolution of the mitochondria.

Embryonic cells: Many, at least, of the facts obtained from observation of cells in tissue cultures may be applied to the interpretation of similar cells developing normally in the embryo.

In conclusion I wish to record my indebtedness to M. R. and W. H. Lewis for the loan of their splendid collection of fixed preparations, and for their valued guidance; also to Dr. F. R. Lillie for the courtesy of a room at the Marine Biological Laboratory at Woods Hole, where some of the work was carried on.

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EXPLANATION OF PLATES.

PLATE I.

- FIG. 1. Area of new growth from No. 42, 8:12:14 (Lewis collection). In the field are six binucleate (*a*) and one quadrinucleate (*b*) cells. The material was heart from a chick which had been incubated for 6 days; growth was of 48 hours' duration, in Locke (0.5 per cent dextrose), fixation by osmic-acid vapor, and staining with iron hematoxylin. (*b*) shows two young daughter cells, the product of a recent mitosis. The guide-line from (*a*) terminates in the centrosphere of a binucleate cell. Retouched photograph. $\times 465$.
- FIG. 2. Elongated nucleus with bilateral constriction—the beginning of direct bilateral nuclear fission. The nucleolus is also apparently dividing. This figure, and also Nos. 3, 4, 5, 11, 12, 13, 14, 15, 16, 17, 18, and 22 are from No. 14, 9:1:15 (Lewis). Heart from 6-day chick; grown in Locke (0.5 per cent dextrose) with a little yolk; fixed on third day of growth in Zenker; stained with Mallory's connective tissue stain. On account of the technique the cytoplasmic details are not represented. This and following drawings, except figures 24 to 35, were outlined by camera lucida. $\times 1,012$.
- FIG. 3. Elongated nucleus almost completely divided; final stage of direct bilateral nuclear fission. $\times 1,012$.
- FIG. 4. Nuclear fission completed; nuclear parts divided and lying in contact. $\times 1,012$.
- FIG. 5. Direct unilateral nuclear fission; initial stage. $\times 1,012$.
- FIG. 6. Direct unilateral nuclear fission; final stage; cell of connective-tissue type; nuclear parts connected only by the merest filament; centrosphere between nuclear sacs; mitochondria streaming across the narrow connecting strand. Drawn from preparation No. 2 (Lewis); 7-day chick heart grown for 5 days in Locke (1 per cent dextrose); osmic-acid vapor and iron hematoxylin. $\times 1,032$.
- FIG. 7. Nuclear fission completed; growth from heart membrane; cell similar to that shown in figure 4, but prepared to show the centrosphere and mitochondria; the single centrosphere contains two centrosomes; its position, opposite the line of contact of the two nuclear portions (below and to the right), is characteristic. No. 42 (Lewis) (see fig. 1). $\times 1,032$.

PLATE II.

- FIG. 8. Final stage of direct bilateral nuclear fission in a cell of connective-tissue type; shows the somewhat unequal nuclear parts joined by a very slender thread, apparently the attenuated nuclear membrane; overlying this are several strands of mitochondria, a similar relationship to that of figure 6; the larger nuclear sac contains two nucleoli; the smaller but one. A large centrosphere, from which many mitochondria radiate, is conspicuous. The entire cell is very thin and shows mitochondria streaming out into the processes. The morphology and arrangement of the mitochondria is characteristic for the connective-tissue cell growing *in vitro*, at periods other than mitosis. No. 17, 24:11:14 (Lewis). 6-day chick stomach; Locke (1 per cent dextrose); 3-day growth; osmic-acid vapor and iron hematoxylin. $\times 1,012$.
- FIG. 9. A binucleate cell from heart membrane; the two parts are somewhat separated, and lying between them a single centrosphere and mitochondria are to be seen; the latter resemble cocci or short bacilli and show the characteristic radia arrangement about the centrosphere. This type of mitochondria is found in cells of membranes growing from chick hearts. No. 42 (Lewis) (see fig. 1). $\times 1,012$.
- FIG. 10. Nucleus of distorted form in cell of connective-tissue type found in a culture growing in a weak alcoholic medium. The nucleoli in this preparation show as aggregations of granules; this appearance of the nucleoli in connective-tissue cells stained in this way is found when differentiation with iron alum is carried too far. Mitochondria are apparently uninjured. No. 23, 24:11:14 (Lewis). 6-day chick stomach grown in Locke (1 per cent dextrose) to which ethyl alcohol had been added to make approximately 1 per cent; 3-day culture; osmic-acid vapor and iron hematoxylin. $\times 1,012$.
- FIGS. 11, 12, 13. These figures show a simple degree of nuclear fragmentation. They were found in a culture in which the cells were otherwise apparently normal. In figure 11 the nucleus is constricted at one end; the larger portion contains two nucleoli of unequal size and irregular shape. In figures 12 and 13 the constriction is farther advanced. No. 14, Lewis (see fig. 2). $\times 1,012$.
- FIG. 14. Prophase of mitosis. Nuclear membrane and nucleoli are disappearing and skein is forming; cell not yet rounded. No. 14, Lewis (see fig. 2). This, and the four figures which follow it, represent the process of mitosis in a mononucleate cell. All drawn from the same preparation. $\times 1,012$.
- FIG. 15. Metaphase. Cell rounded and compact; processes drawn in; cytoplasm granular and stains very densely with hematoxylin; definite spindle with equatorial plate of chromosomes. $\times 1,012$.
- FIG. 16. Anaphase. The chromosomes have separated and the remains of the spindle may be seen as faintly defined streaks connecting the two aggregations of chromosomes; cytoplasm still densely granular and darkly staining, the entire cell contracted; cell-processes small and thread-like. $\times 1,012$.
- FIG. 17. Early telophase. Chromosomes less clearly marked, the chromatin masses breaking up. No evidence of nuclear membranes is to be seen. The cytoplasm is dividing, as shown by constriction about the equator. Markedly granular and darkly staining protoplasm. $\times 1,012$.

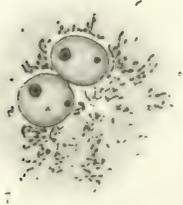
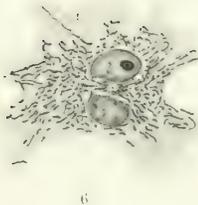
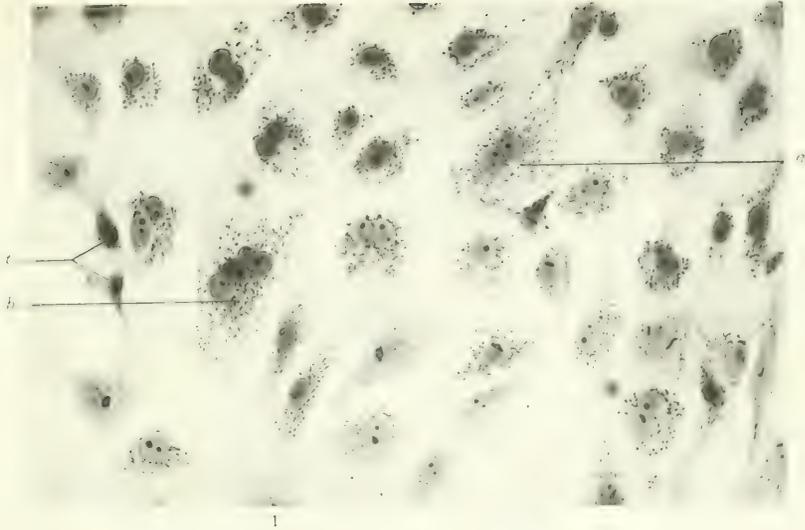
- FIG. 18. Late telophase. The two daughter cells are seen, separated and spread out thinly; protoplasm stains much more lightly; nuclei well defined and contain coarsely granular chromatin. In each daughter nucleus the beginning of a nucleolus is to be seen; but is very small compared with the size of this body at maturity. $\times 1,012$.
- FIG. 19. Late prophase of mitosis, in cell probably of connective-tissue type; two centrospheres at opposite poles; nuclear membrane has disappeared; spireme well marked; mitochondria short and thick. No. 42, Lewis (see fig. 1). Figures 20 and 21 are from the same preparation. $\times 1,032$.
- FIG. 20. Early prophase in a nucleus showing beginning direct unilateral fission; skein forming, nucleoli disappearing; centrosphere still single, situated in the fissure; mitochondria becoming shorter and thicker, and are intermediate in these respects between those seen in figure 8 and those of figures 19 and 21; nuclear membrane has almost disappeared. Cell of connective-tissue type. $\times 1,032$.
- FIG. 21. Late prophase in a nucleus undergoing direct unilateral fission. Skein has formed and nuclear membrane has disappeared; one centrosphere is to be seen in the cleft, and there is some indication of a second on the opposite side of the nucleus, in the area devoid of mitochondria. $\times 1,032$.
- FIG. 22. Prophase in a binucleate cell. Early stage. Skein is forming; membrane and nucleoli are disappearing. Some chromatin has become segregated in the area of contact between the two parts. The method of fixation and staining does not permit of the centrosphere and mitochondria being seen. No. 14 Lewis (see fig. 2). $\times 1,012$.
- FIG. 23. Prophase in a binucleate cell of connective-tissue type; somewhat later stage than figure 22. In each nuclear portion there has been formed simultaneously a skein and the nuclear membrane has disappeared. The chromatin material of the combined double nucleus will form a single equatorial plate of chromosomes, as in figure 67. Only one centrosphere, containing two centrosomes, is seen in the preparation, situated at one extremity of the fusing nucleus, it having come from the interval between the nuclear parts. It is thus probable that the spindle will form parallel with the long axis of the fusing nucleus. Mitochondria are short and thick. No. 18, 21:2:14, Lewis. 7-day chick heart, grown for 2 days in Locke (0.25 per cent dextrose); osmic-acid vapor and iron hematoxylin. $\times 1,032$.

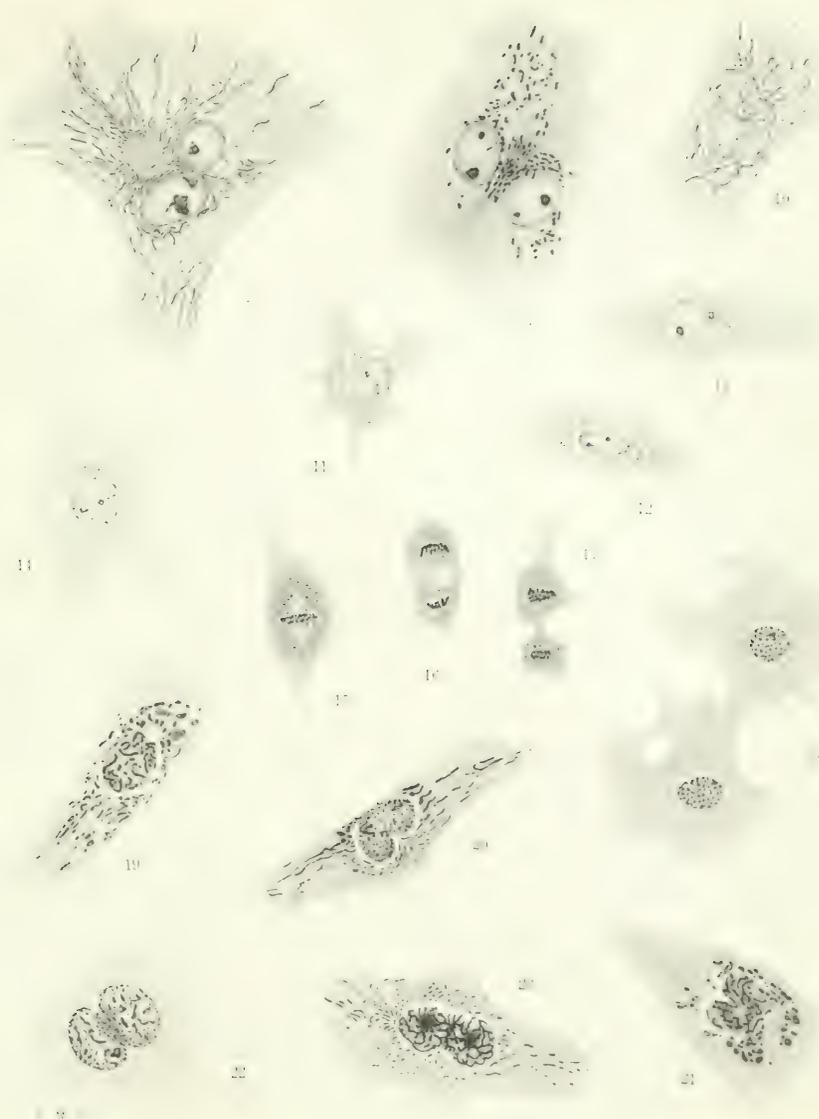
PLATE III.

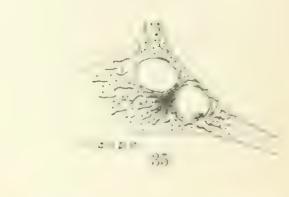
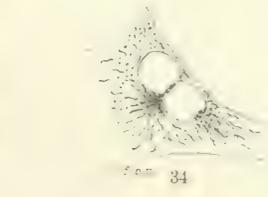
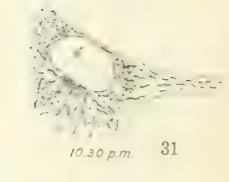
- FIGS. 24-35. A series of drawings from a living connective-tissue cell made at 15-minute intervals for 2½ hours. The nucleus at the start was elongated and notched at one side. It was seen to take various forms, and ended as two separate nuclear parts. The series thus shows direct nuclear fission. It will be seen that the centrosphere is contained within the unilateral cleft, and when the nucleus ultimately divides the centrosphere is situated between the parts of the nucleus; mitochondria stream across the interval separating these two parts. The nucleolar bodies undergo interesting changes. The nuclear outlines, position of nucleoli and centrosphere, the cell outlines, and principal features of the cytoplasm were sketched in fresh hand from direct observation of the living cell. The drawings were afterwards retouched by reference to fixed preparations. Small circles represent fat globules, and short threads mitochondria. *c*, figure 24, marks the centrosphere. 5-day chick heart; 57 hours' cultivation, from No. 7, 9:1:15, in Locke (0.5 per cent dextrose) with extract of chick embryo. \times about 900.
- FIGS. 36-47. Fragmenting nuclei showing probable effect on form of nucleus of prolonged growth in unchanged media; outlines of nuclei very irregular, each has a number of lobes; in some cases separation of these lobes has taken place. Culture shows other evidences of degeneration. No division of the cytoplasm following division of the nucleus was observed. Drawn from various cells selected from No. 23, 12:1:15 (Lewis). 5-day chick stomach in Locke (0.5 per cent dextrose). Zenker; Mallory connective-tissue stain. Culture grown for 6 days in the same media. $\times 1,012$.
- FIGS. 48-58. A collection of nuclei of irregular form, grown in media containing alcohol; centrospheres are sketched in to show their characteristic relationship. Same preparation as figure 10. $\times 1,500$.
- FIG. 59. A regular paired nucleus from the same preparation as that from which the series 48-58 was drawn. Some of the nuclei have escaped distortion. $\times 1,500$.

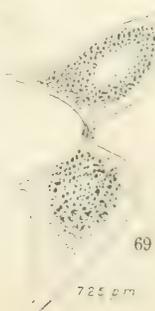
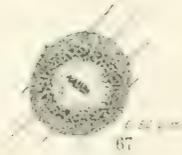
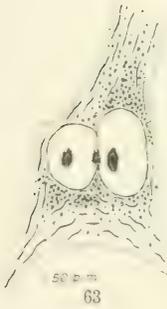
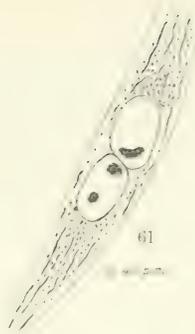
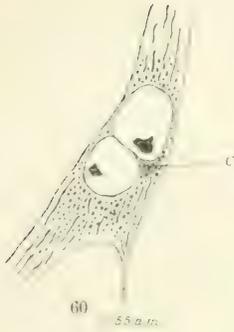
PLATE IV.

- FIGS. 60-70. A series of camera-lucida drawings from a single living binucleate cell of the connective-tissue type, which was observed continuously for 8 hours. At the beginning there were two separate nuclear parts, with one centrosphere; the parts combined to form a single mitotic figure, and the successive stages of mitosis are seen in figures 66 to 70. The ultimate result is two separate mononuclear cells, each containing a single centrosphere. The series brings out the fact that the parts of the "double" nucleus are not independent so far as their reproductive capacity is concerned, but in cell division they combine and act as a single nucleus. *c*, figure 60, represents the centrosphere. 7-day chick heart, grown for 19 hours when the observation commenced. Locke (1 per cent dextrose) with extract of chick embryo. Culture of March 15, 1915. $\times 1,500$.









CONTRIBUTIONS TO EMBRYOLOGY

VOLUME V, No. 14



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CONTRIBUTIONS TO EMBRYOLOGY, NO. 14

THE DEVELOPMENT OF THE CEREBRO-SPINAL SPACES IN
PIG AND IN MAN

By LEWIS H. WEED

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THE DEVELOPMENT OF THE CEREBRO-SPINAL SPACES IN PIG AND IN MAN.

BY LEWIS H. WEED.

I. INTRODUCTORY.

Probably no field in embryology has been less explored than that relating to the meninges. Our knowledge of the transformation of the perimedullary mesenchyme into the three fully developed membranes about the cerebro-spinal axis has been largely of a crude sort, with gross generalities based on inexact or incomplete evidence. The present work was undertaken in the hope that by a study of the various stages in the development of the cerebro-spinal spaces there might be gained some knowledge which would afford a basis for a conception of this dynamic metamorphosis.

Many of the problems centering around the development of the meningeal spaces have recently been expounded by Cushing⁽⁹⁾.* Not only do we lack knowledge as to the method of differentiation of the primitive mesenchyme, but we know little about the establishment of the circulation of the cerebro-spinal fluid. When do the chorioid plexuses begin to secrete? When does the venous absorption of the fluid take place? When does the perivascular system begin to remove waste products from the cerebral tissue? And also, what factors play a part in the formation of the subarachnoid and subdural spaces?

These questions, some of which it is hoped the present study will answer, relate to the field of physiological anatomy. Consideration of the subject, however, serves to convince one that they must be investigated coincidentally with the stages of morphological differentiation; for it may readily be conceived that the physiological use of the meningeal spaces may precede any morphological differentiation of the three membranes, nor indeed is it unlikely that one of the active causative factors in the metamorphosis concerns this filling of the mesenchyme about the nervous system with fluid.

This study, therefore, has been anatomical, but with a broader scope than purely morphological studies would have afforded. Not only has it dealt with the morphological differentiations about the nervous system, but throughout the investigation the relationship of these structures to the possible presence of cerebro-spinal fluid has been considered. As the problem developed it was projected more and more into the difficult realm of "tissue spaces." Interest in these spaces largely concerned their physiology, but many points of correspondence between structure and function were found.

In some measure this work is a development of an earlier study of some of the anatomical and physiological problems of the cerebro-spinal fluid, carried out in the laboratory of Dr. Harvey Cushing at the Harvard Medical School.

*The figures in parentheses refer to the bibliography at the end of this paper.

II. REVIEW OF LITERATURE.

In order fully to understand the problems which confront one in the study of the embryonic cerebro-spinal spaces, a comprehension of the stage to which investigations have brought our knowledge of these fluid-pathways in the adult is necessary. It is with this purpose that the adult relationships are here considered. The inclusion of this material may be pardoned, for it will be seen that unanimity of opinion has by no means existed in regard to any of the problems concerned in the circulation of the cerebro-spinal fluid.

Modern anatomical knowledge of the meninges dates from the work of Axel Key and Gustav Retzius²⁹. These Swedish investigators, in their excellent monograph published in 1875, first conclusively demonstrated the anatomical continuity of the spinal and cerebral subarachnoid spaces. But for years after their publication appeared, a physiological continuity between the subdural and subarachnoid spaces was argued for by many observers, notably by Hill²⁴. Gradually, however, workers in this field have reached the opinion that the subarachnoid spaces (the interrupted but continuous channels between arachnoidea and pia) are functionally the channels for the cerebro-spinal fluid. Between the intra-leptomeningeal and the subdural spaces no anatomical connection exists; physiologically there may be some mode of fluid-passage. Thus Hill²⁴ states that either by filtration or through actual foramina fluid passes readily from one space to the other. Quineke⁴⁶, from observations on animals, somewhat similarly premised a connection between the two spaces, but only in the direction from subdural to subarachnoid. His experiments, based on the results of the injection of cinnabar granules, are open to criticism as indicating a normal passage-way for the fluid; for, as he has recorded, an intense phagocytosis of practically all of his granules occurred. More modern conceptions of the subdural space treat it as a space anatomically closed, lined externally by a polygonal mesothelium. Less error is introduced if it be regarded as analogous in many respects to well-known serous cavities rather than as an essential portion of the pathway for the cerebro-spinal fluid.

The question of the absorption or escape of cerebro-spinal fluid from the subarachnoid space has claimed the attention of many workers. Since the original conception that the meningeal coverings were actually serous cavities, anatomical investigations have furnished many new views. Key and Retzius, by spinal subarachnoid injection of gelatine masses colored with Berlin blue, demonstrated an apparent passage of the injection fluid into the great cerebral venous sinuses through the Pacchionian granulations (die Arachnoidzotten). Their observations were made on a cadaver and the injections carried out under fairly low pressures (about 60 mm. of mercury). A lesser drainage of the fluid into the lymphatics was also shown.

Since the view advanced by Key and Retzius of the absorption of cerebro-spinal fluid, the general trend has been away from the idea of an absorption into the venous sinuses. Quineke's⁴⁶ observations, made on lower animals after the subarachnoid introduction of cinnabar granules, really offer some substantiation of

this theory, but the failure to find the great Pacchionian granulations in infants and in the lower animals caused many workers to reject utterly the conception of the Pacchionian granulations as the functionally active mechanism for the fluid escape.

Physiological evidence, however, advanced by Hill²⁴ from intraspinal injection of methylene blue, indicated that the major escape of the cerebro-spinal fluid was into the venous sinuses of the dura, while a slow and minor absorption took place along the lymphatic channels. Ziegler²⁷, with potassium ferrocyanide introduced into the cerebro-spinal space, likewise found that the venous absorption was much greater and more rapid than the lymphatic. Reiner and Schmitzler²⁸ with the same agent detected the ferrocyanide in the jugular blood-stream after injection. With olive oil these investigators found a similar venous absorption, but with a slowing of the venous blood-stream. Lewandowsky³³, also using ferrocyanide, found this salt in the urine within 30 minutes after its subarachnoid injection. Spina⁵², from observations on freshly killed and living animals, presented somewhat similar evidence of a major venous and lesser lymphatic absorption. Cushing⁹ suggested a valve-like mechanism of escape of the fluid, his hypothesis being based on the findings after the introduction of mercury into the meningeal spaces.

Several theories concerning the absorption of cerebro-spinal fluid into the blood-vascular system have more recently been offered. Mott⁴¹, from a study of dilated perivascular and perineuronal spaces, has advanced the idea of fluid-escape by way of the perivascular system into the cerebral capillaries. Dandy and Blackfan¹⁰, from an analysis of their evidence, consider that the chief drainage of the fluid is into the capillaries of the pia-arachnoid. Opposed to this conception of a major drainage of cerebro-spinal fluid into the blood-vascular system is the view championed by Cathelin⁶, that the lymphatic drainage is the chief method of fluid-escape. Cathelin's contention of a veritable circulation of the fluid has not received support from other workers.

Thus it will be seen that since the work of Key and Retzius the trend of opinion has been away from the view that the Pacchionian granulations carry the cerebro-spinal fluid into the venous sinuses.

In the earlier investigation⁵⁵ carried out in the Harvard Medical School the problems of this fluid absorption were attacked in a somewhat different manner than by previous workers. True solutions of potassium ferrocyanide and iron-ammonium citrate, such as have been used in the present investigation, were injected into the spinal subarachnoid space under pressures but slightly above the normal. The animals (dogs, cats, and monkeys) were kept under anesthesia during the period of injection, which was usually continued for several hours. Complete filling of the subarachnoid channels was secured by this technique, provided the injections were continued for a sufficient length of time. At the conclusion of the experiment the foreign solution was precipitated *in situ* and blocks were carried through for histological purposes.

Many of the anatomical findings in this work carried out as outlined are of interest in the present problem. The complete correspondence of the spinal and

cerebral subarachnoid spaces as demonstrated by Key and Retzius was amply verified. The normal return of the cerebro-spinal fluid to the general circulation by way of the arachnoidal villi into the great dural sinuses was demonstrated. These villi are projections of the arachnoidea through the dural wall, prolonged directly beneath the vascular endothelium of the venous sinuses. Furthermore, columns of arachnoid cells were found, normally affording fluid channels in the dura. In addition to the major escape of cerebro-spinal fluid into the sinuses a lesser drainage was also demonstrated, slower than the primary drainage, out along certain of the emergent nerves into the lymphatic system. No evidence whatsoever was obtained in support of any of the theories of a drainage of cerebro-spinal fluid into either the leptomeningeal or cerebral capillaries, nor could an anatomical valve-like mechanism along the great sagittal sinus be demonstrated. The process of escape of cerebro-spinal fluid from the arachnoid villus unto the great sinus appeared to be a simple one of filtration or of diffusion.

Another of the problems concerning cerebro-spinal fluid, which has been of interest to anatomists and physiologists, is the source of the fluid. Haller⁽²¹⁾ and Magendie⁽³⁵⁾, to whom the greatest credit for work on this subject must be given, believed it to be the product of the leptomeninges. Faivre⁽¹⁵⁾ in 1853 and Luschka⁽³⁴⁾ in 1855 were the first to suggest the chorioid plexuses as the elaborators of this circumambient medium. Since then the view has been generally accepted that these villous structures do give origin to the fluid, but the early evidence was based wholly on the glandular character of the plexus. Cappelletti⁽⁵⁾ and Pettit and Girard⁽⁴³⁾ offered more definite proof of this relationship by the introduction of pharmacological agents which affected the rate of production of the fluid. These latter authors recorded definite histological changes in the cells of the plexus when influenced by these drugs, indicating, in conjunction with the changed rate of production of the fluid, an undoubted relationship of the chorioid plexus to the fluid elaboration. Since these early investigations many observers—Findlay⁽¹⁷⁾, Meek⁽³⁷⁾, Mott⁽⁴¹⁾, Pellizzi⁽⁴²⁾, Ilworostuchin⁽²⁶⁾, and others—have studied the histology of the chorioid plexus with reference to its function as an elaborator of the cerebro-spinal fluid.

In addition to the elaboration of the fluid by the chorioid plexuses, increments are furnished by the nervous tissue itself. This elimination from the nervous system occurs by way of the perivascular spaces. In the previous work referred to⁽⁵⁵⁾ it was found possible to inject the entire perivascular system by continuing a physiological injection of the spinal subarachnoid space, and subsequently causing an extreme cerebral anemia. By this procedure an injection of the system to its termination about the cerebral capillaries and nerve-cells could be secured. From this and other evidence the view was advanced that the cerebro-spinal fluid was derived from a dual source—in part from the perivascular system and in greater part from the chorioid plexuses. This view had already been advanced, but on rather insufficient grounds, by Mestrezat⁽³⁸⁾ and by Plaut, Rehm, and Schottmuller⁽⁴⁴⁾. Recently Frazier⁽¹⁸⁾ has signified his acceptance of this conception of the source of the fluid.

Such, then, is the basis for our present understanding of the meninges, in regard to their characteristic morphology and particularly their functional relationship to the cerebro-spinal fluid. Without a consideration of the circumambient fluid morphological studies of these membranes would be incomplete, for in order to understand the meninges knowledge concerning the cerebro-spinal fluid is necessary.

THE COMPARATIVE ANATOMY OF THE MENINGES.

Sterzi⁵³ has published a comprehensive report of the comparative anatomy of the spinal meninges. From his studies he has advanced hypotheses, supported by observations on a limited number of fetuses, regarding the development of the human meninges. On account of the interest of this subject in relation to the present discussion a brief summary of Sterzi's work will be here included.

In the acrania there is no special envelope of the central nervous system, but rather a fibrous sheath corresponding to the meninges of higher forms. This fibrous sheath is largely made up of circular fibers, except in the median ventral line, where there occurs a ventral ligament of longitudinal fibers. In cyclostomes, however, there is found a single "primitive meninx"—vascular and composed of white and elastic fibrils coursing in a longitudinal direction. Some of these fibrils traverse the perimeningeal spaces (filled with star-like cells, with some fatty tissue) and are attached to the inner surface of the vertebræ. This same general plan of a single "primitive meninx" is likewise found in fishes (elasmobranchs, teleosts, etc.); the membrane here is often pigmented and follows closely the external architecture of the spinal cord. The perimeningeal space is filled by mucus in elasmobranchs, but in teleosts this is replaced by fat. For the most part there are found dorsal and ventral ligaments and two lateral ligaments.

The next stage in the development of a more complete form of spinal covering is found in the urodele amphibia. A "primitive meninx," formed of two layers, often artificially separated from each other, replaces the simpler meninx of cyclostomes and fishes. Of the two layers in this membrane the external is thin and free from pigment; the inner, strongly pigmented, adheres to the spinal cord. The meninx is perforated by the denticulate ligaments.

In amphibia (Anura) Sterzi found the first evidence of a "secondary meninx," corresponding to the pia-arachnoid. Surrounding this membrane, but separated from it, is the dura, thin and transparent; between the two meninges is the intradural (subdural) space. The dura lies in the peridural space. The spinal prolongations of the endolymphatic canals lie in the dorsal part of the peridural space. Both the dura and the "secondary meninx" continue outward along the roots of the spinal nerves and along the filum terminale. Embryologically the perimedullary mesenchyme is differentiated into these two meninges in the Anura.

This arrangement of the two meninges in Anura is followed out in reptiles. The dura, thin as in the amphibia, is covered by endothelium and is vascular. The "secondary meninx" possesses laterally the denticulated ligaments and ventrally the ventral ligament. Both the peridural and intradural spaces are very small.

Likewise in birds Sterzi was able to differentiate only two meninges—the dura and the “secondary meninx.” These membranes are quite similar to those of reptiles. The “secondary meninx” has acquired three layers—an outer endothelial covering, a middle vascular layer, and an inner membrane closely adhering to the cord. This is a distinct approach to the three meninges of mammals. An intradural (subdural) space covered by endothelium can be easily made out. The development of these avian meninges concerns a differentiation of the perimedullary mesenchyme.

The arachnoid, according to Sterzi, first appears as a definite membrane in mammals (marsupials and placentals). In marsupials this arachnoid has become well differentiated and the pia mater possesses denticulated and ventral ligaments. A transformation of the extradural portion of the denticulated ligaments unites the dura to the endorachis. In perissodactyla the differentiation of the three meninges (particularly of the arachnoid) is incomplete. The arachnoid is separated from the pia mater by a peculiar tissue which contains numerous lymphatic lakes, forming the intra-arachnoid spaces. No intradural (subdural) space is apparent, due to the approximation of dura and arachnoid. The subdural space is clothed by endothelial cells; these can not be made out in the intra-arachnoid spaces. The dura is surrounded by a fatty pad.

According to Sterzi the augmentation of the intra-arachnoid (subarachnoid) space is the distinguishing characteristic of the meninges of carnivora. This increase takes place at the expense of the peridural space.

As Sterzi developed the knowledge of the comparative anatomy of the lower forms—of the transition from the primitive meninx of the cyclostomes to the three membranes of mammals—the possible correlation of this analogy to the embryological development in mammals became apparent. He extended his observations to human beings and to human fetuses. His findings will be detailed in the following section.

Farrar¹⁶, in a short discussion of the development of the meninges of the chick, finds in early stages three laminae about the spinal cord, “the middle one of which alone still presents the primitive features of the mesoblastic-sheath.” The inner layer, close to the medullary tissue, is highly vascular; in the outer zone “the connective-tissue elements are assuming elongated forms and crowding together with long axes parallel, giving a very close mesh with long but extremely narrow spaces, in contradistinction to the loose irregular reticulum of the pia-arachnoid.” The outer lamina becomes dura mater, while the inner two zones are considered together as the embryonic pia-arachnoid. Farrar defines the pia-arachnoid as developmentally a single membrane consisting of a loose reticulum, at the outer and inner borders of which limiting membranes are formed.

LITERATURE ON THE DEVELOPMENT OF THE MAMMALIAN MENINGEAL SPACES.

The development of the meningeal spaces in mammals has not been studied extensively, and the literature in regard to it is quite meager. Only a very few workers have touched upon the subject except casually. Reford⁴⁷, working in the Anatomical Laboratory of the Johns Hopkins University, studied the development of these spaces by the method of injection with india ink. His work unfortunately has never been published, but it has been rather extensively referred to by Sabin⁴⁹ in 1912 and by Cushing⁹ in 1914. Their summaries of this work are here included.

Miss Sabin thus speaks of it:

"In a study of the arachnoid made by the injection method in the Anatomical Laboratory of the Johns Hopkins University by L. J. Reford, and as yet unpublished, it has been shown that the thinning out of the mesenchyme around the central nervous system is not haphazard, but that injections of the same stage give the same pattern, and that the form of the arachnoid space changes as the brain develops. That is to say, the arachnoid space has as definite a form as the celom, and it never connects with the lymphatics."

Cushing⁽⁹⁾ gives the following summary:

"It was thought that an investigation of the cerebro-spinal spaces in the embryo would most likely shed light on the subject, and some unpublished studies in this direction were undertaken in 1904 and 1905 by Lewis L. Reford in Mall's laboratory in Baltimore. In living pig embryos of various stages low spinal india-ink injections were made either into the wide central canal or into the subarachnoid space, and the embryos were subsequently cleared. It appeared from the course taken by the injection mass that the full development of the spinal arachnoid preceded that of the intracranial spaces, the impression being gained that the separation of the primitive meninx into its layers occurred later over the cerebral vertex than in the basilar portion of the chamber. Still, I never felt quite convinced that the failure of injection of the meninges over the surface of the hemispheres in many of Reford's specimens was not due to the floating up of the brain against its envelopes by the introduction of the injection mass from below. However this may be, it was nevertheless apparent that a venous injection of the body of the embryo was often produced, and the impression was gained that a communication existed between the basal subarachnoid spaces and the precursors of the sinusoidal veins of the cranial chamber which empty into the jugulars. If due to an artifact from a vascular rupture, at all events the communication always occurred at the same point. Reford, moreover, in agreement with Cruveilhier, Reichert, and Kölliker, came to doubt the existence of the foraminal opening described by Magendie, believing that the opening was an artifact and that the fluid escaped by seepage through a persistent membrane."

It is regrettable that Reford's study has not been published, as it represents the only attempt to solve the problems of the development of the cerebro-spinal space by the method of injection. As stated in subsequent sections of this communication, his apparent failure to control pressures of injection and to use only granular suspensions is unfortunate.

In a study of the development of the blood-vessels of the human brain, Mall³⁶ noted the ease with which an extravasation into the embryonic arachnoid spaces could be brought about by increasing the pressure in a venous injection. In a specimen of 46 mm, an arterial injection with aqueous prussian-blue resulted in a complete subarachnoid spread, due to rupture of the vessels as they perforated the

nervous tissue. In general, it was found that this rule held: an arterial extravasation always took place from the perforating capillaries, while a similar venous rupture occurred in the veins themselves.

Mall made similar observations on living pig embryos from 30 to 80 mm. in length, with analogous results. But when, in these embryos, the arachnoid spaces were completely filled by an intraventricular injection of india ink, no passage of the granular injection into the veins or sinuses occurred. The ventricular injection flowed into the extraventricular spaces "through the medial opening of the fourth ventricle." From the spinal cord the ink extended for a short distance along the main trunks of the spinal nerves. In the larger embryos (above 50 mm.) the ink usually gushed from the mouth, reaching it by way of the Eustachian tube. Using, in the pig embryo, the heart as the mechanism for injecting the ink, extravasation from the cerebral vessels in the arachnoid spaces occurred.

In one human specimen of 90 mm., Mall found both the arachnoid spaces and the cerebral ventricles filled with india ink after an arterial injection of that suspension. He states: "The injection passes through the medial opening into the fourth ventricle (Magendie), and apparently the ventricles are injected through this opening from the arachnoid."

To His⁽²⁵⁾ and to Kölliker⁽³¹⁾ belongs the credit of first having established on a firm basis the development of all the meninges in man from mesenchyme. This perimedullary layer of mesenchyme Salvi⁽⁵⁰⁾ called the "primitive meninx"—a term now used extensively in comparative anatomy. The primitive meninx divides into two layers, the outer forming the dura and the inner the pia-arachnoid. Sterzi⁽⁵³⁾, working on the development of the human spinal meninges, advanced a view similar to that of Kölliker. The perimedullary mesenchyme (the "primitive meninx") divides into two portions, one hugging the inner surfaces of the vertebræ and the other adhering to the cord. This inner layer of the perimedullary mesenchyme, according to Sterzi, should properly be termed the "primitive meninx," as it divides subsequently into dura and the "secondary meninx," which in turn forms both arachnoid and pia. The denticulate ligaments develop in the "primitive meninx." The dura and arachnoid in human embryos are modeled up to a certain point on the cord; then, with the augmentation of the subarachnoid space, they follow the outline of the vertebral canal.

His⁽²⁵⁾ has given information regarding the development of the meninges, with particular reference to the formation of the subarachnoid space. He affirms the mesenchymal origin of all of the cerebro-spinal membranes. His describes the first differentiation of mesenchyme to form the meninges as consisting of two zones of condensation, the outer being closely associated with the developing perichondrium of the vertebral column and the inner facing upon the cord. Between these two zones of condensation the subarachnoid space develops, posterior and anterior spaces first appearing, with later fusion laterally. These appearances were met with in chicks of 10 to 12 days' incubation. Quite soon after this process of space-development a separation occurs which gives rise to a complete subarachnoid space. Later the splitting-off of dura from the vertebral periosteum takes place.

III. METHODS OF INVESTIGATION.

In the study of any problem dealing with the development of fluid-spaces within the body, the method of investigation must of necessity be such as to offer exceptional opportunities for control. In the present work several well-known and generally accepted anatomical procedures were naturally suggested, such as injection of the spaces about the central nervous system, reconstruction from serial sections, or merely study of the various stages by means of serial sections.

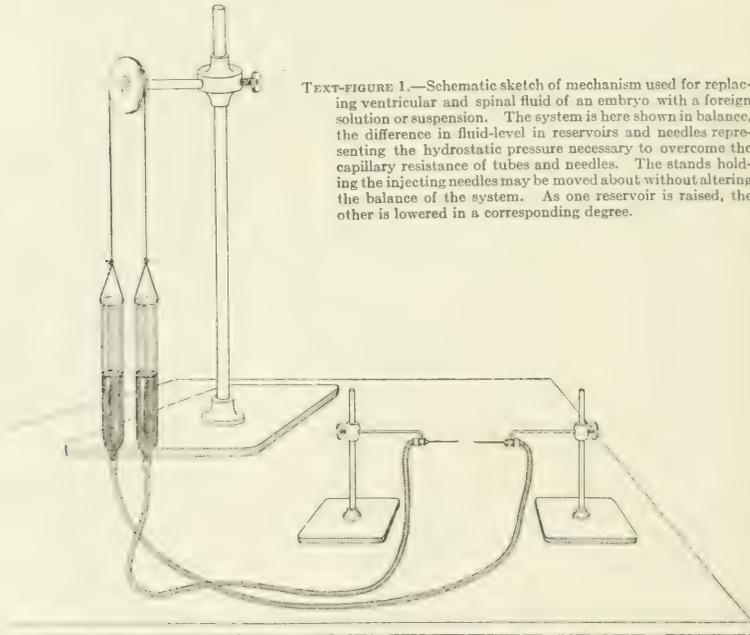
It was ascertained early in the investigation that by injection and serial sections without reconstruction the necessary stages in the process of meningeal differentiation could be established. In regard actually to the physiological aspects of the problem more reliance was placed on the results of injection than on any histological differentiation, for, as explained above, considerations of the pathway and of the flow of the cerebro-spinal fluid were deemed most important. No method of injection, however, holds out much promise in such a problem unless it can be applied, under conditions approximating the normal, within the spaces about the nervous system. The greatest objection to reliance upon injections in this problem is in relation to pressures. From the very nature of the case it will be realized that any ordinary injection into the embryonic central canal or perispinal space must result in an extraordinary increase in the normal tension of the fluid. This objection applies to any method employed, whether that of a simple syringe and needle, the glass tube and bulb devised by Knowler³⁰, or a glass capillary-tube contrivance.

The erroneous conclusions drawn by investigators from the employment of excessive pressures of injection are nowhere more strikingly illustrated than in studies of the circulation of the cerebro-spinal fluid. Many such examples were recently brought forward in a critical review⁵⁵ published in connection with a study of the fluid. In the embryo, with structures and membranes still of very little tensile strength, the consequences of a disregard for the pressures of injection are even more disastrous.

A second criterion for the study of fluid-pathways in the body is necessarily the type of injection mass. Not only should attention be paid to the pressures involved, but the peculiarities of the particular body-fluid concerned must be considered. Adopting for this work on the embryo the same standards followed in the previous investigation on the adult, true solutions were used in place of the customary granular suspensions. Emulsions and viscous solutions were not employed because of their obvious disadvantages in studying the passage through membranes. India ink and process black (in which carbon granules are the particulate matter) were also used, but only for comparison with the standard true solution, as the likelihood of the insoluble granules being phagocyted within the period of experimentation or of being caught mechanically in tissue meshes appeared *a priori* to be too great.

In any study of fluid-pathways in the body, not only must the injection fluid be a true solution, but it must also be one which is not attracted to particular cells (as with many stains). Likewise, colloid stains (such as the benzidine group) could not be employed, because of the fact that certain cells (macrophages, as described

by Evans¹⁴) phagocyte the small colloidal particles. In addition, the true solution must be readily precipitated as an insoluble salt, capable of remaining unchanged in histological technique. After trying many salts in long-continued injections into the adult cerebro-spinal spaces, it was found that solutions of potassium ferrocyanide and iron-ammonium citrate in equal parts were admirably adapted to the purposes of the experiment. By the addition of a mineral acid (preferably hydrochloric) ferric ferrocyanide could be precipitated. This prussian-blue is insoluble in the routine technique and is readily identified in sections. After mounting in damar or balsam the blue granules can be observed unchanged for several months, but after a year there is some deterioration in the specimen, due to a conversion of the blue into indefinite greens.

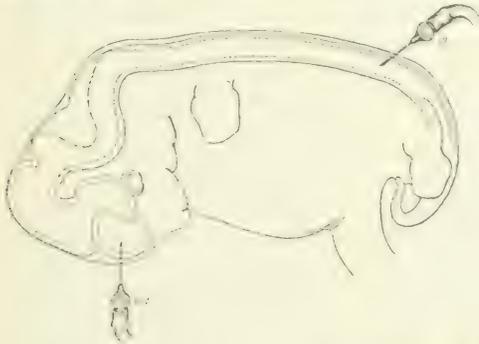


TEXT-FIGURE 1.—Schematic sketch of mechanism used for replacing ventricular and spinal fluid of an embryo with a foreign solution or suspension. The system is here shown in balance, the difference in fluid-level in reservoirs and needles representing the hydrostatic pressure necessary to overcome the capillary resistance of tubes and needles. The stands holding the injecting needles may be moved about without altering the balance of the system. As one reservoir is raised, the other is lowered in a corresponding degree.

In regard to these two major factors in the employment of injections (pressure and true solution) it was found necessary to devise a method of experimentation which would satisfy the requirements of the problem. Solutions of the ferrocyanide and of the citrate were non-toxic within the central nervous system and afforded an excellent histological means for following the fluid-pathways. It was hoped at first that a simple "replacement" type of injection might be employed, as in the adult animals. In this procedure a given amount of fluid was withdrawn from the subarachnoid spaces and immediately replaced by an equal quantity of the injection fluid. The method was successfully tried on fetal cats of considerable size, but

was impracticable on small embryos. After such a replacement the animals were allowed to live for varying periods of time (up to 3 hours) and then killed.

It was soon ascertained that the essential circulation of the cerebro-spinal fluid was established in pig embryos of less than 30 mm. in crown-rump measurement. Hence the ordinary method of replacement had to be discarded for some more delicate system. With the realization that a simultaneous withdrawal and introduction in a living embryo would be far preferable to a two-stage procedure, the extremely simple apparatus pictured in text-figures 1 and 2 was employed. This device consists of two glass tubes of uniform and like bores, suspended from above by a string running over a pulley. To the tapering lower ends of these reservoirs are attached rubber tubes which connect the reservoirs to two needles. These needles are held at the same level by two metal brackets which can be moved at will on a level glass plate.



TEXT-FIGURE 2.—Diagrammatic representation of the method of replacing the cerebro-spinal fluid in a living embryo. The spinal needle is inserted into the central canal of the spinal cord, while the cerebral needle is introduced into one of the cerebral ventricles. The canal of the spinal cord and the cerebral ventricles are represented by the interrupted lines. The foreign fluids are introduced by the spinal needle and withdrawn by the cranial.

The apparatus is employed as follows: Both tubular reservoirs are filled up to the point where the fluid is just ready to fall from the needle in a drop. This point is easily obtained by filling the reservoirs slightly in excess and allowing this excess fluid to run off from the needle. With the system thus in balance the needles lie in the same horizontal plane and can be moved without altering the balance of the solutions. The injection is made by inserting one needle into the central canal of the spinal cord and the other into one of the lateral ventricles; then as the reservoir connecting with the spinal needle is raised the other is lowered, so that an amount of fluid equal to that introduced into the spinal canal is withdrawn from the cerebral ventricles. In this way the whole contents of the cerebral ventricles and central canal of the spinal cord can be slowly withdrawn without increasing the pressure in the central nervous system. The initial pressure necessary to secure this flow is only that required to overcome the capillary resistance of the medullary-canal system. In practically all cases this can be accomplished by using a positive pressure of less than 60 mm. of water (associated with a negative pressure of the same degree).

In the present study the above procedure was the routine method of injection employed. Pig embryos, brought from the abattoir, contained in the uterus, were found to be wholly satisfactory material. If not permitted to cool excessively in transit the embryos lived for at least two hours in a 38° incubator. On being received at the laboratory a section of the uterine wall containing the placenta was excised, with the embryo left connected by the umbilical structures. As soon as the technical preparations for injection were completed the amnion was opened and the embryo placed upon a padded block at the proper level. The first needle was then inserted into the easily discernible central canal of the spinal cord and the second into the left cerebral ventricle or into the mesencephalic ventricle. By elevation of the reservoir connected with the first needle the cerebro-spinal fluid was replaced by the injection solution. As soon as the replacement was complete the needles were withdrawn and the embryo and its uterine portion replaced in the incubator. The heart of the embryo could be easily observed in the smaller forms and served as the index of a continued circulation.

The incubation of the embryos was continued for varying periods of time, but it was soon ascertained that a period of over 30 minutes generally resulted in a complete spread of the injection solution. For comparison the period of incubation was lengthened and shortened, but the best results were usually obtained with a 45-minute incubation after the replacement.

Injections of the necessary true solutions were made, in the routine experiment, with a 1 per cent concentration of potassium ferrocyanide and iron-ammonium citrate in distilled water. By a 1 per cent solution is meant a salt concentration of this amount (potassium ferrocyanide, 0.5 gm.; iron-ammonium citrate, 0.5 gm.; water, 100 c.c.). The resultant true solution should be practically isotonic with the body-fluids. In this way any injurious consequences due to hypertonic or hypotonic solutions were apparently overcome. The factors of osmosis and diffusion also had to be considered in this connection.

Other concentrations of the so-called "ferrocyanide mixture" were used, but only for the sake of comparison or for the purpose of investigating some particular phase of the problem. The results obtained by the use of these concentrations were not relied upon as affording standards for the normal pathway of the cerebro-spinal fluid.

In addition to the replacement type of injection, many observations were carried out on pig embryos, with a simple syringe-injection of the ferrocyanide solution into the central canal of the spinal cord or into the cerebral ventricles. It proved to be a very simple matter to regulate the pressures by this method, and three arbitrary standards (mild, moderate, and strong) were found to be of value in a comparison of the extent of the spread obtained by replacement and by injection.

The prussian-blue reaction (formation of ferric ferrocyanide) was obtained in these experiments by fixing the whole embryo in an agent containing hydrochloric acid. For histological study the best results were obtained by immersing the specimen from 1 to 10 minutes in a 10 per cent formaldehyde solution containing

1 per cent hydrochloric acid. After this primary procedure, during which the ferrocyanide was precipitated, the embryo was transferred to Bouin's fluid (saturated aqueous picric acid, 75; formaldehyde 40 per cent, 20; glacial acetic acid, 5). The specimens were allowed to fix over night and were then dehydrated in graded alcohols. From 30 per cent alcohol, use was made of 4 per cent changes up to 60 per cent; and from this point to absolute the changes were by 2 per cent gradations.

In addition to the technique outlined above, Carnoy's solution and 10 per cent formol were employed. The Carnoy fluid, containing acid (absolute alcohol, 60; chloroform, 30; glacial acetic acid, 10; hydrochloric acid, 1) proved to be of particular service in the study of specimens cleared by the Spalteholz method; histologically, however, it has not been as valuable as Bouin's fluid.

Besides the ferrocyanide solution, two other injection masses were constantly employed. Solutions of silver nitrate in concentrations of 0.5 per cent were injected into the central canal of the spinal cord and into the cerebral ventricles. This method, with reduction of the silver salt in the sunlight, gives very pleasing preparations. It is, however, subject to obvious limitations. The intraspinal toxicity of the silver, together with its action as a precipitant of albuminous substances, renders its use unsatisfactory in replacement experiments. Furthermore, it reacts apparently with any protein tissue, irrespective of the true function of that tissue (as, for example, its coagulation of the lining ependyma of the ventricles).

India ink, the other substance employed, is of extreme value in anatomical studies. Because of the suspension of carbon granules it possesses the disadvantages already commented upon for the study of any true pathway of fluid. It has been of service, however, in the present work in showing marked differences in spread from that of true solutions and in furnishing information in regard to fluid passage through a membrane.

This investigation has been carried out on the basic idea of correlating the physiological spread of the embryonic cerebro-spinal fluid with the gradual transformation of the perimedullary mesenchyme into the three fully formed meninges. This has necessitated a histological study of the embryo. Pigs for the most part were the animals used, but the findings have all been verified by a study of the same regions in the human embryos in possession of the Carnegie Institution of Washington. In addition, certain structural characters have likewise been identified in sections of chick, rabbit, and cat embryos.

It was early apparent that the material to be of value must be free from any great shrinkage about the central nervous system. Comparative freedom from this artifact was obtained by fixing the embryo alive in Bouin's fluid and dehydrating by 2 and 4 per cent gradations of alcohol. The material was chiefly cut in paraffin after being embedded by means of xylol.

The methods of investigation outlined in the foregoing paragraphs have been followed throughout the major portion of the work. In many minor instances other procedures not commented upon have been employed: these will be detailed in appropriate subdivisions of this paper.

IV. INJECTIONS AND REPLACEMENTS IN THE CEREBRO-SPINAL SYSTEM.

RESULTS OF REPLACEMENTS IN THE VENTRICULAR SYSTEM OF TRUE SOLUTIONS.

The results of experiments carried out on embryo pigs by the technical procedures outlined in the previous section will be detailed here. The study was made on this animal because of the facility with which it could be obtained living and in good condition and also because it exhibits the characteristic meningeal anatomy of all mammals. The chick could not be used in this investigation on account of the dissimilarity between the avian and the mammalian meninges.

The chief problem concerned here was the actual physiological extent of the cerebro-spinal spaces. This apparently could be ascertained by the replacement of cerebro-spinal fluid by the ferrocyanide mass. But there was also to be considered the passage of fluid from the ventricles out into the periaxial* spaces, corresponding exactly to a similar passage in the adult.

If into the central canal of the spinal cord of a living pig embryo of 9 mm., crown to rump measurement, an injection of the ferrocyanide solution be made under very mild syringe-pressure, the ventricles can be fairly well filled without rupture of any element. Incubation of this experimental embryo with its circulation continuing almost unabated for an hour should cause a further spread of the fluid throughout the normal canals. If at the end of this time the whole embryo is fixed in an acid medium the ferrocyanide will be precipitated *in situ*.

Such a specimen, subsequently cleared by the Spalteholz method, is represented in figure 1.† In this drawing the spread of the injection solution is clearly shown. Running upward from the point of introduction, wholly within the central canal of the spinal cord, it reaches the bulbar region and extends outward into the large fourth ventricle, appearing as a dense collection of the prussian-blue. Cephalad from this region it spreads in diminishing intensity until it is finally lacking in the diencephalon.

The injected solution, then, in spite of the unavoidable increase in the normal intramedullary pressure, is contained only within the medullary-canal system (central canal of spinal cord and cerebral ventricles). There is no evidence of any spread outwards, either from the third or fourth ventricle.

In the next stage of meningeal development the replacement method can be used, as the embryo is no longer too small for its employment. In figure 2 is represented an embryo of 13 mm., in which the circulation continued for 90 minutes after the replacement. The same general picture shown in figure 1 results. The whole medullary-canal system is filled with the precipitated prussian-blue, which is densest in the region of the fourth ventricle. The roof of the ventricle, however, shows a striking difference from that of the ventricle in the embryo of 9 mm. Just posterior to the cerebellar lip is a regular oval, which is covered from within by a dense collection of prussian-blue granules, causing it to stand out in clear contrast to the thinner and more evenly distributed blue lining of the remainder of the

*Throughout this paper the term "periaxial" has been used in the sense of "around the central nervous system" or "around the cerebro-spinal axis."

†Throughout this work the reference "figure" 1, etc., refers to plate illustrations; the word "text-figure" refers to the illustrations inserted in the text.

roof. This oval area is comparatively large and comprises a portion of the superior or anterior half of the ventricular roof. This area, differentiated from the remainder of the rhombencephalic roof, is clearly shown in figure 2, a drawing of a cleared specimen of this stage.

With the exception of this strikingly dense area in the rhombic roof, the injection spread in an embryo of 13 mm., subjected to replacement of the cerebro-spinal fluid by the ferrocyanide, differs in no way from that in the embryo of 9 mm. Careful inspection of figure 2 is convincing that the spread still remains within the medullary canals, with no extension of the fluid into the spaces outside of the cerebro-spinal axis. It seems justifiable, then, to speak of the cerebro-spinal spaces at this stage of development as being only intramedullary in type, with no indication as yet of a meningeal fluid cushion (corresponding to the adult subarachnoid space).

With the use of larger embryos, however, for the medullary replacement with ferrocyanide and citrate, the picture gradually changes. The first indication of a more advanced stage of development is obtained in embryos whose length exceeds 14 mm. Figure 3, of a pig embryo of 14.5 mm., is included here as representing this further extension of the injection fluid. The cerebro-spinal fluid of this specimen was replaced, by the compensating mechanism, by a solution of potassium ferrocyanide and iron-ammonium citrate. The embryo was then kept alive (as judged by the heart-beat) for a period of one hour. At the end of this time it was fixed in an acid medium and subsequently cleared in oil of wintergreen after careful dehydration.

The essential differences between an embryo of this stage and one of the stage represented in figure 2 concerns the spread of the injection fluid from the roof of the fourth ventricle. Both specimens show a complete filling of the intramedullary system (cerebral ventricles and central canal of the spinal cord) with the precipitated prussian-blue granules. The specimen of 13 mm. (fig. 2) is characterized by a dense oval collection of the prussian-blue on the upper and inner surface of the rhombic roof. In the specimen shown in figure 3, in contradistinction to this localized aggregation of granular matter, there is a delicate extension of the injection fluid caudalwards from the roof of the fourth ventricle. This fusiform projection is here readily made out, lying beneath the skin over the ventricular roof and separated quite distinctly from the easily discernible line of the roof. This outward extension of the fluid has a fairly wide and deep origin from the upper portion of the roof, but tapers caudally to a sharp point with considerable rapidity.

At the stage of 14 mm. the roof of the fourth ventricle shows the small depression which marks the formation of the chorioid plexuses. With this depression occurring transversely the relation of the external surface of the embryo to the ventricular roof necessarily alters somewhat in this region. The chorioidal depression of the roof gradually becomes separated from the skin; and it is into this area between the skin and the ventricular ependyma that the first spread from the cerebral ventricles occurs. At this stage, illustrated in figure 3, the injection is intramedullary in type, with but slight extension into the pericerebral tissues.

The pericerebral spread may be made out in nearly all replacements in embryos of 14 mm., but in a few cases the injection has remained intramedullary in type. In embryos of 16 mm. the spread into the pericerebral tissues is invariably found. Often, with this extension of the replacement solution outside the ventricles, the oval area noted in the stage of 13 mm. persists. (This phenomenon is especially well shown in a simple injection of silver nitrate, illustrated in figure 11.)

The next stage of importance in the development of the cerebro-spinal spaces is represented in figure 4, a drawing of a pig embryo of 18 mm. in which a typical intramedullary replacement of the cerebro-spinal fluid with a solution of potassium ferrocyanide and iron-ammonium citrate had been made. Here, with the exception of the region of the roof of the fourth ventricle, the replaced fluid is contained solely within the central canal of the spinal cord and within the cerebral ventricles. The roof region, however, exhibits a new phenomenon, which distinguishes it from the stage shown in figure 3. The chorioid plexus invagination has become strongly developed, dividing the roof into two parts. These roof divisions have been termed superior and inferior, the former lying anteriorly and orally from the chorioid fold. The general surface outline is but little changed, due to the mesenchyme filling up the area between roof and skin. From two areas in the entire roof of the fourth ventricle the foreign fluid has escaped into the pericerebral tissue. These points of fluid passage lie in the two divisions of the ventricular roof. The superior area of escape corresponds to the oval outlined by the prussian-blue in figure 2 and to the point of emergence of fluid shown in figure 3. The lower area of fluid escape is in the inferior half of the ventricular roof, where the ependymal lining and its supporting tissue are developing into a well-marked dorsal distension. This area corresponds to Blake's³ caudal protrusion, though, as Heuser²³ has pointed out, the shape of the structure in the pig in no way resembles the "finger of a glove."

The extraventricular spread of the injection fluid in this specimen is considerably greater than in the pig embryo of 14 mm. (fig. 3). On the whole, however, the distribution of the replaced fluid is not extensive as compared with the adult relationship, where the central nervous axis is entirely surrounded by its subarachnoid cushion of cerebro-spinal fluid. From the superior area of fluid passage the replaced solution (as shown by the resultant precipitation of the prussian-blue) has passed both superiorly and inferiorly. In the median line, and extending laterally but slightly, a projection of the blue may be seen occupying a large portion of the extraventricular area formed from the chorioidal invagination. This area of fluid passage occupies at this stage about one-third of the total transverse diameter of the ventricular roof. From it the blue tapers caudally, diminishing in all directions. Above, the precipitate may be made out extending superiorly over the cerebellar lip. Its extension into the pericerebellar tissue is not marked; here again it tapers from the area of fluid passage, its midline prolongation stretching farthest anteriorly. This relationship is easily made out in figure 4, a frank lateral view of such an experimental replacement. The granules which result from the introduced ferrocyanide solution are found only in the central canal of the spinal cord and not in any perispinal arrangement.

In the pig embryo of 18 mm., shown in figure 4, the replaced solution has been carried somewhat farther than in the embryo of 14 mm. (fig. 3). The chief point of differentiation lies in the fact that in the latter stages two areas have apparently become permeable to the intraventricular fluid, so that a larger periaxial spread has resulted. Then, too, the extension of the ferrocyanide solution from the superior area is considerably greater, overlapping the cerebellar lip and filling in some degree the pericerebral tissue in the chorioidal invagination.

With a definite periaxial spread established for the cerebro-spinal fluid in pig embryos of 14 to 18 mm., it seemed not unreasonable to expect a gradual increase in the extent of the future subarachnoid distribution in more advanced stages. The earliest extension of the fluid into the peribulbar tissues occurred with the inception of the infolding of the ventricular roof to form the chorioid plexuses of the fourth ventricle. Its further extension, particularly its passages through a second area, occurred with the greater development of the chorioidal invagination (*i. e.*, 18 mm. stage). A still more extensive pericerebral flow of the ferrocyanide and citrate is illustrated in figure 5. Here the cerebro-spinal fluid in a living pig embryo of 19 mm. was replaced by the ferrocyanide solution. The embryo was kept alive for about an hour after the replacement and was then fixed *in toto* in an acid fixing medium, which caused the precipitation of the prussian-blue. On clearing subsequently by the Spalteholz method the spread of the solution was found to be somewhat more extensive than in the stage of 18 mm. (*cf.* figs. 4 and 5). In figure 5 the whole periaxial area over the roof of the fourth ventricle is shown to be completely filled by a dense aggregation of the prussian-blue granules. The separation of the two areas of fluid passage can not be made out in such a specimen. This dense periaxial extension almost completely covers the cerebellar lip, not only in the medial region but laterally to the limit of the ventricular roof. The injection precipitate lies directly beneath the skin in this area, but more posteriorly its separation from the skin becomes more marked. Tracing this dense periaxial injection posteriorly, it is seen (fig. 5) to end somewhat abruptly in the region of the cephalic flexure. The line of termination of the denser mass, to the ventral surface of the medulla, tapers somewhat anteriorly. This extraventricular spread is medial to the otic vesicle, but extends peripherally along the caudal cerebral nerves, reaching outward as far as the peripheral ganglia. The periaxial spread also closely covers the ventral surface of the medulla and extends in this plane around the pontine flexure for a short distance upwards along the basilar surface of the mid-brain.

Examined from its dorsal aspects, the superior portion of the spinal cord is found to be covered (in a perispinal relation) by a fine deposit of the prussian-blue. This is shown in figure 5. Caudally from the higher cervical region there is no evidence indicating a further spread in the perispinal tissues. Such a spread from above downward is wholly at variance with Reford's⁴⁷ conception of a development of the spinal meningeal spaces before the cerebral. The complete filling here of the central canal of the spinal cord and of the cerebral ventricles with the replaced fluid,

with no evidence of a periaxial spread except in the region of the fourth ventricle, indicates that in the pig embryo the adult human relationship between the cerebral ventricles and the subarachnoid spaces endures. There is apparently in this embryo no evidence of the foramina of Bichat and of Mierzejewsky, a finding in accord with the observations of Dandy and Blackfan⁽¹⁰⁾.

In the slightly larger embryos the further extension of the embryonic extraventricular spaces progresses rapidly. Figure 6 represents such an extension in a pig embryo of 21 mm., in which the normal cerebro-spinal fluid was replaced by a dilute solution of potassium ferrocyanide and iron-ammonium citrate. In this specimen the central canal of the spinal cord and the cerebral ventricles are completely filled with the precipitated prussian-blue. But in addition there is almost a total filling of the periaxial spaces. Viewed laterally the densest aggregation of the blue granules is again in the region of the roof of the fourth ventricle. As in the embryo of 19 mm. (fig. 5), the whole extraventricular tissue posterior to this ventricular roof is filled with the granules precipitated from the foreign solution. The spread from this region is similar to that in the previous specimen, except in its far greater extent. The granules may be traced caudalwards in the perispinal spaces to the point of injection. The arrangement of the precipitated material, both within the central canal of the spinal cord and surrounding it in the perispinal relationship, is well shown in figure 7, a frank dorsal view of the same specimen represented in figure 6. The greater density of the perispinal granules in the upper region of the cord, as contrasted with the granules in the thoracic region, is probably of importance in indicating the direction of the flow from above downwards. The increased amount of the injection fluid in the region about the point of insertion of the spinal needle is in all likelihood due to a local spread from the needle, such as frequently occurs in a very limited area. The phenomenon may, however, be due to an actual increase in the size of the potential perispinal space, though observations upon other embryos of the same stage of development argue against this view. The segmental outlining of the caudal portion of the perispinal space is to be noted in this figure.

The cephalic regions in the specimen of 21 mm. show a quite extensive spread (fig. 6), and there is the same general distribution of the granules about the medulla, as in the specimen shown in figure 5. The rhombencephalon is completely surrounded by the blue, the ventral sheet inclosing it tightly. Laterally the prussian-blue is shown in a dense mass, in intimate relation to the cranial nerves as they join the brain-stem. The cerebellum is practically completely covered by the precipitate; from the ventral portion of the pericerebellar granules the replaced solution (as evidenced by the granules of prussian-blue) spreads forward and surrounds a portion of the mid-brain. Only the ventral surface of the posterior half of the mid-brain is circumscribed by the granules; anteriorly it is wholly surrounded by the periaxial injection; more anteriorly the extension is limited to the mesial structures, leaving unrounded the cerebral hemispheres, although creeping between the hemispheres and the mid-brain.

The peculiar avoidance by the replacement fluid of the extreme dorsal half of the mid-brain is also to be made out in the dorsal view of the specimen (fig. 7).

The two lateral extensions from the ventral sheet of the injection granules approach on either side this mesencephalic eminence. The peculiar appearance of the injection spread caused by the chorioidal invagination of the roof of the fourth ventricle is also here illustrated.

In this specimen, then, of a pig embryo 21 mm. the periaxial spread is almost complete, the only areas not entirely surrounded being the anterior mesencephalon and the cerebral hemispheres. In an embryo but a few millimeters larger this periaxial extension of the solution is complete. The mesencephalon first becomes entirely covered by the prussian-blue precipitate, with later extension over the hemispheres. This complete periaxial injection occurs usually in replacements in embryos varying in length from 24 to 28 mm.

A specimen exhibiting a complete extension of the replaced solution around the central nervous system is shown in figure 8. This specimen was prepared by replacing the cerebro-spinal fluid in a living embryo of 26 mm. and then keeping the embryo alive for an hour. After fixation in an acid medium, dehydration, and clearing, the injection was found to occupy the whole medullary-canal system and also to surround completely the cerebro-spinal axis, as shown in the lateral view. The striking features of this stage are similar to those observed in the younger specimens -- the dense accumulation of granular material in the region of the roof of the fourth ventricle, the surrounding of the central portion of the caudal cranial nerves, and the thin pericerebral covering by the replacement mass. In addition the specimen exhibits in the thoracic region an extension of the granular material laterally along each spinal nerve. An observation of this peculiarity reveals the prussian-blue extending outwards only as far as the ganglia on the posterior roots.

The relationships, then, observed in an embryo pig of 26 mm. are those which exist in the adult; the cerebro-spinal axis contains cerebro-spinal fluid within its cerebral ventricles and within the central canal of the spinal cord, while in turn it is completely surrounded by cerebro-spinal fluid within the subarachnoid space. Communication between the ventricles or intra-medullary system and the peri-spinal spaces occurs only in the region of the fourth ventricle. Here again the adult human relationship holds. The evidence, therefore, from a study of the fluid spread in a replacement experiment with the use of true solutions, indicates that in pig embryos of about 26 mm. an adult distribution of cerebro-spinal fluid occurs.

THE RESULTS OF INJECTIONS OF TRUE SOLUTIONS.

In the preceding section there have been detailed the results of experiments on living pig embryos in which the cerebro-spinal fluid of both the central canal of the spinal cord and the cerebral ventricles has been replaced by a dilute solution of potassium ferrocyanide and iron-ammonium citrate. After the replacement, carried out so as to avoid any increase in the normal tension, the embryos were incubated for varying periods of time so that the normal current of the fluid might cause an extension of the foreign solution. In the experiments which will be recorded in this section the same true solution was injected from an ordinary syringe and the

salts immediately precipitated as prussian-blue. The purpose of these observations was solely to ascertain the effect of injections at pressures above the normal tension, so that the conclusions drawn from the replacement method might be more fully substantiated.

It was soon ascertained that the pressures caused by injections with a simple syringe could be fairly well controlled and that several degrees of tension might be employed. Thus it was found to be simple and serviceable to designate the injections as those made with mild, moderate, or strong syringe-pressure. Most of these injections were made into the central canal of the spinal cord, but occasionally into the perispinal spaces or cerebral ventricles. Injections under equivalent pressures in the central canal of the spinal cord or into the cerebral ventricles always gave corresponding results. It is necessary to record that the injections, even under strong pressure, were not carried to the point of macroscopic rupture.

The so-called mild syringe-pressure, making use of solutions of potassium ferrocyanide and iron-ammonium citrate, resulted in extensions of the prussian-blue wholly similar to those obtained in the replacement experiments which were carried on for 30 minutes and over. This similarity indicates a complete filling of the available cerebro-spinal system in the replacement method, for certainly (even in the mildest syringe injections) the intraventricular pressure must be excessively increased. Figure 1 shows a specimen under such conditions, with a marked thinning of the injection mass in the region of the fore-brain. This finding is customarily present in the injections under mild pressure, due to the pushing upwards of an existent ventricular fluid.

When moderate pressures are employed with the syringe the picture gradually changes. The essential difference in the results obtained by moderate syringe injection and by the replacement method lies in the greater extension of the foreign solution in the smaller embryos. Thus in figure 9 the spread of the injection precipitate in a pig embryo 16 mm. is shown to be about as extensive as that obtained by the replacement method in an embryo of 19 mm. (fig. 5). The extraventricular distribution of the injected solution around the medulla, the extension (even more marked here) along the central roots of the caudal cranial nerves, and the localized perispinal spread are easily made out in this specimen of 16 mm.

This general rule applies to all of the results obtained with the use of syringe-pressures above the mildest. Dependent upon the degree of syringe-tension, the spread extends in simple ratio. Thus, by the use of moderate pressures of injection into the central canal of the spinal cord, a complete intramedullary and periaxial spread was secured in a pig embryo of 22 mm. somewhat earlier than the equivalent stage was obtained by the use of the replacement method.

With the highest syringe-pressures (insufficient, however, to cause macroscopic rupture) the same general type of injection spread was obtained, bringing the more complete stages down into smaller and smaller embryos. Most of these embryos, however, on microscopic section showed obvious rupture of some part of the central nervous system.

The most important feature of these findings in the embryo pig injected with true solutions under moderate pressures from a syringe concerns the fact that the extension of the injection coincides, except as to the size of the embryo, in every instance with that obtained by the replacement method. Thus similar and analogous spaces are filled by injections under syringe-pressures in small embryos and by the solution under normal tension in larger embryos. It must be assumed, then, that the pressure of injection is sufficient to dilate potential cerebro-spinal spaces which normally would not be concerned in the pathway taken by the cerebro-spinal fluid. No evidence of new or abnormal pathways for the fluid is afforded by the observations made with the increased pressure; these phenomena indicate great potential strength in the tissues which limit the immature cerebro-spinal spaces.

Injections with a simple syringe may be made with such a degree of pressure that gross rupture of the tissues becomes apparent. In such an injection into the central canal of the spinal cord the infundibular region ordinarily ruptures in the smaller embryos (under 15 mm.), while in larger embryos rupture usually occurs into the subcutaneous tissues of the back of the neck over the fourth ventricle.

In discussing the effects of the introduction of solutions of ferrocyanide under pressures higher than normal into the central canal of the spinal cord, it may be appropriate to record observations made in the attempt to inject the cerebro-spinal spaces from the perispinal space. In embryos under 15 mm. in length it is quite difficult to make a perispinal injection. As the embryos exceed this measurement the injection becomes increasingly easy, but not until a length of 20 mm. is attained can it be made under the mild pressure advisable. These observations tend to substantiate the findings already recorded in both the intramedullary replacements and the injections under mild pressure.

RESULTS OF INJECTIONS OF NITRATE OF SILVER.

In a number of experiments a dilute solution (0.5 per cent) of nitrate of silver was injected into the central canal of the spinal cord and the salt then reduced in the sunlight. This solution, although a true one, is wholly unsuited for the replacement type of injection, on account of its great toxicity and its power to coagulate protein. It was employed here only for the simple type of injection.

The results obtained by this intraspinal injection of solutions of nitrate of silver were of but little value in the determination of a pathway for the cerebro-spinal fluid, but they vividly present certain aspects of the problem. Thus, in figure 11, a drawing of a specimen (pig) of 16 mm., the area through which fluid passes in the superior portion of the roof of the fourth ventricle is clearly outlined by a denser deposition of the silver. This specimen was prepared by introducing the solution of nitrate of silver into the central canal of the spinal cord under the so-called moderate syringe-pressure. The drawing shows a slight, cone-shaped extraventricular spread of the injection fluid. This spread takes place solely from the superior area of fluid passage, a result in accord with the finding that the solution of potassium ferrocyanide and iron-ammonium citrate passed first through the superior area. Of course it is realized that the precipitant action of the silver may

have exerted a more potent action on the structures constituting the lower area of fluid passage.

Another interesting phenomenon of the injections of silver nitrate is shown in figure 12. The embryo of 13 mm. here represented was injected under strong syringe-pressure with a solution of silver nitrate into the central canal of the spinal cord. On subsequent reduction and clearing it was found that the excessive pressure had resulted in a complete intramedullary injection with a localized pedunculate spread into the tissues from the roof of the fourth ventricle. This bulbous extravasation into the extraventricular tissue has not been observed in any specimens except those into which the solution of silver nitrate was injected. Such a spread is probably to be accounted for by an immediate coagulation of the surrounding tissue.

The extensive use of solutions of silver nitrate as a means of demonstrating vascular channels naturally suggests a careful comparison of the results obtained from its use and those obtained from the employment of other available true solutions, in regard to the evidence afforded by the two methods of intraspinal injections. The chief objection to the use of silver nitrate, as has already been mentioned, is its power to coagulate protein. This is illustrated by many features of the specimen shown in figure 11—by the sharp outlining of the area of fluid passage, the markings on the caudal process of the fourth ventricle, and the delimitation of the cerebellar lip. But much more marked are the evidences of this coagulative power as shown in figure 12, the pedunculated extraventricular spread, the transverse corrugation of the cerebellar lip (amounting to circumscribed indentations), and the peculiar outlining of the roof attachment to the bulb. These phenomena obtained by the intraspinal injection of solutions of silver nitrate must be classed as artifacts. The different degrees of this corrosive action of the silver probably result from the varying rates of reduction of the salt to the metal, a factor which is not easily regulated. The findings, therefore, with this method are worthless unless controlled.

Many embryos of varying sizes were injected with the silver nitrate. In the main these observations followed the course of development of the cerebro-spinal spaces as evidenced by the replacement experiments with the ferrocyanide. The injections required moderate pressure in the syringe in order to secure more than a local extension from the roof of the fourth ventricle, and to secure the same extent of spread it was generally necessary to use embryos a few millimeters larger than those required in the replacement experiments; but this is to be expected, in view of the probability of a constant precipitation of the albuminous tissues by the injection fluid.

Specimens prepared by the intraspinal injection of silver nitrate, then, afford but little reliable evidence in this problem except of a substantiative sort. The findings by this method indicate that the perispinal and pericerebral spaces, in pig embryos of 25 mm. and upward, could be filled by an injection of silver nitrate under moderate pressures into the central canal of the spinal cord. The point of passage of the fluid from the intramedullary to the periaxial system was in the region of the roof of the fourth ventricle.

THE INJECTION OF INDIA INK.

The objections to the use of any fluid of insoluble particles in suspension have already been discussed in considering the methods of injection which were possible for use in this study; but for comparison with results obtained by more promising methods and to ascertain to what extent injections with india ink are reliable they will be further considered here. No granular substance other than india ink (carbon granules) was employed in this investigation. In every way this suspension possesses advantages over other possible masses—in its ease of preparation, in the small size of the granules, and the insolubility in the reagents used for microscopic technique.

Suspensions of india ink (diluted from 4 to 10 times) were introduced first into the medullary-canal system of living pig embryos by the replacement method. In no case, however, even though the circulation of the embryo may have continued for 90 minutes, was there any evidence of an extension of the replaced mass outward into the periaxial spaces. The carbon granules remained wholly within the ventricles, a striking difference from the results obtained by the ferrocyanide replacements. It would appear, then, without the further evidence afforded by microscopic section of the specimens, that there is an existing mechanism which prevents the passage of the carbon granules from the fourth ventricle into the periaxial spaces. This finding was found to be constant in all the living embryos subjected to the cerebro-spinal replacement.

Quite similar to these results by the replacement method are those from the injection of a suspension of india ink under mild syringe-pressure. In no instance, provided the pressure was maintained at a low enough degree, was there any passage of the granular material into the periaxial tissue. In embryos of over 30 mm., however, even with the lowest pressure, it becomes increasingly difficult to prevent a sudden spread into the periaxial spaces. The type of spread indicates a sudden release of some restraining agent and suggests a rupture of a membrane. This spread is usually local and takes place from the roof of the fourth ventricle.

With moderate and strong syringe-pressures, however, it is possible to secure a periaxial spread, but this is quite different from the distribution of the injections by the use of ferrocyanide solutions. Figure 10 illustrates a specimen of a pig embryo of 21 mm. into whose central spinal canal india ink was injected under strong syringe-pressure. The resultant spread of the injection is easily discerned: the cerebral ventricles are quite filled with the carbon, while from the superior portion of the roof of the fourth ventricle a dense but localized periaxial spread is made out. This extraventricular extension of the ink is well defined: it stretches caudalwards for a slight distance, curving about the bulbous caudal portion of the ventricle and extending lateralwards but a short distance. The median portion of the cerebellar lip is covered by the granules. Evidences of the excessive pressure at which the injection was made are shown by the lines of invasion of the spinal cord and mid-brain. A comparison of the spread of this injection mass with the extension of a ferrocyanide replacement in an embryo of the same size (21 mm.) is afforded by figures 10 and 6. With such a divergence in the results obtained by the two

methods of approach it is not surprising that observations such as Reford's⁽⁴⁷⁾ fail to coincide with these findings. The unsuitability of suspensions of granular material in the investigation of the cerebro-spinal spaces has been many times verified in this work.

In the further study of the course of the spread with injections of india ink it was found that, in pig embryos of approximately 22 mm. and over, a partial periaxial injection could be secured by plunging the syringe-needle into the perispinal spaces. The carbon granules could subsequently be seen filling the perispinal spaces and also mounting upwards in partial pericerebral relationships, particularly around the medulla. This result was obtained by the use of strong syringe-pressures. Apparently the resistance to the spread of the ink in injections or replacements in the medullary-canal system occurs in the passage of the fluid from the roof of the fourth ventricle into the periaxial spaces. So far as is known, Reford⁽⁴⁷⁾ did not control his injection pressures. These results with the injection of india ink under strong pressures coincide with the idea of his observations afforded by the abstracts given by Sabin⁽⁴⁹⁾ and Cushing⁽⁹⁾. Suspensions of india ink, then, injected under mild syringe-pressure or by the replacement method, offer no evidence, in the pig embryo, of a passage of the cerebro-spinal fluid into the periaxial spaces. Only by employing pressures much above the normal tension can such evidence be obtained.

V. UNDESCRIBED STRUCTURES IN ROOF OF THE FOURTH VENTRICLE.

The results of the replacement of the existing cerebro-spinal fluid by a true solution of potassium ferrocyanide and iron-ammonium citrate in a living pig embryo indicated, as detailed in the foregoing section, that the fluid passed from the ventricular system into the periaxial tissues in the region of the roof of the fourth ventricle. This important transit of the fluid, agreeing with the established conception of the relationship in the adult, was first observed in an embryo pig of 14 mm. (fig. 3). At this stage the exudation of the replaced fluid occurred in one defined area, seemingly corresponding to the dense oval in a smaller embryo shown in figure 2.

Such a passage of fluid from ventricle to periaxial tissue is necessarily a physiological phenomenon, and it was in the hope of finding an anatomic basis for this phenomenon that the roof of the fourth ventricle was studied histologically. It was realized that failure to demonstrate anatomically differentiated structures would not vitiate the physiological observations, but that a correspondence between function and structure was most desirable. Hence observations were undertaken to determine, if possible, an area of histological differentiation in the roof of the fourth ventricle which might be concerned in the primary passage of fluid from the cerebral ventricles into the periaxial tissues. The investigation concerned first the examination of this region in pig embryos of 14 to 15 mm., at which stages the fluid passes from a single area. Subsequently, similar studies were undertaken in regard to the second, more inferior area (shown in figure 4). The results of these studies will be given here.

AN UNDESCRIBED AREA IN THE SUPERIOR PORTION OF THE ROOF OF THE FOURTH VENTRICLE.

THE AREA MEMBRANACEA SUPERIOR IN THE PIG EMBRYO.

Examination of the roof of the fourth ventricle in a pig embryo of 14 mm. revealed a peculiarly differentiated area in the superior portion. The general topography of this area is shown in the rectangular area marked off in figure 32—a median sagittal section from a pig embryo of this critical stage. In figure 33 this rectangular area is enlarged to show the morphology in greater detail.

In this figure the densely staining ependyma lining the fourth ventricle approaches from both sides. The superior portion of the ependyma ends abruptly, while the inferior line of the layer tapers more slowly. Between these two points is an area having none of the characteristics of the ventricular lining at all other points. The comparatively smooth contour of the ependymal cells is replaced by an irregular cell-border. The pyknotic nuclei of the cells have been replaced by less densely staining, elongated, spindle-like nuclear bodies. The cell-layer lining the ventricle is here really only of a single cell in thickness, although blood-capillaries closely applied to it suggest a greater thickness. The mesenchyme between this layer and the peripheral epidermis is quite thin, but resembles in every way the mesenchyme in the immediate neighborhood.

There is, therefore, as pictured in figures 32 and 33, an area in the roof of the fourth ventricle which is morphologically dissimilar to the characteristic ependyma lining the cavity. Is this the result of some distortion in fixation or in the routine histological technique? Is it a constant finding and, if so, what is its history? Does it arise at a definite period and persist throughout intra-uterine life only or through adult life also?

The question of the actual existence of this area, or of its being caused by technical manipulations, is one which must be answered. That this differentiated portion of the roof of the fourth ventricle is not an artifact is verified by the general history of its formation, by its invariable occurrence (not only in the pig but in other animals), and by its general histological appearance. Moreover, the physiological importance of this area undoubtedly inclines one completely from the possible explanation that it is due to an artifact. No single finding wholly excludes such a possibility; rather is one convinced, by many features, of its actual occurrence.

Considering the fact, then, that this differentiated structure in the roof of the fourth ventricle may be found in all embryo pigs at the stage of 14 mm., it becomes necessary to ascertain at what time in the development of the embryo it first appears and how it is formed. Obviously the most satisfactory method is to trace the area through the lower stages and also through the older embryos. For the sake of greater clearness, however, a description of the area will be given from its first differentiation through its maximum transformation to its final disappearance—for the structure is only temporary.

In pig embryos of 8 mm. and less in crown-rump measurement, the roof of the fourth ventricle is formed of cells morphologically and tinctorially different from

those lining other parts of the ventricular cavities. These cells are quite unlike the deeply staining ependymal cells, which can be so readily identified as the lining cells in older embryos. In this younger stage of 8 mm., the entire ventricular roof is composed of several layers of cells with round or somewhat oval nuclei and fairly abundant cytoplasm. The cell-boundaries are not well defined. The nuclei are not deeply tinged with hematoxylin. The chromatin material is sparse and irregularly distributed. Nucleoli are prominent. The cytoplasmic border lining the ventricular cavity is rough and ragged at times, often blending with the coagulated albumen of the cerebro-spinal fluid. Altogether, these lining cells bear a much greater resemblance to the epithelial cells than to the ependymal.

These characteristics of the lining cells of the roof of the fourth ventricle are shown in figures 24 and 25, from a pig embryo of 8 mm. The close association of the roof cells to the surface epithelium is easily made out in figure 25, as well as the general character of the lining cells.

At the stages of 8 mm. and under, in the pig embryo, the roof of the fourth ventricle is relatively quite large. In its whole extent it is formed of the peculiar lining cells described above. With the growth of the embryonic nervous system, the roof of the fourth ventricle is subjected to alterations in form and position; to some extent these changes influence the cells which line the cavity in the early stages.

In pig embryos between 8 and 12 mm. in length the roof of the fourth ventricle undergoes a change. The ependyma, which from comparison with later stages is regarded as typical, begins to encroach upon the epithelial-like cells which are so numerous in the 8 mm. stage (fig. 25). The area occupied by these cells diminishes, not only relatively but absolutely. It becomes smaller and the cells gradually change their character. These changes are shown in figures 26 and 27, from a pig embryo of 11 mm. Figure 26 gives the location, in a sagittal section near the mid-line of the area in figure 27, taken at a higher magnification.

In figure 27 the densely staining lips of ependymal and nerve cells are seen approaching each other. For a considerable space in the central portion of the photograph there is an area similar to that shown in figure 33. But considered in connection with figure 25 this area represents the epithelial-like cells of the roof of the fourth ventricle. This relationship is more clearly shown in figures 28 and 29, taken in a more lateral plane from the same embryo (11 mm.). Examination, however, of the area in figure 29 shows the epithelial-like cells again apparent in the roof of the fourth ventricle.

The process of transformation, then, as shown in these photographs from an embryo pig of 11 mm., concerns a gradual encroachment upon the area of epithelial-like cells by the more densely staining and more closely packed ependymal cells. Gradually the epithelial-like cells in the central portion of the area lose their former character (fig. 27), while around the periphery, especially on the lateral sides, the epithelial-like appearance persists (fig. 29).

On the lateral side of this area, just as the typical ependymal lining is about to become isolated (fig. 29), the epithelial-like lining cells form a several-celled layer.

The nuclei are poor in chromatin material and the cytoplasm somewhat small in amount. The inner cytoplasmic border lining the ventricle is in contrast, by its ragged outline, with adjacent smoother ependyma on both sides. At this stage of the pig embryo the characteristics of the epithelial-like cells are still to be made out, but a gradual transformation is becoming evident.

The metamorphosis becomes much more marked in the central portion of the area, as shown in figures 26 and 27. In these figures the whole central area seems to have lost some of its former character as an intact cell-layer. Closer examination, however, under higher power demonstrates that it still possesses an intact surface as a lining for the ventricle. Delicate cytoplasmic strands stretch in a continuous line across the whole area between the lips of denser typical ependyma. The nuclei in this differentiated area are seemingly altered from their rounded form and have elongated almost into spindles. The inner cytoplasmic border is characteristically rough, with small amounts of coagulated albumen adhering to the processes. The area, then, in its central portion, at the stage of 14 mm., has assumed the character of the stage of 14 mm. (fig. 32). On the periphery, however, the cells still resemble those of smaller stages (8 mm.).

From the pictures presented by the intermediate stages (figs. 27, 28, and 29) the differentiation goes on very rapidly, so that in the pig embryo of 13 mm. there is rarely any evidence of the epithelial-like cells. Figures 30 and 31 are photomicrographs of a sagittal section of an embryo pig of 13 mm.; here there are no evidences of the epithelial-like cells. The whole area, pictured in figure 31 as sharply delimited from the tongues of typical ependyma above and below, has become well differentiated. The cell-character observed in figures 27 and 33 (elongated nuclei and scanty strands of protoplasm) has become very obvious. The ragged and roughened intraventricular border, the coagulated albumen, and the abrupt transition from the neighboring typical ependyma are well shown in the photomicrographs of this specimen.

The differentiation of this area in the roof of the fourth ventricle of the pig embryo proceeds at a very rapid rate, so that within the growth of a few millimeters (from 8 to 13 or 14) a great histological change occurs. Figures 32 and 33, already described, show the extent of this metamorphosis in a pig embryo of 14 mm. The process, however, continues, modified possibly by the changing of the roof of the fourth ventricle. For this roof structure is subjected to marked alteration in stages of 14 mm. and upwards, both by the lateral development of the chorioid plexuses and by the readjustment of the cervical and pontine flexures. Its maximal differentiation may be said to appear at a stage of 18 mm.; this is maintained through several millimeters, until undergoing final retrogression.

This maximal change in the roof of the fourth ventricle is shown in figures 34, 35, 36, and 37. Several points of interest are brought out in these photomicrographs. Figure 35 represents an enlargement of the rectangular area in figure 34, taken from transverse sections of an embryo pig of 18 mm. The area is particularly well shown in this figure, in which, from the right, the typical ependyma, in a fairly

smooth single-cell layer, approaches the differentiated cells in the central portion. On the left, too, similar typical ependyma is shown. In the central area, which has been repeatedly described, the elongated nuclei, the strands of protoplasm, and the ragged, irregular intraventricular surface are well presented. The photomicrograph has been reproduced to show the relation of this differentiated area to the various blood-channels in the supporting mesenchyme. Apparently the whole ventricular roof is, at this stage, a site for an extensive capillary plexus; from both sides, as shown in figure 35, vessels (one of great caliber) approach the central area of differentiation. Directly beneath this area smaller capillary channels can be made out, from which, apparently, a slight extravasation of red blood-cells has occurred. Here, as in the greater part of the basilar pericerebral region, extravasation of the blood-cells is very frequent. This phenomenon has already been pointed out by Mall⁽³⁶⁾.

The large extent and the great differentiation of this peculiar area in the roof of the fourth ventricle are well shown in figures 36 and 37, taken from a transverse section of a pig embryo of 18 mm. In the photomicrograph of higher magnification the two sharp tongues of typical ependyma are quite striking. Their abrupt termination in the wide, differentiated area has nowhere been more convincingly shown. The resemblance of these lining cells in the central area to the mesenchymal elements adjoining is here also seen. The most interesting of all the phenomena exhibited in this reproduction, however, is the attachment, apparently by precipitation, of the coagulated albumen of the cerebro-spinal fluid. This coagulation, in this specimen, delimits the differentiated area in the roof of the fourth ventricle. The phenomenon is seemingly only an amplification of a similar attachment of small fragments of the albuminous precipitate shown in other figures.

Beyond the stage of 18 mm., which may be termed the maximal stage, the differentiated area in the roof of the fourth ventricle undergoes a regression. This is apparently due to the morphological alterations in this rhombic roof. The chorioid plexuses in embryos over 18 mm. long deeply invaginate the fourth ventricle, possibly drawing some of the true roof with them, but surely encroaching upon the mid-line with their lateral tuftings. This growth tends to decrease the available extent of the differentiated area, but an even more potent factor is the rapid development of the cerebellum. The caudal growth of the cerebellar lip soon largely occupies or replaces the superior half of the roof. These two factors, the cerebellar growth and the enlargement of the chorioid plexuses, render the persistence of the differentiated area impossible, so that a regression or disappearance is to be expected.

With these considerations before us, the study of sectioned pig embryos of a greater length than 18 mm. becomes important. The process of disappearance, however, does not occur at once. Thus, in an embryo pig of 19 mm. (figs. 42 and 43) the differentiated area is as large and as characteristic as in the stage of 18 mm. This same appearance and maintenance of size may be observed through the next several millimeters' growth, but in pig embryos of 23 mm. the chorioid plexus has

usually developed to such an extent that a continuation of the former size becomes impossible. This is shown in figures 44 and 45. Figure 45, the enlarged squared area from figure 44, is a photomicrograph from a pig embryo of 23 mm. The differentiated area, due to the factors favoring its regression, now appears in close proximity to the chorioid plexus. It has more the appearance of a degenerating area at this stage than in any of the younger embryos, but it still shows a characteristic delimitation of both edges—on the one from the typical ventricular ependyma, and on the other from the differentiated ependyma of the chorioid plexus. The cytoplasmic strands of the area which forms the ventricular border do not show to advantage in the photomicrograph, but the same ragged character with the covering of coagulum may be made out. The process of regression, mechanical as it perhaps is, has begun at this stage in the pig, and in the course of the next few millimeters' growth will become even more active.

With the encroachment of the chorioid plexuses and the downward growth of the cerebellar lip, the superior portion of the ventricular roof soon disappears, and is practically non-existent in embryos of 30 mm. and more in length. The differentiated area thus encroached upon from the sides and above becomes a mere vestige of its former size. Thus in a pig embryo of 32 mm. (figs. 46 and 47) it appears as a very small break in the lining continuity of the ventricular ependyma. Without the intermediate stages such a picture would undoubtedly be considered as an artificial erosion of the ependymal lining of the ventricle, but when studied in connection with figure 45 the true vestigial character of the area becomes established.

The final fate of this differentiated area in the roof of the fourth ventricle is a complete disappearance, with the occupation of the region by chorioidal epithelium and cerebellum. In this study it was impossible to find traces of the differentiated areas in pig embryos of over 33 mm. in length: vestiges may persist, but so small as to present difficulties of decision. The persistence of such a differentiated vestige in rare instances would not be surprising; the transitory character of the area and the method of disappearance make this seem not unlikely.

This transitory area of differentiation in the roof of the fourth ventricle of the pig has not, so far as can be determined, been noted or described by any previous author. His²⁵, in a retouched photomicrograph of a sagittal section of a human embryo of 17 mm., reproduced the area as differentiated from the roof, but he has made no comment upon it. I have called this differentiated area in the superior portion of the rhombic roof ventricle the "area membranacea superior ventriculi quarti." This terminology is based on the anatomical character of the area as a continuous membrane, but chiefly on its physiological significance. For, as will be shown in the succeeding section of this paper, the transit of embryonic cerebrospinal fluid from ventricle to periaxial tissue occurs in this area, which functions apparently as a physiological membrane. With such a physiological conception of the area, the term "area membranacea" seems most suitable, inasmuch as it also meets the anatomical requirements.

THE AREA MEMBRANACEA SUPERIOR IN THE HUMAN EMBRYO.

The finding of the differentiated area in the superior portion of the roof of the fourth ventricle in the embryo pig suggested the value of a study of the same region in the human embryo in the further solution of the problems underlying its occurrence. Hence this region in the roof of the fourth ventricle has been examined in the sectioned human embryos of the Department of Embryology of the Carnegie Institution of Washington. It was found that a similar area occurred in the human embryo of approximately the same age.

The study of the roof of the fourth ventricle is usually more difficult in the human embryo than in the pig. This is due to the fact that the roof of the fourth ventricle quickly suffers from poor fixation and dehydration—collapse or inversion of the whole structure being commonly met with. It is rarely possible, in the younger embryos, to secure the most satisfactory fixation, whereas in the pig these factors may be controlled as desired. Furthermore, the undue pressures to which the human ovum is frequently subjected in abortion may cause crushing of the more delicate parts of the nervous system.

It is probably best, in the human embryo as in the pig, to trace the formation of the area membranacea superior ventriculi quarti from its beginning, through the various differentiations.

In a human embryo of 4 mm. (No. 836 of the Collection of the Carnegie Institution of Washington) the entire roof of the fourth ventricle is composed of cells with round or slightly oval nuclei and palely staining cytoplasm. The nuclei of the cells are poor in chromatin material as contrasted with the pyknotic character of the typical ependymal cells. The lining tissue is of the thickness of several cells. The ventricular cytoplasmic border is fairly smooth at this stage. This characteristic ventricular lining is shown in figures 40 and 41, both taken from embryo No. 836. The whole picture is similar to that exhibited by the pig embryo of 8 mm. (figs. 24 and 25).

A similar accumulation of epithelial-like cells is found in a human embryo of 7 mm. (No. 617 of the Carnegie collection). This is pictured in figures 48 and 49. The photomicrograph of higher magnification shows these poorly staining cells heaped up in a rather localized part of the ventricle, fairly sharply delimited from the adjoining ventricular lining. This accumulation of cells in the roof of the ventricle invariably occurs, and it must not be considered as being due to the distortion of the ventricular roof. The reason for the asymmetry of the rhombic roof shown in these figures lies in the fact that in this embryo, as in practically all the embryos of similar stages in this collection, some degree of distortion of the roof of the fourth ventricle is present. Photomicrographs (figs. 50 and 51) taken more posteriorly (from embryo No. 617) give strong evidence of this distortion. They are reproduced not only to show the possible distortion, but also to give a further picture of the lining of the ventricle, with its epithelial-like cells in several layers (fig. 51).

Similar accumulations of these epithelial-like cells are to be found in human embryos of 9 mm. Reproductions of a much fragmented specimen of this size

(No. 721) are given in figures 52 and 53. In the latter figure the complete occupation of the ventricular roof by these cells is well illustrated. Moreover, the specimen shows the many-layered stage to a degree but seldom found. It is unfortunate that such a degree of fragmentation and distortion is found throughout this specimen.

Thus far, in human embryos up to and including 9 mm. in length, the roof of the fourth ventricle has shown the same architecture as appears in the pig. As will be recalled, the first evidence of a further differentiation of these cells in the pig embryo was found at a stage of 11 mm. (figs. 26 and 27). In one human embryo of this stage (No. 544) a distinct break in the roof of the fourth ventricle can be made out. This is shown in two photomicrographs (figs. 54 and 55). The picture in this case is somewhat obscured by the shrinkage and distortion of the ventricular roof, but a distinct differentiation of the lining epithelium can be made out. On the caudal side of figure 55 considerable nervous tissue is seen. Just superior to this (toward the left) the lining tissue is almost lacking, a few nuclei, only, preserving the contour of the ventricle. Above this area appears again the ventricular lining of many layers of cells. It has been quite difficult to interpret these findings. The area under discussion shows a rather typical adherence to the coagulated albumen; there is evidence of its extension also into the adjacent mesenchyme, a finding observed in no other similar stage. The caudal position of the opening, the character of the tissue approximating the ventricular cavity, and the presence of the albumen in large amount in the adjacent mesenchyme—all indicate that in great measure the pictures presented in this specimen are largely artifacts. It seems most likely, though, that some differentiation of the tissue in this area has occurred.

In a human embryo of 14 mm.,* as in the pig of the same stage, the area membranacea superior has attained a great degree of differentiation. This is particularly well shown in figures 56 and 57, the latter being an enlargement of the squared area in the former. These photomicrographs are from embryo No. 144 of the collection of the Carnegie Institution of Washington. Figure 57 shows a characteristic which distinguishes the area membranacea from that of the pig, although in the later stages of the pig embryo (figs. 45 and 47) this feature is present. This concerns the marked decrease of cellular tissue in the membranous area. In figure 57 the deeply staining typical ependyma is shown approaching from below. These cells end abruptly at the border of the area membranacea; the ventricle in this area is lined by cells possessing small elongated nuclei and long cytoplasmic processes, which unite to form a ventricular lining. The oval nuclei along the ventricular border become more closely massed together in the superior portion of the area, but nowhere is there the same architecture as in the equivalent stage in the pig (fig. 33). A feature of the histological appearance of the membranous area in the pig embryo is also shown in figure 57; this is the marked adherence of the coagulated albumen of the cerebro-spinal fluid to the area membranacea superior.

The roof of the fourth ventricle in the human embryo is subjected to the same factors causing changes in the form and relationships which were commented upon

*Measured on the slide after mounting.

in the pig; but these play little part until the chorioid plexuses become of sufficient size to divide the ventricle into a superior and inferior portion. In the human embryo, as in the pig, the superior half of the ventricular roof is sacrificed to the greater growth of the cerebellum.

In human embryos of 17 mm., however, these factors have not begun to influence the membranous area. This is shown in figures 58 and 59, photomicrographs from embryo No. 576. The section is somewhat to the side of the midline, but in the superior portion of the roof of the fourth ventricle the differentiated membranous area can be made out. The sharp delimitation of this area from the denser typical ependyma on both sides is quite apparent. The ragged character of the ventricular border, with its few elongated spindles, seems wholly in keeping with the transverse view of this area afforded by figure 37.

Embryo No. 576 exhibits one characteristic of the area membranacea superior very frequently seen in human embryos, but almost invariably absent in these stages in the pig. Along the lateral margins of the superior membranous area are dense borders of the many-layered epithelial-like cells which lined the ventricular roof in younger stages. This feature is well shown in figures 60 and 61, the latter figure being a higher magnification of the former. The cellular border of the superior area reaches transversely only through a few 15-micron sections, but it extends throughout the whole cephalo-caudal diameter of the area. It seems likely that this represents purely a survival of the epithelial-like cells in the younger embryos. In rarer instances the whole area membranacea superior may be surrounded by such a border of many-layered cells, but even in these cases the superior and inferior margins are quite thin.

No apparent agencies favoring the disappearance of the superior membranous area in the roof of the fourth ventricle of the human embryo are apparent in stages up to the fetus. Thus, in human embryos of 18 mm. this differentiated area in the roof has reached its maximal differentiation. A section from an embryo of this size (embryo No. 409) is reproduced to show the distortion and its influence upon the topography of the area membranacea. The two photomicrographs (figs. 62 and 63) show the extreme collapse and distortion of the roof of the fourth ventricle. In the figure of higher power (No. 63) the membranous area appears facing posteriorly, due to the shrinkage; the proper leader runs to this area. It shows the differentiation from the adjoining typical ependyma which is characteristic of the fully developed area membranacea superior.

In a beautifully preserved and sectioned human embryo of 21 mm. (No. 460) in the collection of the Carnegie Institution of Washington the area membranacea superior appears as a sharply delimited area (figs. 64 and 65). These figures give a very good idea of the definiteness of the area when the fixation and dehydration approach the perfect. The tissue of this membranous area lining the ventricle here appears to be wholly lacking in an epithelial covering; the mesenchyme seems to serve as the ependymal lining. Study of this area, however, through different stages argues most strongly against such a view.

The process of regression of the area membranacea superior in the human embryo differs somewhat from that described in the pig. This alteration in the mode of disappearance is largely due to the fact that in the period of growth from 20 to 35 mm. the superior portion of the roof of the fourth ventricle in the human embryo is not sacrificed to the cerebellar lips; for in the human the cerebellum grows largely into the fourth ventricle, enlarging beneath the superior part of its roof. Thus, the attachment of this part of the roof is not greatly interfered with by the rapid development of the cerebellum. The total extent, then, of the superior portion of the roof is hardly altered in these stages in the human, while in the pig embryo the roof is shortened by its attachment to the inferior portion of the cerebellar lip, which retains its earlier characters. These differences in the relationship of the superior portion of the ventricular roof in human and pig embryos may be seen by comparison of figures 74 and 89.

Another factor which renders the mode of disappearance different in the two embryos concerns the greater tufting and development of the chorioid plexuses of the fourth ventricle in the pig. This greater size and complexity of the plexus causes an encroachment upon the roof structures which, in the pig embryo, seems of considerable importance in the final closure.

In the human embryo, however, it has been found very difficult to explain the final disappearance of the superior membranous area on the same mechanical factors which seemed so well to account for its transitory characters in the pig; but at approximately the same stage of growth the process of regression occurs in the human fetus. The area maintains a fair size in stages up to a length of 23 mm. Thus, in figures 89 and 90 (No. 453 of the Carnegie collection) a sagittal section from a human fetus of this size is illustrated. In the higher power (fig. 90) the superior membranous area is shown, rather sharply delimited on its superior border by the typical, dense ventricular ependyma. Below, its edge is irregularly formed by the deeply staining ependyma over the invagination of the chorioid plexus. The cell-character of this area resembles that shown in the photomicrographs from the specimen of 21 mm. (figs. 64 and 65). There is left in the area no indication of the cellular architecture which characterized the original ventricular ependyma; the cells with their elongated cytoplasmic processes here have the oval nuclei which are found almost invariably in this membranous area.

In the human fetus of 26 mm. (No. 1008 of the collection of the Carnegie Institution of Washington) there is but slight evidence of a superior membranous area in the upper portion of the roof of the fourth ventricle. The evidence present in this specimen consists in a localized thickening of the lining cells of the ventricle in the situation of the area in other stages. This thickening is illustrated in figures 91 and 92; it consists of several layers of epithelial-like cells, similar in all respects to the many-layered border shown in figure 83. The picture is somewhat obscured by the vascular plexus directly beneath the ventricular lining.

There is difficulty in determining exactly when the last evidences of the superior membranous area in the roof of the fourth ventricle may be found. This is

due to the likelihood of artifacts disturbing the character of the ventricular lining in human material, where the freshness and fixation of the specimen may not be ideal. In the larger specimens in the collection of the Carnegie Institution, which are well fixed and sectioned, the existence of the area membranacea superior could not be wholly verified. Thus, in specimen 405 (26 mm.) the presence of the area seemed probable though not definite. In another embryo of this same size (No. 782) the existence of this area was still more questionable. In a larger embryo (30 mm., No. 75) the presence or absence of the area could not be assured; many indications suggested its existence, but the resemblance to an artificially separated ependyma was strong. In all specimens of human embryos of over 30 mm. examined, no evidence of the area membranacea superior could be found. It appears likely, then, that the final disappearance of this differentiated area in the roof of the fourth ventricle occurs at a slightly earlier stage in the human embryo than in the pig.

The final disappearance of the area membranacea superior in the human embryo is not accompanied by the same ingrowth of typical ependyma that characterizes the process in the pig. There is a great tendency, in the human, as indicated in figure 92, for a replacement of the area by the same type of epithelial-like cell which comprised the whole ventricular roof in the earlier stages (fig. 41) and later formed lateral borders for the superior membranous area (fig. 83). Thus, in a human embryo of 24 mm. (No. 632 of the Carnegie collection) there is evidence of a very small membranous area surrounded by a border of epithelial-like cells. In a slightly larger specimen (No. 840, 24.8 mm.) the whole membranous area is occupied by the epithelial-like cells. The frequent association of these cells with the area indicates that in disappearing the area membranacea is probably replaced first by these cells, which in turn disappear, so that the whole roof is finally composed of the typical, densely staining ependyma.

THE AREA MEMBRANACEA SUPERIOR IN OTHER ANIMALS.

In order to ascertain whether the area membranacea superior existed in other animals examinations of serial sections of the rabbit, cat, sheep, and chick of suitable stages were made. All of these animals were found to possess a differentiated area in the roof of the fourth ventricle.

Opportunity was afforded for the study of serial sections of the head of a chick* of 121 hours' incubation. The head was carefully dehydrated and embedded by Dr. E. R. Clark, and was subsequently sectioned by Dr. C. R. Essick. The material was beautifully fixed and dehydrated, showing practically no evidence of shrinkage. Typical portions of the superior membranous area are reproduced in figures 66, 67, 68, and 69. Figure 67, taken near the crown of the embryo and representing the squared area in figure 66, shows the two dense masses of ependyma separated by the more lightly staining area membranacea. The cellular character of this differentiated zone resembles more the histological features of the similar area in the pig than those of the human embryo. This resemblance is also to be seen in figure 69, taken

*This chick measured 14 mm. in 40 per cent alcohol.

more posteriorly than the two preceding figures. The dense ependyma approaching on both sides is sharply delimited at the edge of the broad membranous area. This is composed of cells having elongated, chromatin-poor nuclei, and long cytoplasmic processes, which form the ventricular roof. The adherence of the albuminous coagulum occurs here also.

In the rabbit the occurrence of the superior membranous area was verified as in the other species studied. In a rabbit embryo of 13 mm. (series x in the embryological collection of this laboratory) the area was well differentiated from the surrounding typical ependyma. The cells of the area resembled those of the adjacent mesenchyme. The ventricular surface was roughened by the projection of numerous protoplasmic processes. An albuminous coagulum was attached to the cells of the membranous zone.

One sheep embryo from the collection of this laboratory was also studied. The sections, although labeled as an embryo of 10 mm., resembled in every way a pig embryo of 18 mm. The area membranacea was easily identified in the roof of the fourth ventricle; it is similar in every respect to the same area in the pig and the human embryo.

In a cat embryo of 10 mm. a small but highly differentiated area membranacea superior was made out. The most striking feature in this specimen is the great adherence of the coagulated albumen to the cells of the area and the resemblance of these cells to the mesenchymal elements adjacent. The edges of this differentiated area are sharp and clear-cut.

No attempt was made to identify the area membranacea superior in other animals—as further suitable material was not immediately available. The chief study has been made on pig embryos and on human embryos. The occurrence of the area in the cat, sheep, and rabbit probably indicates its existence in all mammals. The finding of such an area in the chick is also suggestive.

GENERAL CONSIDERATION OF THE AREA MEMBRANACEA SUPERIOR.

The occurrence of a definite area of differentiation in the superior portion of the roof of the fourth ventricle has been pointed out in preceding subdivisions of this paper. It has been described in detail in the pig embryo and in the human embryo; it has been identified also in cat, sheep, rabbit, and chick embryos. It remains here to discuss the general characteristics of this area.

No description of such an area of differentiation in the ventricular roof has been found in the literature. It may be that the distortion of this structure in the course of the usual embryological technique has rendered its discovery less likely. His²⁵, in his description of the ventricular roof, has not commented upon the occurrence of this membranous area, even though in a retouched photomicrograph of his fetus C-1 (a human specimen, of the beginning of the third month) the area membranacea superior can be made out. Likewise in his description of the plica chorioidea he fails to mention any differentiated areas in the roof, although plate I, in his "Die Entwicklung des menschlichen Rautenhirns, von Ende des ersten

bis zum Beginn des dritten Monats," shows a slight irregularity in the roof. Practically all of the contributions to the anatomy of the roof of the fourth ventricle deal with the lower half of the structure, with particular reference to the occurrence of the foramen of Magendie.

The general biological process involved in the formation of the area membranacea superior concerns a differentiation of the epidermal elements which line the ventricular cavity. This differentiation, both in human and in pig embryos, first begins with the occurrence in the ventricular roof of an area of epithelial-like cells. These, in the course of enlargement of the roof, become more or less isolated in the superior portion of the structure, and then undergo a metamorphosis into the typical cells of the membranous area. They are characterized by oval or elongated nuclei (rather poor in chromatin as compared with the nuclei of the typical ependymal elements) and by cytoplasmic strands (in which the cell-boundaries are very poorly marked) which compose the ventricular border. The ventricular surface in the area membranacea is more ragged and irregular than where lined by typical ependyma. In many instances, as in figure 57, from a human embryo of 14 mm., this transformation has proceeded to such an extent that the epithelial character of the lining cells is almost wholly lost, and the ventricle seems, in this area, to be lined by mesenchyme. Study of the membranous area in many stages convinces one that such an hypothesis is untenable; in every case the ventricle must be considered as being lined by epidermal elements, no matter to what extent the process of differentiation has proceeded. There is no real evidence to support the view that the ependymal lining of the ventricle has been replaced by mesenchymal elements to form the area membranacea superior.

In general the area membranacea superior is a rounded oval. Its measurement is quite difficult except when fixation and dehydration have been excellent, because of the highly abnormal distortion of the ventricular roof which frequently occurs in the technically poor specimens. Measurements have been made in a considerable number of favorable specimens, both of human and pig embryos. With the history of this area in mind, it will be realized that the size of the structure necessarily varies with the length of the embryo, attaining its greatest dimensions at about the length of 18 or 20 mm. Herewith is a short table of the measurements taken.

Dimensions of area membranacea superior.

Species.	No. of specimen.	Length of embryo.	Width of area.	Length of area.	Species.	No. of specimen.	Length of embryo.	Width of area.	Length of area.
		<i>mm.</i>	<i>mm.</i>	<i>mm.</i>			<i>mm.</i>	<i>mm.</i>	<i>mm.</i>
Pig.....	98	12	0.37	0.5	Pig.....	121	16	0.6	0.48
Rabbit.....	107	13	0.95	0.4	Human.....	576	17	1.5	0.9
Human.....	144	14*	1.25	1.1	Sheep.....	108	18 (?)	0.8	0.8
Pig.....	119	14	0.45	0.6	Pig.....	45	18	0.9	0.4
Chick.....	106	14	0.65	0.85	Pig.....	84	22	0.8	0.7

In a rough way, then, we may consider the area membranacea as an oval; in some cases the longitudinal diameter exceeds the lateral, and in others the reverse

*Measured on slide after mounting.

holds. The measurements given above were taken from mounted sections and are probably somewhat disturbed by the histological technique which was followed.

The borders of this oval area membranacea are usually fairly regular and smooth, but in some instances they are irregular, due to the fact that small extensions of the area run into the bordering ependyma. These extensions are more commonly met with at the stage when the area has reached its maximum size, as in figures 38 and 39, photomicrographs from an embryo pig of 19 mm. The higher power of these two photographs shows two areas in the smoother ependymal wall. These are extensions of the area membranacea, and within a section or two directly connect with the differentiated area. Both of these small spots on the circumference resemble technical errors; their ragged appearance, the relative excavation of their surface, and the intact ependymal borders would seem to encourage such a view; but when considered in connection with the character of the whole area membranacea they assume a definite relationship in this regard. Other similar areas, rather rare in occurrence, are found separated entirely from the main area membranacea. These isolated areas are of the same size as those shown in figure 39. In significance and character they are probably identical with the larger area membranacea superior.

Most of the general features of the area membranacea superior have been commented upon in descriptions of the various stages of differentiation in both pig and human embryos. The characteristics most commonly observed concern the differentiated character of the cells of the area, the sharp borders of the typical ependyma, the ragged ventricular surface throughout the whole extent, and the peculiar adhesion of the albuminous coagulum from the embryonic cerebro-spinal fluid to the lining cells. The area membranacea superior should be considered, then, as a transitory focus of differentiation of the typical ependymal lining of the roof of the fourth ventricle.

AN UNDESCRIBED AREA IN THE INFERIOR PORTION OF THE ROOF OF THE FOURTH VENTRICLE.

With success attending the effort to find in the superior portion of the rhombic roof an anatomically differentiated area which would furnish a morphological basis for the physiological phenomenon of the extraventricular passage of the cerebro-spinal fluid, attention was necessarily directed to the inferior portion of this roof (considering the whole roof structure to be divided by the chorioid plexuses). The spread of the replaced injection fluid (fig. 4) into the periaxial tissues through two points in the roof of the ventricle suggested a study of this stage (pig embryo of 18 mm.) as the basis of the investigation. As a histologically differentiated area in this inferior portion of the roof is easily made out, the complete history of the area will be given chronologically. It has been termed the "area membranacea inferior ventriculi quarti," the terminology being based on the same physiological and anatomical features which led to its adoption in the case of the analogous area in the upper portion of the roof.

THE AREA MEMBRANACEA INFERIOR IN THE PIG EMBRYO.

The inferior portion of the fourth ventricle shows no evidence of a differentiation from the typical lining ependyma until the length of 15 mm. is reached. In this development consideration must be given to the factors concerned in the process. It will be recalled that in the younger embryos, both pig and human, up to and including a length of 9 mm. the whole roof of the ventricle is occupied by the epithelial-like cells. With rapid growth of the medulla and corresponding enlargement of the fourth ventricle the roof becomes elongated and widened. This process results in the isolation of the area composed originally of the epithelial-like cells and the subsequent formation of the superior membranous area. The epithelial-like cells remain in the superior portion of the enlarged ventricular roof, while the whole inferior half is composed of the densely staining, typical ependyma. The division of the roof by the laterally developing chorioid plexuses becomes evident in pig embryos of 14 mm. At this stage the whole inferior portion shows a ventricular lining composed of the typical ependyma.

The first indication of a differentiation in this inferior half of the roof was found in a pig embryo of 15 mm. This is illustrated in figures 70 and 71. The sagittal section from which these photomicrographs were taken is near the mid-line of the embryo, as is indicated by the partial section of the central canal of the spinal cord (fig. 70). The division of the ventricular roof into two parts is also indicated in figure 70 by the invagination of the chorioid plexus. The squared area in the lower half is reproduced in figure 71 under higher magnification; here the first evidence of an ependymal differentiation is observed. The dense line of the typical ependyma appears from both sides, but in the center of this ventricular lining a small area of differentiation is seen. This area, isolated by the abruptly terminating pyknotic ependymal elements, is composed of two or three layers of less deeply staining cells. The nuclei are round, rather larger than those of the adjacent mesenchyme, and contain little chromatin. The cytoplasm stains fairly well with eosin and is not scanty in amount. The cells resemble those epithelial-like elements which so largely make up the ventricular roof in the earlier stages. No albumen is found near this point of differentiation, although the whole ventricular cavity is filled with the normal amount. In figure 70 the marked zone of the area membranacea superior may easily be seen.

After this initial indication of a differentiation in pig embryos, the further differentiation of the tissue proceeds but slowly until the length of 18 mm. is attained. Thus, in a similar specimen from an embryo pig of 18 mm. the area of differentiation is not greatly increased in size. This is shown in figures 72 and 73. In the higher-power figure (fig. 73) both the superior and inferior membranous areas can be made out by the attachment to these areas of the protein coagulum of the ventricular cerebro-spinal fluid.

In the higher-power figure (fig. 73) of the squared area from figure 72, the area membranacea inferior shows the same character as exhibited by the specimen of 15 mm. (fig. 71). The opening maintains the same approximation to the lateral lip

of the medulla, but the area is larger and the histological character more nearly approaches the permanent feature of the tissue. The nuclei in this zone are paler than those of the adjoining ependymal elements and contain less chromatin. The cytoplasm is not scanty, nor is it very abundant in amount. The area is also characterized by the occurrence of the cells in a layer, two or three cells in thickness.

In view of the very slow differentiation of the area membranacea inferior in the growth of the embryo from 15 to 18 mm., the enormous enlargement of the region within the next few millimeters' growth is very astonishing. This period, as has been pointed out, is a critical one in the extension of the embryonic cerebro-spinal fluid from a ventricular to a periaxial relationship. Apparently, in the course of the embryo's growth during these next few millimeters the whole inferior roof of the ventricle undergoes a transformation and enlargement, so that the differentiated area membranacea comes to occupy practically the whole inferior half of the roof. This portion of the roof, persisting, enlarging, and suffering no extension of nervous tissue upon it, becomes the *tela chorioidea inferior*.

The rapid differentiation of the whole inferior half of the roof of the fourth ventricle is a very interesting process. Apparently the typical ependymal elements, visible on both sides of the membranous area in figure 73, undergo a very rapid alteration, so that in the course of a few millimeters' growth the cubical lining of the ventricle is replaced by a low-type cell, with round or oval nuclei, staining much less densely than do the ependymal elements. The whole area membranacea rapidly becomes a membrane in the true sense of the word; it is a continuous, intact layer of cells, generally only one cell in thickness, closing in the fourth ventricle from the choroid plexus above and the bulbar lips on the sides.

The general characteristics of this transformation are seen in figures 74 and 75. These photomicrographs are taken from a sagittal section of a pig embryo of 23 mm. On one side of the sharply delimited membrane shown in figure 75 is a tongue of nervous tissue of the medulla; on the other is the differentiated ependyma of the choroid plexus; between these two structures stretches uninterruptedly the area membranacea inferior. The flattened cells of the membrane, with their oval nuclei and almost continuous cytoplasm, effectually close the whole ventricle. The photomicrograph also shows an interesting characteristic of this membranous area which is universally present in the larger forms; this is the relatively unsupported character of the membrane. The highly vascular mesenchyme posterior to the area has gradually developed, during growth, larger and larger interstices between the cytoplasmic processes. The phenomenon is not due to shrinkage, but is intimately connected with the formation of the future *cisterna cerebello-medullaris*. This phase of the mesenchymal differentiation will be more fully considered in an appropriate section of this paper. It will suffice here merely to record the lack of support of the membrane.

Another phenomenon of importance in the cerebro-spinal fluid relationships of this stage is shown in figure 75. In the mesenchymal spaces directly beneath the membranous area there is a large amount of albuminous coagulum. This phenome-

non does not occur to any appreciable extent in earlier stages or in other parts of the mesenchyme, except about the nervous system. The close association of the coagulum from the ventricular cerebro-spinal fluid with the inner border of the area membranacea (shown in figure 75 as a slight roughening of the border) is of very great significance in this connection. In one point in the membranous area (fig. 75) the albumen can be traced almost without interruption from the ventricle into the wide spaces of the mesenchyme (cf. fig. 8). This observation strongly suggests that the embryonic cerebro-spinal fluid, which is rich in protein material, is passing, in this stage of embryonic growth, from the ventricle into the periaxial mesenchyme; and such an interpretation becomes established by the comparative findings in the embryo of the same stage in which a replacement of the cerebro-spinal fluid by the ferrocyanide solution had been effected. These comparable findings are surely of the utmost importance for the final solution of the problems centering about the embryonic cerebro-spinal fluid.

In the later stages of development of the area membranacea inferior in the pig embryo the same structural relationships persist that are shown in figure 75. Figures 76 and 77 are photomicrographs taken from a sagittal section of a specimen of 32 mm. In the enlargement of the squared area, from the first of these figures, the continuity and completeness of the membrane are well established. The photograph shows well the flattened character of the cells comprising the membrane and its sharp differentiation from the nervous tissue and ependyma below and from the ependyma and chorioid plexus above. Most important in this case is the distribution of the albuminous coagulum. Within the ventricular cavity this appears in considerable amount, and in several places it is in close adhesion to the lining area membranacea. This albuminous precipitate may likewise be traced in some places apparently through the cellular membrane into the periaxial spaces. For here, as indicated in figure 75, the clotted albumen from the cerebro-spinal fluid apparently exists in large amounts in the space just posterior to the membrane—the future *eisterna cerebello-medullaris*. Delicate strands of mesenchyme are still observed running through the wide space, but in general the whole tissue has returned to the line of the future arachnoid. The relative lack of substantial support of the membrane is well brought out in figure 77. A characteristic feature of this membrane, which Blake³¹ has championed, and which is indicated in figures 76 and 77, is the posterior bulging of the roof—"the caudal process like the finger of a glove."

Another section from the same pig embryo, taken more laterally, is represented in figures 78 and 79. In the photomicrograph of higher power the flattened character of the lining cells, the intactness of the membrane in isolating the ventricular cavity, the unsupported freedom of the membrane, and the relation to the albumen coagulum on both sides are of particular interest.

The ultimate fate of the area membranacea inferior will not be more fully entered into until the early history of the similar area in the human embryo has been detailed. For in this connection the occurrence of the foramen of Magendie requires discussion, and it seems best to delay the further consideration of the present topic until the whole question can be reviewed.

THE AREA MEMBRANACEA INFERIOR IN THE HUMAN EMBRYO.

The same process in the formation of an area of differentiation in the inferior portion of the roof of the fourth ventricle may also be followed in the human embryo. Unfortunately, however, human embryological material can rarely be subjected to the immediate fixation and preservation which yield excellent histological results in the more plentiful specimens. It does not seem strange, therefore, that the determination of the exact stage at which an area of differentiation can be made out in the ventricular roof should be practically impossible; for, in poor technical procedures, the roof of the fourth ventricle suffers almost more than does any other portion of the specimen.

In a human embryo of 13 mm. (No. 695 in the collection of the Carnegie Institution of Washington) there is slight evidence of a differentiation in the lower portion of the rhombic roof. The changing character of cells in this specimen is not marked, but as the central portion of this inferior roof is reached the ependymal cells seem to assume gradually a more cubical morphology. Associated with this change in shape, there is also a slight loss of the deeply staining character of their nuclei. The whole differentiation, however, is slight and would be commented upon only from the conception of this area in the pig embryo.

The first definite evidence of differentiation in the inferior portion of the ventricular roof was found (specimen 390 in the Carnegie collection) in a human embryo of 15.5 mm. This initial differentiation occurs, then, in the human embryo of approximately the same length as in the pig. The specimen showed the same change in character of the lining ependyma as was found in the pig. The deeply staining ependymal elements are replaced in a limited central area in the inferior portion of the roof by cells with more elongated nuclei, poorer in chromatin, and resembling somewhat the epithelial-like cells which early filled the ventricular roof. These cells tend to compose a layer of more than one cell in thickness—a feature particularly noticeable in the peripheral portions.

The size of the area membranacea inferior observed in specimen 390 suggested that the earliest evidence was probably to be observed in somewhat smaller specimens. This could not, with the material at my disposal, be verified, but it is probably safe to assume that the first signs of an ependymal differentiation will be found in human embryos of about 15 mm. This time of appearance of the area in the human would coincide with its time of primary differentiation in the pig embryo. In this limitation of the first appearance of the area membranacea inferior, the standard has been an unmistakable differentiation of ependyma and not an isolated change of a lining-cell or two which might have been the result of the technical procedure. Such a criterion was necessitated by the very marked changes in the ventricular borders observed in specimens in which distortion of the chorioidal roof had occurred.

The area membranacea inferior very rapidly increases in extent after the onset of the process of ependymal differentiation. This was likewise observed in the pig embryo, although perhaps more stages could be made out. In a human embryo of

16 mm. (No. 406 of the collection of the Carnegie Institution) the area membranacea inferior is quite extensive, as is shown in figures 80 and 81. In the photomicrograph under higher power (fig. 81) the densely stained ependyma approaches the membranous area (*ami*) as tongue-like processes from above and below. These tips gradually lose their dense character and are prolonged as a delicate membrane, lining, in this localized area, the ventricular cavity. The nuclei of the cells here are not heavily laden with chromatin: they are oval and somewhat larger than the more densely packed nuclei of the typical ependymal element. Unfortunately, the middle portions of the membranous area in this specimen are surrounded by extravasated red blood-cells obscuring somewhat the structure (fig. 81). The process, though, of the differentiation of these ependymal elements into paler and larger epithelial-like cells is quite apparent.

As in the pig, the tendency of the differentiated ependymal cells forming the area membranacea inferior to lose in some degree their distinctive appearance and to approach in character the undifferentiated mesenchymal element is apparent in the human embryo very shortly after the original steps in the process of differentiation have occurred. Photomicrographs from two human embryos of 17 mm. have been included to show this phenomenon. Thus, in figure 88, an enlargement of the blocked area from figure 58, the area membranacea inferior (*ami*) is well defined. The sagittal section from which this photomicrograph was taken is from embryo No. 576, in the Carnegie collection. Above and below the dense line of ependyma may be made out; this tapers quite abruptly, to be succeeded by the cells of the area membranacea inferior. These cells, products of ependymal differentiation, have lost much of their epithelial-like appearance; they now show rather small, oval or rounded nuclei, poor in chromatin. The cytoplasm of the cells is small in amount, but not disproportionate for the size of the nucleus. The ventricular border of these cells (fig. 88) exhibits a rather characteristic phenomenon, the adherence of a slight albuminous coagulum. The fine processes of this coagulum fuse with the cytoplasmic borders of the cells and render these borders vague and indefinite. Beneath the cells of this inferior area small vascular channels may be made out. These tend to make the membrane appear denser than its cellular character warrants.

In another section from this same embryo (No. 576) the inferior membranous area is shown in relation to the tufted chorioid plexuses (figs. 82 and 83). In the reproduction under higher magnification (fig. 83) the ependymal lining may be traced caudalwards to a gradual fusion into the area membranacea inferior. From the rather high cubical cells in the immediate proximity to the plexuses the ependymal elements become reduced in size and in height, and then rather abruptly the pyknotic character of the ventricular lining is lost. This loss of the deeply staining character coincides with the superior border of the area membranacea inferior (*ami*). The membrane of this area shows the same cell-character as already described for this embryo. On the superior side of the plexuses (fig. 83) the lateral border of the area membranacea superior (*ams*) is shown composed of epithelial-like cells.

The apparent tendency of the cells composing the inferior membranous area to lose the epithelial-like character, as shown in the figures from embryo No. 576, is not an invariable phenomenon. Rather is an aggregation of epithelial-like cells met with in human embryos very commonly in this area, not only in embryos of small size, but also in small fetuses. This phenomenon is illustrated in figures 84 and 85, reproductions of photomicrographs from a human embryo of 18 mm. (No. 409 in the collection of the Carnegie Institution). In figure 85 the total transverse extent of the area membranacea inferior (*ami*) is illustrated, with the villous chorioid plexuses appearing to the left. Although this membranous portion of the embryo has been distorted somewhat by the technical procedures to which the specimen was subjected, the cellular character of the membranous area is well indicated. The most striking feature, apart from the characteristic tinctorial differentiation from the typical ependymal elements, consists in the marked clumping of the cells in certain parts of the membrane. On one lateral extent the membrane is thickened into a bulbous swelling several cells in thickness. These cells have palely staining nuclei, poor in chromatin, with an oval or round form. In other places in the membrane smaller but no less characteristic clumps of similar cells may be made out. Between these cellular aggregations the membrane stretches in a continuous line with but few nuclei.

Analogous clumps of cells, with pale, rounded or oval nuclei, may be made out in figures 86 and 87, taken from a human embryo of 19 mm., No. 431 in the collection of the Carnegie Institution. Only a small portion of the membrane is reproduced in the figure under higher magnification, but a characteristic clump of epithelial-like cells (*epc*) is shown. These cells of the differentiated ependyma here again have oval and rounded nuclei, poor in chromatin, similar to those which have been pointed out many times in the foregoing pages. A second broadened area in the inferior membrane is also shown in figure 87.

The further development of the area membranacea inferior proceeds in the human embryo in a manner very similar to that described for the pig. In the stages but slightly above those already described the differentiation goes on slowly, but within a few millimeters the cellular pictures resemble those given for the embryo of 17 mm. (figs. 82, 83, and 88). The cellular clumps which appeared quite frequently in the embryos under 20 mm. have not been found in the larger forms. Thus, in an embryo of 23 mm. (No. 453 in the collection of the Carnegie Institution) the inferior membranous area (*ami*) appears as an extensive membrane comprising almost wholly the inferior portion of the chorioid roof. The membrane is here of a single cell in thickness; these cells are rather small, with oval nuclei, simulating in some measure those of the surrounding mesenchyme. The most interesting phase of the membranous area at this stage of 23 mm. concerns its completed cellular differentiation and its rather slow increase in size.

Wholly similar pictures of the inferior membranous area of the roof of the fourth ventricle are afforded by a human fetus of 26 mm. (figs. 91 and 92). These photomicrographs were taken from embryo No. 1008 in the collection of the Car-

negie Institution. In this specimen (fig. 92) the fourth ventricle seems almost to lack a lining of ependymal (epidermal) elements in the area membranacea inferior (*ami*). The cells of this area are small, inconspicuous in their distinctions from the underlying mesenchyme. The whole character resembles that of the superior area membranacea shown in figure 57.

The appearances exhibited by the inferior membranous area in the stages above 26 mm. are modified in great part by the development of the great cisterna cerebello-medullaris. As in the pig, the breaking-down of mesenchyme to form this cistern results finally in the almost total isolation of the inferior membranous area. The cistern is fairly rapidly formed when once the process begins, and so in an embryo of 35 mm. (No. 199 in the Carnegie collection) the isolated character of the area membranacea inferior (*ami*) may be easily made out. This is shown in figure 94, an enlargement of the blocked area in figure 93. The general architecture of the membrane, particularly its intact character, appears in this photomicrograph, but its finer structure is obscured by the albuminous coagula which adhere on both surfaces. The cell structure of the area membranacea resembles closely that described in the embryos already pictured.

Discussion of the final disposition of the area membranacea inferior will be undertaken in the following subdivision of this paper, in order that the findings in the pig and in the human embryo may be correlated.

GENERAL CONSIDERATION OF THE AREA MEMBRANACEA INFERIOR.

The ependymal lining of the caudal portion of the roof of the fourth ventricle undergoes a process of differentiation which results in the formation of the area membranacea inferior. This transformation has been observed in pig and human embryos; in both, the first definite evidence of the cellular change has been observed in specimens of 15 mm. The essential phases of the process are identical in the two embryos. The tendency of the deeply staining typical ependymal elements is to lose their highly pyknotic character; the nuclei become poorer in chromatin and the cytoplasm somewhat more abundant. In the first stages of the metamorphosis the lining cells come to assume epithelial-like appearances, but in the final change the nuclei become small oval bodies, poor in chromatin, resembling to some degree the nuclei of the adjoining undifferentiated mesenchyme. In the human embryo, a tendency for the epithelial-like characters to persist in isolated cellular aggregations is apparent.

After the initial process of differentiation has begun, the area membranacea inferior increases rapidly in extent and the differentiated cells which characterize it come to occupy the greater portion of the caudal part of the chorioidal roof. In the somewhat later stages the area membranacea is almost wholly unsupported by other tissues, due to the development of the cisterna cerebello-medullaris. As soon as the cistern forms, the area membranacea serves as practically the sole dividing membrane between the ventricular system and the future subarachnoid spaces.

The ultimate fate of this area membranacea inferior is necessarily involved in the distribution of the tela chorioidea inferior. Likewise it necessitates a discussion of the possible formation of the so-called foramen of Magendie and its mode of origin from the "caudal process" of Blake. It is proposed to discuss briefly some of these questions in the hope that some phases of the problem may be brought forth.

It must be clearly understood that the questions of the ultimate fate of this area membranacea inferior probably differ considerably in the different species of mammals. In the horse and in the pig the absence of the medial foramen of Magendie is fairly well established, but in man its existence seems to rest on equally firm grounds. While, primarily, this investigation has not been concerned with the possible existence of the foramen of Magendie, the question has been presented many times in regard to the pig and human embryos examined.

As far as can be determined, no descriptive study of the development and differentiation of the inferior portion of the rhombic roof has been published. Heuser's²³ studies on the form of the cerebral ventricles of the pig have afforded a very good conception of the gradually changing relationships in this region. Hess²² has devoted attention to the histological appearances of the inferior roof in the embryo. One of his interesting observations concerns the caudal portion of the rhombic roof in a fetal cat of 10 cm., where he noticed a very sudden interruption in the epithelial lining of the ventricle, with a complete closing by a fibrous net. This description by Hess is the only comment upon the histological appearance of the ventricular roof that has been found. His²⁵ pictures, without comment, in a retouched photomicrograph, a differentiated area in the proper situation in his fetus C-1 (beginning of the third month).

The many writers in embryology have commented upon the roof of the fourth ventricle. Minot⁽⁴⁰⁾, in 1892, stated regarding it:

"Several writers have thought that the membrane was broken through at several points, but it probably is really continuous throughout life. The fourth ventricle is to be regarded, then, as an expansion of the central canal permanently bounded by the original medullary walls."

Kollman³², on the other hand, advances the view that during the third month the rhombic roof is broken down to form the foramen of Magendie and the two foramina of Luschka. Streeter⁵⁴, in his chapter on the development of the nervous system in the Keibel-Mall Handbook of Embryology, advances a similar view. The majority of investigators to-day incline to the belief that the roof of the fourth ventricle in man is perforated to form the median foramen of Magendie.

Hess²² has advanced a conception of the foramen of Magendie that is supported by numerous observations. To test Kölliker's statement that the fourth ventricle remained closed during human embryonic life, Hess sectioned the region in human fetuses, new-born infants, and in adults. The lengths of the fetuses cut were as follows: 7, 12.5, 15, 16, and 17 cm. In the 47 cases the roof showed a medial opening (Magendie), except in one case, in which it was closed by a "thin pial membrane." Hess's conception of the process of formation of this membrane

was that in early embryological life the rhombic roof was bordered by a regular, meshed tissue. Later the small meshes in this tissue fused to form the larger foramen of Magendie.

Blake's³ hypothesis of the formation of the medial foramen has been quite extensively quoted in the more recent publications on this subject. In a study of the chorioidal roof Blake found a caudal bulging of the inferior velum; this out-pouching became more and more extensive in the older embryos. In man this pouch became sheared off at its neck, leaving the foramen of Magendie.

In addition to the few studies referred to above, there have been in the past 25 years a great number of articles (notably those of Wilder⁽⁵⁶⁾ and Cannieu⁽⁴¹⁾) offering evidence that this median foramen of the fourth ventricle is an existent, functional opening. Into this literature it is not proposed to go in the present communication; it may be stated that in the larger part the views presented have been in favor of the consideration of the true occurrence of the foramen of Magendie.

The material on which this study is based has been purely embryological in type, so that no reliable data regarding the foramen of Magendie could be obtained. But even in the largest fetuses examined, there was no evidence which indicated a breaking-down or a shearing-off of the inferior roof of the fourth ventricle. In the largest human fetus at my disposal, in which the histological material was good enough to permit an accurate examination of the chorioidal roof (embryo No. 448, 52 mm. in the Carnegie collection) the area membranacea inferior appeared as an intact membrane supported only by a few pial cells. In the pig the material at hand has been such that accurate study of the roof could be made in specimens up to 20 cm.; in all of these later fetal pigs the roof has been wholly without foramina. If, however, in these larger stages the histological procedures have not been of the best, ruptures and other artificial separations are very frequently found.

The area membranacea inferior, then, may be regarded as a region of ependymal differentiation. Whether it persists as an intact membrane or undergoes, in certain animals, a perforation to form a foramen of Magendie can not be here answered; this study has been concerned solely with the embryology of the cerebro-spinal spaces, and it affords no evidence in favor of or against the existence of such a foramen. Nor has any study been made of the two foramina of Luschka, the two openings from the lateral recesses of the fourth ventricle into the subarachnoid spaces. It can be stated, however, that these foramina are not in existence at the time of establishment of the circulation of the cerebro-spinal fluid. This phenomenon, as recorded in the previous section, occurs in pig embryos of 26 mm.; at this time the lateral recesses are anatomically and physiologically closed.

VI. PASSAGE OF FLUID THROUGH ROOF OF THE FOURTH VENTRICLE.

On pages 20 to 30 is a description of the passage of a true solution, substituted without increase in pressure for the embryonic cerebro-spinal fluid, through the roof of the fourth ventricle into the extraventricular or periaxial spaces. This extension of fluid occurred in two localized areas, one in the superior half and the other in the inferior half of the rhombic roof. Histological study of these regions revealed a localized differentiation of the ependyma, both in the upper and lower halves of the ventricular roof. It becomes necessary, then, to correlate, if possible, the areas of this fluid-passage to the anatomical differentiations pointed out.

THE ACCUMULATION OF INJECTION-MASSSES IN THE SUPERIOR MEMBRANOUS AREA.

It has already been recorded that the first evidence of a change in the reaction to a replacement injection occurred in an embryo about 13 mm. long (fig. 2). This stage was characterized by a dense collection of the precipitated granules in a definite area in the roof of the fourth ventricle. At this stage also the area membranacea superior is well differentiated (fig. 31). That the site of the granular accumulation is this membranous area is easily proved by an inspection of figure 117, which represents an enlargement of the squared area in figure 116. In the low-power photomicrograph the prussian-blue granules are not represented, but are found scattered through the ventricles, with a definite collection in the posterior region of the fourth ventricle. Under a higher magnification (fig. 117) the blue can be traced in but small quantity along the normal ependymal lining (shown to the left in the figure), but as soon as the differentiated area (area membranacea superior) is reached the granular material is heaped up in a dense mass, which extends as a thickened pad into the ventricle.

The same phenomenon of the accumulation of the injection fluid in the superior membranous area is shown in figures 112 and 113, the second photomicrograph representing the area outlined in the first, but reproduced under much higher magnification. In this specimen (an embryo pig) a dilute solution of silver nitrate was injected into the central canal of the spinal cord. On histological examination the accumulation of the silver also shown in figure 11 was found. Thus, in figure 113 the ventricular epithelium can be made out in the upper right-hand corner, while below (in the area membranacea superior) the silver is densely accumulated.

The explanation of this phenomenon of accumulation in the superior membranous area is not wholly clear. It occurs only in stages in which the histological differentiation of the ventricular roof has proceeded to some degree and in stages where the fluid-passage into the periaxial tissues is not wholly unobstructed. This aggregation of the precipitated granules of prussian-blue and of the reduced silver in a localized area certainly suggests a physical explanation, as in these cases the physical laws of precipitation and reduction must hold. The many figures of the superior membranous area of the ventricular roof show that in the stage under consideration the cell-outlines projecting into the ventricles are rough and ragged as contrasted with the smoother and more regular surface of the adjoining ependyma.

Could not these roughened, irregular cell-surfaces become the site of the first and most extreme precipitation of the prussian-blue and of the reduction of the silver? Certainly they would serve much more efficiently as the foreign substances about which precipitation would occur in greatest amount. This physical explanation finds many arguments for its support in these studies.

Another explanation of the phenomenon concerns the normal flow of the fluid and the relation of the direction of this flow to the roof of the fourth ventricle. As has already been emphasized, it is difficult to assume that there is any marked production of cerebro-spinal fluid before the periaxial spread occurs. Such an assumption would argue against the development of any special current toward the roof of the fourth ventricle in any stage smaller than that represented in figure 3, and would vitiate the explanation of the occurrence of the granular accumulation shown in figure 2 (a pig embryo of 13 mm.). In the later stages (16 mm., *cf.* fig. 11) this explanation would probably suffice for the phenomenon exhibited.

THE SITES OF FLUID PASSAGE THROUGH THE ROOF OF THE FOURTH VENTRICLE.

With consideration of the evidence presented as to the accumulation of the precipitates of the injected fluid about the area membranacea superior during certain stages in the development of the cerebro-spinal spaces, it would seem that the same area must be concerned in the passage of fluid from the ventricular cavities into the periaxial tissues. This view receives support from the reproduction of a cleared specimen (fig. 11) in which an injection of silver nitrate had been made into the central canal of the spinal cord. The pressure employed was great enough to force the fluid into the periaxial spaces, but the resultant picture clearly showed the oval outline of the area membranacea superior.

The study of the passage of fluid from the ventricular to the extraventricular spaces can best be made by simple histological serial sections. In these observations pig embryos in which the cerebro-spinal fluid had been replaced by the compensating device, supplying a true solution of potassium ferrocyanide and iron-ammonium citrate, were sectioned and examined with reference to the sites of fluid passage. The results of these studies are given here in order that the whole question of the connection of the cerebral ventricles with the subarachnoid spaces may be discussed.

In the stage represented by figure 3 (in which fluid passes from one area in the roof of the fourth ventricle into the extraventricular tissues) histological sections show that the point of fluid passage is localized and concerns solely the area membranacea superior. The replaced fluid (as demonstrated by the subsequent precipitation of the prussian-blue) passes through this entire membranous area into the adjoining mesenchyme. The process is wholly confined to this area; the adjoining ependyma is entirely impervious to the ferrocyanide. This phenomenon of passage of the replaced fluid through the superior membranous area is well shown in figures 14, 18, and 23.

The distribution of the minute granules of prussian-blue in the cells of the superior membranous area is of importance in any discussion of the passage of fluid through a membrane; for this area (in the superior portion of the roof of the

embryonic fourth ventricle) must be considered as a membrane permeable in certain degrees to the fluids bathing it. That the area membranacea is intact and does not contain stomata or other minute foramina has been demonstrated histologically. Further evidence of the entire lack of intercellular stomata is afforded by the distribution of the prussian-blue granules precipitated *in situ* after the replacement of the cerebro-spinal fluid by the ferrocyanide solution.

Figure 14 is a reproduction of the superior area from a transverse section of a pig embryo in which the routine replacement had been made. The position of the area is shown by the squared outline in figure 13. On both sides the impermeable ependyma is seen, with granules of the blue adhering to the ventricular border of the cells, but not penetrating them at all. To the left of the drawing the few ependymal cells possess, beneath their central border, a chain of the granules which have entered from the abrupt edge of the area membranacea. In the cellular border between the two lips of the ependyma, the area membranacea superior, the passage of the replaced fluid is easily made out by the resultant blue granules. The area is roughly delimited by a ventricular collection of the blue granules. Examination of these cells shows that the prussian-blue is present within the cytoplasm, avoiding the nuclei with perfect precision. Some of the cells are rounded and almost free from the granules; others, particularly those whose cytoplasm is elongated, are completely filled with the granules, the nuclei standing out in a blue granular cytoplasm.

The question of the passage of the fluid between the cells must also be answered by the histological evidence. In the same drawing (fig. 14) in one or two places there are indications of a slight stream of granules between the cells of the area membranacea superior. This apparent transit of the fluid through intercellular passages is particularly clear in the small areas where the cellular cytoplasm is relatively free from the granular deposits. But upon careful examination of these areas under oil immersion it is always apparent that the adjoining cytoplasm is also involved in the granular precipitation, indicating that the cells, although almost free from the deposit, are also engaged in the process of the fluid passage. Compared to the whole area of fluid transit, the points indicative of a passage through possible intercellular stigmata are almost negligible. It seems not unlikely that the outlining of canals between cells may be a physical phenomenon, as in most cases no cellular borders (as demonstrated by the precipitated granules) can be made out. These peculiarities of fluid passage may be seen in figures 14, 18, and 23.

Consideration of all the evidence afforded by histological examinations of the essential character of the area membranacea superior and of the passage of fluid through it inclines one inevitably to the belief that this area functionates as a cellular membrane. The fluid passes through it as through any permeable living membrane. Histologically the passage is for the most part through the cytoplasm of the cells, but occasionally an intercellular course is suggested. Both processes are wholly compatible with the accepted view of a cellular membrane devised for the passage of fluid through it.

The same phenomenon of the passage of fluid from the fourth ventricle into the periaxial spaces is beautifully illustrated in figure 23. This drawing is from a transverse section of a pig embryo (23 mm. in length) in a stage when the superior membranous area is rapidly being encroached upon by the developing cerebellum and by the caudal chorioid plexuses. Between the deeply staining ependymal cells on either side the membranous area is densely outlined by the deposition of the granules of prussian-blue in the cytoplasm of the cells of the area membranacea superior. The avoidance of the nuclei of these cells by the ferrocyanide is well demonstrated in this reproduction, as is also the impenetrability of the ependymal cells. In a specimen of this nature the question of the passage of the injection fluid through possible intercellular foramina loses its significance; for the drawing shows clearly the importance of considering the entire area membranacea as a functioning whole—a permeable, living, cellular membrane.

It has been shown in a foregoing section of this memoir that histologically the area membranacea superior decreases to an almost negligible remains in specimens of embryo pigs over 30 mm. long. This same rule apparently holds for its functional importance, as determined by the relative and absolute amount of prussian-blue granules deposited in the cells of the superior membrane. This decrease in the functional importance may be inferred from figure 47, a photomicrograph from a pig embryo of 32 mm. Apparently the size of the membrane determines in large measure the amount of the replaced fluid which passes through it.

Thus far we have been concerned solely with the passage of fluid through the area membranacea superior. In the earlier stages of from 14 to 23 mm. the importance of the superior membrane functionally is great, but in the later stages the inferior membrane assumes far greater significance. This is demonstrated not only by the structural history of the two areas, but by the functional index afforded by the replacement of the cerebro-spinal fluid by a foreign solution.

In the foregoing section the first evidence of any histological differentiation in the inferior portion of the roof of the fourth ventricle was shown to occur in pig embryos of 15 mm. in length. From this stage upwards (figs. 4, 5, etc.) a portion of the inferior roof allows fluid to pass through it. The exact point of fluid passage is the localized ependymal differentiation forming the area membranacea inferior. This relationship is easily verified by reference to figure 18. In this drawing of a median sagittal section of a pig embryo the two localized points of fluid passage into the periaxial tissue are readily identified; they are quite limited in comparison to the extent of the periaxial spread.

Figure 16 represents the inferior membranous area of the roof of the fourth ventricle from a pig embryo of similar size (18 mm.). The histological character of the inferior area is well shown in this drawing. It will be seen that, except in small areas, the histological differentiation of the ependyma has not proceeded to any great extent; the fluid from the ventricular cavity (as traced by the precipitated granules) closely follows the points of greatest cellular differentiation. There is no possibility of an interpretation of the findings concerned with the existence of

intercellular stomata; the passage of fluid is here again to be looked upon as a transit through a cellular membrane.

The same general phenomena of the passage of fluid through a localized area (the area membranacea inferior, in the caudal portion of the roof of the fourth ventricle) that have been observed in the superior portion of the roof are shown in figure 18. Chief among these phenomena is the careful avoidance by the precipitated granules of the ependymal lining of the ventricles and the adherence of the granules to the lining walls at the points of fluid passage. The ependymal lining, except in the two areas of differentiation, is everywhere impenetrable to the solution of the ferrocyanide.

As the size of the embryo increases the functional importance of this more caudal area becomes much greater (*cf.* figs. 3, 4, 5, and 76). The whole caudal half of the fourth ventricle becomes an area of ependymal differentiation and of fluid passage. It serves everywhere as a complete diffusing membrane, unbroken by the occurrence of stomata. Through this whole membrane the replaced solutions of potassium ferrocyanide and iron-ammonium citrate pass with apparent ease, as demonstrated by the precipitated granules of prussian-blue (fig. 18). From stages of 24 mm. and over, the lower membranous area is the only one of significance in the total fluid passage.

The areas, therefore, through which the replaced solution of potassium ferrocyanide and iron-ammonium citrate passed, in the experimental pig embryos, are the two areas of histological differentiation in the roof of the fourth ventricle—the area membranacea superior et inferior. There is no evidence whatsoever of any other point of escape of the fluid from the ventricular system into the periaxial spaces. The precipitated prussian-blue does not penetrate any of the lining cells of the ventricle except in the two areas under consideration. Nor is any evidence afforded by histological study of the escape of ventricular fluid through the described foramina of Bichat and of Mierzejewsky.

FACTORS CONCERNED IN THE EXPERIMENTAL FLUID PASSAGE.

It becomes necessary to discuss the question of the passage of the replaced fluid through the two cellular membranes in order to ascertain to what extent the results obtained by the method may be relied upon. Naturally in such questions the factors concerned in the normal transit of body-fluid through such structures must be considered.

Probably the most essential element in obtaining reliable results in any injection is the control of the pressure at which the foreign fluid or mass is introduced. This matter has been fully discussed in the résumé of the methods employed; it is sufficient to reaffirm here that, in these observations, the normal cerebro-spinal tension has not been disturbed because of the use of a compensatory replacement. Other experiments, carried out under increasing pressures of injection, have been made, in order to compare the results with those furnished by the replacement-method.

Consideration must next be given to the factors of diffusion, filtration, and osmosis in the passage of fluid through the roof of the fourth ventricle. The third factor, however, may be largely excluded, owing to the fact that the solutions of potassium ferrocyanide and iron-ammonium citrate employed were for the most part practically isotonic with the body-fluids. Furthermore, the use of hypertonic solutions apparently gave no different results (except in the increased density of the resultant precipitate) from those obtained by the employment of the isotonic solutions. Finally, it was found to be of service to use hypotonic replacement solutions in order to obtain very slight precipitates; in these experiments also the spread of the replaced ferrocyanide solution was similar to the standard result afforded by the isotonic solution. These observations with varying concentrations of the foreign solutions replacing the cerebro-spinal fluid serve to indicate that osmosis plays but little part in the passage of fluid through the roof structures of the fourth ventricle. Undoubtedly the factor of osmosis can not be ignored in any consideration of the passage of fluid through a cellular membrane, but it seems unlikely that with solutions of practically the same salt-content it should be of great importance.

The influence of diffusion in this passage of the solution of the ferrocyanide and citrate from the cerebral ventricles into the extraventricular space is probably great. The whole plan of the experiment concerns the introduction of salts foreign to the body-fluids, even though in analogous concentrations. It seems not unlikely that as soon as the replacement of the existent cerebro-spinal fluid is effected the ferrocyanide and citrate must immediately begin to diffuse out into the periaxial tissues and the normal salts return to the ventricles. Probably, however, this same phenomenon plays a normal rôle in the human body. Jacobson's⁽²⁷⁾ extensive and important studies on the chemistry of cerebro-spinal fluid have shown that the ventricular cerebro-spinal fluid is not identical with the subarachnoid fluid. The differences in the two fluids are probably to be accounted for by the fact that the ventricular fluid represents the pure elaboration of the chorioid plexuses, whereas the lumbar subarachnoid fluid is composed not only of the products of the chorioid plexuses but also of the fluids from the perivascular system. In this transference of the ventricular fluid to the subarachnoid space diffusion may play some part, the relative importance of which can hardly be estimated.

But will diffusion alone account for the passage of the experimental fluid in the ventricle through two well-defined areas into the periaxial tissues? Will diffusion account for the varying extent of the injection in different stages of embryonic development? There are several arguments against according diffusion a maximal importance in the process. In the first place, an injection of the solution of the ferrocyanide under mild syringe-pressure will give a spread similar in every respect to those obtained by the replacement method. This indicates that the course taken by the two solutions is not necessarily the result of diffusion, but rather of the capabilities of the tissues for fluid-spread; and similarly the passage of this true solution through the roof areas need not be solely a diffusion process, but may be accounted for by the true flow of the fluid in this direction. Again, in the stages represented

in figure 2 one would expect as extensive a spread of the replacing solution into the periaxial tissue were diffusion the active force in the movement of the fluid. Instead of such a periaxial spread the injection fluid remains wholly within the ventricular system, indicating that other forces than that of diffusion play an active rôle, in the more advanced stages, in the movement of the fluid. Finally, if diffusion is to be considered the sole agent in the distribution of the replacing fluid, why does not the ferrocyanide penetrate all the cellular structures lining the ventricular cavity? Surely it would be expected that diffusion between the body-fluids and the ferrocyanide solution would occur in each ependymal cell—a phenomenon observed only in the cells comprising the ventricular surfaces of the membranous areas of the rhombic roof.

While acknowledging that diffusion and osmosis may play important parts in the process of the passage of fluid from the fourth ventricle into the periaxial tissues, it seems apparent that some other factor or factors must be the determining agent or agents. It is not unlikely that the formation of cerebro-spinal fluid by the cells of the chorioid plexus may cause, in the replacement experiments, further passage of fluid into the extraventricular regions. Such an elaboration of fluid, with the ventricles filled with the experimental solution, would result in an increase in the normal ventricular tension. If this be the real explanation, the passage of the fluid into the extraventricular spaces would result in part from the increase in pressure on one (the ventricular) side of the membrane. The process, then, would be one of filtration through the membrane from the point of higher to that of lower pressure. This explanation best seems to cover the results obtained by the replacement method, and is supported by the histological examination of the developing chorioid plexuses and by many other features which are dealt with in other sections of the paper. This view is also strongly supported by the results of injections under mild syringe-pressure.

On the basis that the passage of fluid from the fourth ventricle into the periaxial tissues is in large measure a process of membrane filtration, the phenomenon of the fluid transit of the replaced solutions may be taken as a real index of the circulation and distribution of the cerebro-spinal fluid. It may be assumed, therefore, that the resulting distribution of the prussian-blue granules represents the course and extent of the fluid channels of the embryonic cerebro-spinal fluid.

The discussion of the fluid passage outward from the cerebral ventricles into the subarachnoid spaces has thus far been concerned with the processes involved for the transit of the true solutions of the salts. There is, however, an undoubted passage outward, as has already been indicated in a foregoing section, of the protein content of the normal cerebro-spinal fluid. This occurs in specimens in which a truly definitive membrane, intact throughout, can be seen inclosing the chorioid roof. The explanations which suffice for the passage outward of the true solutions will not serve for this phenomenon.

The cells of the body probably are equipped to handle colloidal solutions in several ways, but two methods seem possible as explanatory of the problem at hand.

In the first place, it is conceivable that the cells in the differentiated area membranacea could phagocytose the colloidal albuminous particles of the ventricular fluid and excrete them into the subarachnoid spaces on the other side of the membrane; but it does not seem probable that this explanation is correct. Much more likely is it that the colloidal masses may follow the same laws of fluid-passage as the true solutions. But in such a passage through a cellular membrane the rate of passage will be much slower with the colloid.

These two theories regarding the passage of the albumen of the ventricular cerebro-spinal fluid into the subarachnoid spaces are not based on any findings presented in this article, but are ventured as being in keeping with current physiological explanations of such phenomena. On the basis of the second hypothesis, the failure of granular material to pass through the cellular membrane of the choroidal roof must be explained as being due to the inability of the cells to handle the foreign material except in sizes which could be absorbed. The fact that the original unit was not phagocytosed or passed through the membrane probably depended on the size of the molecule and the specific character of the lining-cells.

THE PASSAGE OF SILVER NITRATE AND INDIA INK THROUGH THE MEMBRANOUS AREAS IN THE ROOF OF THE FOURTH VENTRICLE.

Thus far in the discussion of the passage of the experimental fluids through the ventricular roof, true solutions of potassium ferrocyanide and iron-ammonium citrate only have been considered. This solution, as has been pointed out in this and in a previous article⁵⁵, is non-toxic and is not taken up by the cells. With the dilute solutions (0.25 to 0.5 per cent) of silver nitrate, a far different problem is presented. Replacement experiments with this salt are rendered impossible by its intraspinal toxicity and by its precipitating action upon protein; but beautiful preparations may be made by this method by the simple injection with a syringe into the central canal of the spinal cord.

With mild syringe-pressure the result of such an injection with silver nitrate is in all cases a simple ventricular spread, with no extension into the periaxial tissues. This general rule holds in all stages in which the central canal can be definitely entered without causing a spread into the perispinal tissues. This failure of the spread to extend into the periaxial tissues under mild pressure is undoubtedly due to the coagulating effect of the silver, which renders further passage of the fluid impossible. The reduced silver collects about the superior membranous area in the roof of the fourth ventricle, outlining it distinctly. This phenomenon is illustrated in figure 115 (a transverse section of a pig embryo of 19 mm.). At this stage the replacement of cerebro-spinal fluid by a ferrocyanide solution results in a quite extensive spread (*cf.* fig. 5).

With increased pressures of injection the silver may be pushed into the periaxial tissue through the roof structures of the fourth ventricle. The transit of the injection-mass occurs in the area membranacea superior in practically all cases (*cf.* fig. 12). The inferior membranous area, in the earlier stages, is almost invariably impermeable

to the silver (unless the injection-pressure is extreme). When the superior area is examined after such an injection under high pressure the silver is found deposited throughout the cells of the area, extending only a short distance into the adjacent tissue. This feature of the injection is pictured in figure 113. In these injections the high pressure undoubtedly suffices to force the silver through the coagulated area membranacea. Its coagulating effect on the ependyma is almost equally marked, but the point of least resistance is apparently in the membranous area, allowing the fluid to pass through it.

Replacements of the cerebro-spinal fluid with diluted solutions of india ink within the medullary-canal system of small pig embryos never result in any extension of the granules into the periaxial tissues, for under the normal tension in the ventricles of the pig the area membranacea are impermeable to the passage of granular material. After such a replacement the carbon masses may be found everywhere throughout the ventricles, but not in the periaxial tissues. However, india ink may be forced into the periaxial tissues by the use of high pressures of injection, as shown in figure 10. In this specimen of a pig embryo (21 mm. in length) the periaxial spread occurred solely from the superior membranous area. This is analogous to the results obtained with silver nitrate, shown in figure 12. Without doubt in the earlier stages the superior area is much more permeable than the inferior. Histological examination of these specimens after an injection of india ink under high pressure reveals that the carbon granules gain the extraventricular space only through the area membranacea superior; some cells in this area are crowded with the granules, but for the most part extensive intercellular stomata have been made. The whole process must be viewed as a result of the excessive pressure of injection.

In the more advanced stages of the pig embryo (30 mm. and upwards) the pressure necessary to occasion an extraventricular spread of the india ink after intraspinal injection decreases somewhat, so that with mild syringe-pressure a local periaxial spread from the fourth ventricle may be obtained from an injection into the central canal of the spinal cord. This is in accordance with the observation of Mall³⁶, who found that the injection flowed "through the medial opening of the fourth ventricle." The opening in these cases is in the area membranacea inferior, and in many instances subsequent examination showed rupture of the membrane with escape of the ink, even though the injection-pressure was moderate.

Taken as a whole, then, the findings are against the passage of solutions of silver nitrate or suspensions of india ink from the ventricles into the periaxial tissues, except when injected under pressures far above the normal intraventricular tension.

RELATION OF THE EPENDYMAL DIFFERENTIATION TO THE PASSAGE OF FLUID.

Under this heading it is proposed to discuss the relationship, if any, existing between the stages of differentiation of the ependyma of the roof of the fourth ventricle and the passage of fluid through the two membranous areas. The discussion must necessarily be of a temporal character, with an attempt to consider possible factors in the process.

The most important question in this connection is whether the ependymal differentiation is necessary for the passage of fluid through it. In the pig embryo of 13 mm. the area membranacea superior has reached a stage of marked differentiation (fig. 31), but at this same stage (fig. 2) there is no evidence of any passage of fluid through the roof of the fourth ventricle into the periaxial tissue, only an outlining of the oval membranous area. Here, then, the histological differentiation has definitely preceded the assumption of function on the part of the area membranacea superior. The passage of fluid through the lower area occurs at a relatively earlier stage than it does through the superior opening. The first evidence of differentiation of the inferior roof of the fourth ventricle was observed in pig embryos of 15 mm. in length. At 18 mm., even though the process of differentiation was far from complete, some of the replaced fluid was able to pass through the lower area (figs. 4, 16, and 18).

A consideration of these observations leads to the assumption that some histological differentiation of the ependyma is necessary for the extraventricular passage of the replaced fluid. In the case of the superior area the differentiation occurs at a considerable developmental interval before fluid passes through it; in regard to the inferior area the assumption of function occurs at a somewhat earlier period in its differentiation. This slight difference between the two areas may possibly be explained on the basis that as soon as the stage of 14 mm. is attained (by the pig embryo) a greater amount of cerebro-spinal fluid is produced than can be cared for by the more slowly enlarging ventricular cavities. As soon as this disproportion occurs the excess of fluid is poured into the periaxial tissues through the already differentiated area membranacea superior; therefore, when the inferior area first shows evidence of formation there is still this excess of fluid in the ventricles. The fluid apparently avails itself almost at once of the new opening and its functional existence becomes immediate. It is apparent, moreover, that the capacity of the membranous areas for the passage of fluid is considerably in excess of the demands made upon them, and furthermore, that the provision for the passage of increasing amounts of fluid is completed before the demand arises.

In the passage of fluid from the ventricles into the mesenchyme, there is one other factor which has not as yet been considered. This concerns the potentiality of the adjacent mesenchyme to afford channels for the fluid poured into it. Were resistance offered to the flow of solutions through the mesenchymal tissue spaces, fluid could escape from the ventricles in only very small amounts, if at all; as soon, however, as easily traversed fluid channels became established, the cerebro-spinal fluid could readily escape through the two membranous areas. The question as to what part the embryonic cerebro-spinal fluid plays in the further development of the meningeal spaces also arises in this connection. It is at present impossible to assign to any one of these factors a specific rôle in the passage of fluid from the fourth ventricle into the periaxial spaces, but it is important to consider them as possible determining agents. The evidence all indicates that the rate of production of the embryonic cerebro-spinal fluid is the most important factor, by far, in the extraventricular escape of the fluid.

VII. GENERAL HISTOLOGICAL DIFFERENTIATION OF THE CEREBRO SPINAL SPACES.

The general problems concerned in the formation of the meninges and of the spaces inclosed within them deal with the gradual adaptation of a primitive undifferentiated mesenchyme to the anatomical and physiological requirements of the adult. Originally the meninges were held to be derived from the same epidermal infolding which gave origin to the central nervous system; then, with increasing knowledge of the structure, the dura alone was said to be a product of the middle germ-layer; and finally, by the researches of His²⁵ and of Kölliker,³¹ the mesenchymal origin of the three meninges was established. The general process of the differentiation and the stages in this transformation have not been reported in great detail; here, too, the investigations must have an outlook for physiological anatomy as well as for pure morphology.

It may be well to comment briefly on the relationships of the three meninges found in adult mammals. The dura is well established as the fibrous-tissue envelope of the leptomeninges and the central nervous system. But there is a tendency to regard the arachnoid and pia mater as constituting one structure—the leptomeninges or “pia-arachnoid,” in the terminology of Middlemass and Robertson³⁹. This difference of opinion in regard to the two inner meninges is due to their structural and intimate relationships. The arachnoid may well be assumed to be a single membrane, worthy of being regarded as a single structure if one considers only its outer continuous membrane as the essential structure. But the inner surface of this membrane sends processes inward to fuse with the pia mater, which is so closely applied to the nervous tissue. These processes divide the subarachnoid space (included between arachnoid and pia) into the well-known meshes in which the cerebro-spinal fluid circulates. From the standpoint of these channels (the subarachnoid spaces) the arachnoid constitutes the parietal and the pia the visceral layer. Thus the intimate structural unity of the two membranes seems, in the opinion of many investigators, to warrant their designation as a single membrane. This view, however, has been strongly opposed by Poirier and Charpy⁴⁵, who considered the distinction of three meninges very essential. Hence, in considering the transformation of tissues in the embryo, regard must be had for the dura as a well-differentiated structure, and for the leptomeninges as units, but certainly to be regarded from the standpoint of the subarachnoid spaces. In this connection Sterzi's⁵³ observations on the comparative anatomy of the meninges are of interest. It will be recalled that the dura in lower forms becomes well established before the leptomeninges emerge from a primitive mesenchyme.

THE PERIAXIAL MESENCHYME.

Surrounding the central nervous system in young embryos is a rather thick cushion of undifferentiated mesenchyme, similar in all respects to the undifferentiated tissue in other parts of the embryo. But very soon in the course of development the nuclei in this mesenchyme increase along the clear marginal zone of the

spinal cord and basilar structures, forming the initial indication of the pia mater. This phenomenon is indicated somewhat in figure 40, a photomicrograph taken from a human embryo (No. 836) of 4 mm., the earliest stage here illustrated.

The next essential change in the great differentiation of the meninges concerns a blastemal condensation of this same mesenchymal tissue to form ultimately the bony covering of the central nervous system and a portion of the dura; but between these two zones of differentiation the mesenchyme remains for a time almost unaltered. A portion of this tissue will go to form the arachnoid membrane and the trabeculae which mark off the subarachnoid spaces. This process in the formation of the arachnoid will be discussed here; the formation of the pia mater and dura will be detailed in succeeding divisions of the paper. The differentiation will be discussed as a general process, in regard to both human and pig embryos, for in no respect has any essential difference between the two been observed.

The general character of the periaxial mesenchyme may be commented upon here. The tissue is of a very loose and typical structure, forming a syncytial network of rather small mesh, but fragile. The nuclei of the cells are oval, with a definite chromatin content; the cytoplasm is largely devoted to the maintenance of long processes which connect with adjacent cells. Adhering to the cytoplasmic processes are very tiny albuminous coagula, of such small amount as to be hardly noticeable; also in the meshes of the mesenchyme very small quantities of this albumen may be identified. These albuminous coagula undoubtedly represent the protein of the tissue fluids in the undifferentiated stages.

THE FORMATION OF THE ARACHNOIDEA.

A general consideration of the problems here involved will surely shed light on some of the various factors concerned. It must be noted that in its development this membrane proceeds from an undifferentiated but small-meshed mesenchyme into the adult structure which contains the relatively large cerebro-spinal channels. Then, too, the enlargement of the tissue meshes in certain places—as the future cisternae—must be enormous. Besides this necessary dilatation of the spaces in the periaxial mesenchyme, the outer portion of the tissue must separate from the future dura and form the outer surface of the arachnoid membrane. Here the process must be one of tissue condensation and proliferation. A similar agency is involved in the growth of the mesothelial cells which cover the outer surface of the arachnoid and also the inner subarachnoid spaces.

The general process, then, in the formation of the arachnoid membrane concerns a thinning and readjustment of the primitive mesenchyme in certain areas, while in others the process is reversed, the membrane reaching the adult form through proliferative and condensing phenomena. Such alterative processes must naturally result from the application of certain mechanical or vital agents in the growth of the embryo. Is the mere growth of the central nervous system sufficient to furnish these alterative agents, or must we likewise trace the corresponding development of the bony coverings of the brain and spinal cord? Neither factor seems

relatively of great importance when compared to the possible influence of the presence and circulation of cerebro-spinal fluid on this periaxial tissue. This seems to be the most important factor, an internally-modifying influence to which the periaxial mesenchyme is subjected in the formation of an arachnoid and its subarachnoid spaces. It will therefore be from this standpoint that the development of the spaces will be discussed; for, as has already been pointed out, the periaxial mesenchyme becomes a functionally active tissue for the circulation of the cerebro-spinal fluid at a stage when differentiation has not begun. On this basis, the lack of differentiation shown in the periaxial mesenchyme in the stages before the ventricular cerebro-spinal fluid is poured into the mesenchyme in the neighborhood of the roof of the fourth ventricle is not surprising. The character of the periaxial mesenchyme in the early stages is reproduced in numerous photomicrographs (figs. 25, 49, 51, and 53). The mesenchyme is here characterized by a rather dense meshwork of cytoplasmic processes, interspersed by a considerable number of oval nuclei. The content of the interstices in albumen, as judged by the persisting coagula, is very small. This picture of the periaxial mesenchyme persists until cerebro-spinal fluid is poured from the ventricle through the area membranacea superior.

As will be seen in figure 3, the first indication of an extraventricular spread of the replaced fluid in the ventricles occurred in a pig embryo of 14 mm. At this stage the membranous area in the superior portion of the roof of the fourth ventricle has already become well differentiated. The fluid from the ventricles, however, does not reach any considerable spread until after a length of 18 mm. is attained; the periaxial spread during this period of growth is wholly confined to the peribulbar tissues. It is quite important in this connection that the first obvious differentiation of the mesenchyme for the formation of the arachnoid should appear during this period and should involve the peribulbar tissues.

The first change to be noted in the transformation of primitive mesenchyme into the future arachnoid is an obvious thinning of the structure with a decrease in the number of nuclei per unit-volume. This is made out in a photomicrograph (fig. 57) of a section from a human embryo 14 mm.* long, when contrasted with a similar mesenchymal area posterior to the ventricular roof (fig. 53). In the pig embryo this thinning of the mesenchyme is as obvious at this early stage.

The process of dilatation of the mesenchymal spaces at this stage hardly seems to concern a direct disruption of the syncytial strands, but resembles more the spreading of the cell-bodies by the introduction of more fluid into the tissue spaces. This process would certainly result in an appearance similar in every way to that represented by figures 35 and 57. It probably also concerns other factors, as, possibly, the growth of the whole embryo without a corresponding degree of mesenchymal proliferation.

In a human embryo of 17 mm. (No. 576) evidences are apparent of such a thinning of the mesenchyme about the medulla. Thus, in figures 58 and 59, from

* This embryo measured 14 mm. on the slide.

this specimen, the cellular decrease can be made out both in the region of the roof of the fourth ventricle and around the basilar surface of the medulla. It will be noted that the differentiation (*i. e.*, the thinning) about the roof has proceeded more rapidly than along the anterior bulbar surface. This is perhaps to be expected in view of the initial pouring-out of the cerebro-spinal fluid into the mesenchyme just posterior to the roof.

In this mesenchymal differentiation a slightly increased amount of albuminous coagulum may be noticed. The truth of this is made obvious by an examination of figure 61, a photomicrograph from a human embryo of 17 mm. The almost entire freedom of the mesenchyme from albuminous detritus is most noticeable at earlier stages.

As was pointed out in the description of the results of replacing the cerebro-spinal fluid, a marked change in the rate of development of the cerebro-spinal spaces in the pig-embryo ensues just after attaining the length of 18 mm. Within the growth of 2 mm. the injection spreads completely down the spinal cord and about the basilar structures of the cerebral cavity. This rapid extension finds its analogous process in the equally rapid changes which may be traced in the periaxial mesenchyme. Thus, in figure 72, a photomicrograph from a sagittal section of a pig embryo of 18 mm., the whole nervous tissue appears surrounded by a very thin, lightly staining tissue; this is the periaxial mesenchyme, which is undergoing its rapid metamorphosis. It will be noticed in this figure that the posterior structures (rhombencephalon) are surrounded by a much less dense mesenchyme than are the anterior (mesencephalon). This relative differentiation between the bulbar tissue and that around the mid-brain is only of temporal character; the mesenchyme about the medulla, as has already been pointed out, begins to differentiate first, the differentiation of the mesenchyme about the other nervous structures following somewhat later.

Figure 73 is a photomicrograph of higher power, taken from the squared area in figure 72. It shows to what a surprising degree the mesenchymal differentiation has proceeded during a few millimeters' growth. Two striking features of the process are brought out in this reproduction. In the first place, many of the mesenchymal trabeculae have apparently been broken down, sacrificed to a few larger remaining strands. The cells connected with the destroyed trabeculae appear to recede until one of the heavier surviving strands is met with, when they adhere and apparently aid in the future development of a permanent arachnoid trabecula. The second feature of importance in figure 73 concerns the large amount of albumen seen in the periaxial space. There is here a much greater amount of albumen than is ever found in the periaxial mesenchyme before the differentiating process which results in the future subarachnoid space has become definite. The occurrence of this large amount of albuminous coagulum is apparently related directly to the outflow of the embryonic cerebro-spinal fluid, for the embryonic fluid is very rich in protein material, as can be readily seen by the partial filling of the embryonic cerebral ventricles with the clotted albumen.

This process of the breaking-down of the mesenchymal spaces to form fewer and larger spaces goes on very rapidly in pig embryos as they exceed the length of 18 mm. Thus, figure 75 (from a pig embryo of 23 mm.) shows a marked decrease in the mesenchymal elements about the medulla; the strands are becoming fewer in number, and the albumen-filled spaces are increasing rapidly in size, but decreasing in number. About the mesencephalon, however, the process has only just begun (also shown by fig. 74). In this photomicrograph (fig. 75) the mesenchymal elements have broken down somewhat; the spaces are becoming enlarged, and a fine albuminous coagulum fills the interstices between the mesenchymal processes. The whole picture conveys an excellent idea of the forces which convert the many-spaced mesenchyme into the much fewer cerebro-spinal channels.

This general plan of the formation of the larger subarachnoid canals reaches its maximum in the formation of the various cisternæ for cerebro-spinal fluid. The process is probably best illustrated in the case of the cisterna magna, which persists in the posterior cerebello-bulbar angle. Figures 74 and 75, taken from an embryo pig 23 mm. long, give an idea of the initial formation of the cisterna cerebello-medullaris. The mesenchymal strands, as shown in figure 75, are already broken down in part, and are profusely covered with albuminous coagula. The process has not proceeded to any extent in this specimen of 23 mm., but in the course of the next 10 millimeters' growth extensive changes occur, as are shown in figures 76 and 77, photomicrographs from an embryo of 32 mm. In the space outside the inferior membranous area the mesenchymal trabeculae have almost disappeared; the space—or cistern, as it should now properly be called—is almost completely filled with the clotted albumen. The mesenchyme is seen running through this embryonic cistern as a few isolated strands, but most of the tissue appears now as a fairly definite membrane on the outer side of the space. This membrane will go to form the inner surface of the dura and the continuous outer layer of the arachnoidea, as it furnishes a visceral layer for the subdural space.

More laterally in this same specimen the formation of the cistern has progressed to an even greater extent. In figures 78 and 79 the total freedom of the lower portion of the cistern from trabecular strands is seen; above, the mesenchyme still sweeps down as a supporting structure for the chorioid plexus. A definite differential line of mesenchymal condensation indicates the future outer border of the arachnoid as it incloses the cisterna cerebello-medullaris. This general process of mesenchymal breaking-down, altering the original small spaces into the larger arachnoid channels, holds as the embryo develops into larger forms.

In addition to this formation of the subarachnoid spaces in the adult through the enlargement of the embryonic mesenchymal spaces, the perimedullary mesenchyme undergoes in these same localities condensations which result ultimately in the formation of the arachnoid membrane and the trabeculae dividing up the cavity subarachnoideale. Mention has already been made of the adhesion of the cell-bodies of the disrupted mesenchymal elements to the persisting strands—the initial step apparently in the ultimate differentiation of the mesothelial cells which line

these spaces. Gradually with the increasing growth of the embryo these cells seemingly become arranged in definite columns covering the persisting arachnoidal trabeculae. At the same time a differentiation of these primitive mesenchymal elements occurs, the cells ultimately being transformed into the very low cuboidal mesothelium of the subarachnoid spaces. This differentiation begins first in the basilar portions of the cranium and spreads upward, in a way similar to the course of development of the cranium and of the enlargement of the pericerebral spaces.

While such a general process as outlined accounts for the formation of the arachnoidal trabeculae and the subarachnoid spaces, it has but little bearing on the development of the outer intact membrane of the arachnoidea. This portion of the arachnoidea (which might be termed the arachnoid membrane as distinguished from the arachnoid trabeculae) first appears as a distinct line of mesenchymal condensation separating the mesenchyme into the primitive arachnoid and dura mater, as in figures 76 and 77, *dmc*. This rather thin zone of cellular density in reality represents not only the outer surface of the arachnoidea, but also the inner surface of the dura mater. At first these develop in close fusion with a later separation of the two membranes. With this cleavage of the two surfaces, the arachnoid membrane rapidly differentiates, forming an intact layer over the subarachnoid spaces. The cells covering the surface membrane seem to change gradually into the low cuboidal type, similar to those covering the arachnoidal trabeculae. The details of these processes may be most easily studied in the region of the cerebral hemispheres; in this situation the transformation of the tissues occurs at a later period than in the basilar regions, for the differentiation of this mesenchyme follows the general plan of development of the cartilaginous and bony cranium.

The greatest problem in connection with the development of an external arachnoid membrane naturally concerns the separation of this leptomeningeal tissue from the pachymeninx. In the solution of this particular problem gross dissections have been found of benefit. For this purpose, pig embryos of larger size were used, and attempts were made to ascertain at what stage of development a true anatomical separation of the two membranes occurred. It was found that in embryo pigs of about 40 mm. the dura over the calvarium could be well separated from the arachnoid, but areas of unseparated tissue still persisted at this stage. This was also found to be true in pig embryos of 50 mm.; on the inner surface of the dura at this stage a mesothelial cell pattern could be demonstrated, although areas of attachment to the arachnoid existed. However, the differentiation of the periaxial mesenchyme into the adult arachnoid does not occur coincidentally with the possibility of a foreful separation of the dura from the surface of the brain; but before this separation of the pachymeninx can be made the mesenchyme which will go to form the arachnoid must undergo some differentiation. This process involves a condensation or accumulation of mesenchymal elements directly in the secondary dural thickening; the cells, with oval nuclei, soon form a continuous membrane of two or three cells in thickness. Apparently soon after the cellular accumulation has been accomplished, a separation of the dura from the arachnoid may be made. In certain areas, varying

greatly in size, there is still an intimate connection between dura and arachnoid. These connections are particularly prominent over the developing cerebral hemispheres, and it is with this differentiation in the formation of the arachnoid spaces that we will now deal.

In a human fetus of 76 mm. (No. 1134) the arachnoid was found to constitute, in the region about the great sagittal sinus, a cellular layer which adhered quite closely to the dura, even though a line of differentiation between the two meninges could be made out. This adhesion could undoubtedly be separated, even by gross dissection, although the tendency to adhesion was stronger than the attachment of the pia to the cortex. From its cell-character and general histology the arachnoid at this stage must be considered as a formed membrane, but in a primitive state.

A somewhat similar but more advanced stage in the formation of the arachnoid membrane is seen in a human fetus of 100 mm. (No. 928-1) and in a fetal pig of 114 mm. In both the arachnoid membrane is very cellular, adhering to the dura only along the superior longitudinal sinus and in certain isolated areas. The cells comprising the arachnoidea possess oval, rather large nuclei which stain palely with hematoxylin. No typical arachnoidal trabeculae could be made out in specimens in this cortical region.

The cellular character of the arachnoid persists in the larger embryos and fetuses as a layer, several cells in thickness, constituting the outer arachnoid membrane. In a fetal pig 190 mm. in length the membrane was practically differentiated, its outer wall being covered by mesothelial cells with large nuclei lying about a small fibrous-tissue base. The arachnoid trabeculae were developed only in the larger sulci, where they appeared as typical cellular cords about a core of fibrous tissue. At this stage, too, the vessels traversing the arachnoid spaces were found covered with similar cells. These may now be justly termed the mesothelial cells.

Quite similar stages of arachnoidal differentiation occur in human fetuses of 200 (No. 870) and of 240 mm. (No. 1131). The arachnoid has everywhere practically become adult in character, except for a further decrease in the number of the peripheral layers of mesothelial cells. The fibrous tissue underlying this covering membrane possesses, as in the adult, almost a minimum of support.

In certain areas, however, the differentiation of the mesenchyme into the adult arachnoidea does not keep pace with the general process. In the present study this phenomenon of unequal development was especially well shown in fetal pigs of 150 mm. and upwards. It concerns the development of arachnoid trabeculae in the cerebral sulci. As is well known, the arachnoid membrane bridges the cerebral fissures, while the pia follows the cerebral contour. In the fetal pigs of the stages specified above, certain furrows showed a typical adult relationship with the covering arachnoid membrane and lining pia, the intervening space being traversed by definite arachnoid trabeculae. Other of the sulci were filled with an almost embryonic type of mesenchyme—a loose meshwork of cytoplasmic processes containing rather small oval nuclei. The explanation of this embryonic type of tissue seems to be that it occurs in the newly developing sulci and that some time must elapse in this

formation before the tissue fully differentiates into the adult arachnoid membrane. Strangely enough, a similar collection of an embryonic type of tissue is sometimes met with, in these stages, between the two hemispheres.

The general process, then, of the formation of the arachnoidea involves both a breaking-down (or thinning-out) of the mesenchymal spaces and a condensation of the cells. The first of these processes results in the transformation of the interstices of the periaxial mesenchyme into the larger subarachnoid spaces, divided off by arachnoid trabeculae; the second finds its final accomplishment in the development of the outer arachnoid membrane which, covered with mesothelial cells, forms the inner surface of the subdural space. The transformation begins in the basilar regions of the cranium and spreads upward over the hemispheres.

THE CIRCULATION OF FLUID THROUGH THE SUBARACHNOID SPACES.

In view of the processes of differentiation involved in the formation of the arachnoidea and the subarachnoid spaces, the circulation of fluid through this peculiar membrane must be considered. It seems important to ascertain, if possible, the relationships between the beginning of the passage of the cerebro-spinal fluid and the onset of the histological changes.

The conceptions of the development of the circulation of the cerebro-spinal fluid which are presented in this communication are dependent, in large measure, upon the results of the replacement of the fluid, in living embryos, by the ferrocyanide solution. Additional evidence was obtained from the identification of albuminous coagula in the periaxial tissues. The correlation of these findings with the development of the chorioid plexuses and with the results of injections under low pressures, from a syringe and so forth, gave evidence of their correctness.

The differentiation of the mesenchyme into arachnoid membrane may be said to keep pace with the establishment of the periaxial channels for the cerebro-spinal fluid. In the main, the passage of this fluid into the undifferentiated mesenchyme about the nervous system precedes the process of histological change. This phenomenon is shown in figure 14, from a pig embryo of 18 mm. The replaced fluid is seen passing out into the mesenchyme through the two membranous areas in the roof of the fourth ventricle. The mesenchyme at this stage has already differentiated somewhat, but hardly in proportion to the length of time during which the fluid has been passing into the space.

There are several features of interest in the course of the fluid through the periaxial spaces. In sections of embryos in which the cerebro-spinal fluid has been replaced by a foreign solution the granules of the precipitated salts may be identified in the periaxial mesenchyme in situations corresponding exactly to the extent of the spread shown in the cleared specimens (figs. 1 to 9). The exact location of the prussian-blue granules is of importance in this connection, as the exact form and distribution of the periaxial spaces and their relation to the adult subarachnoid spaces may thus be determined.

Examination of serial sections from an embryo in which the embryonic ventricular fluid has been replaced by the ferrocyanide will reveal, if the embryo exceeded

14 mm. in length, granules of prussian-blue in the peribulbar mesenchyme (fig. 14). The granules are not found in any cell-bodies in this tissue; they are made out, in large measure, adhering to the mesenchymal cell-processes or lying free in the mesenchymal interstices. The granules do not penetrate the pia mater or the dura mater, a finding which will be discussed more fully in the sections dealing with these membranes. Everywhere the transit of fluid into the nervous tissue seems to be prohibited by the pia; in some areas, however, the outer condensation of mesenchyme to form the dura-periosteum has not yet occurred. This is shown particularly well in the region of the roof of the fourth ventricle (fig. 18), where the epidermis offers the only barrier to the passage of fluid from the pericerebral spaces.

In the earlier stages in which the phenomenon of fluid passage about the central nervous system may be observed, the outer layer of the arachnoid is not at all differentiated. Here the barrier to the fluid is the blastemal condensation of mesenchyme (fig. 16). In the later stages, when the outer layer of the arachnoid is beginning to appear as a mesenchymal thickening, the fluid (as indicated by the precipitated prussian-blue) is confined strictly within the immature arachnoid membrane.

The course, then, of the fluid which has replaced the cerebro-spinal fluid in the embryo follows that of the adult cerebro-spinal fluid (as shown by the resultant blue granules). It is everywhere contained within spaces which topographically and embryologically correspond to the subarachnoid spaces in the adult. The spread of the replaced solution from the embryonic ventricle into the peribulbar tissue is analogous in every way to the passage of cerebro-spinal fluid from the fourth ventricle of the adult into subarachnoid spaces.

VIII. A CONSIDERATION OF THE EMBRYONIC PIA MATER.

Our present conceptions of the embryology of the pia mater are largely due to the work of His²⁵ and of Kölliker³¹, who first firmly established the idea that this inner leptomeninx was mesodermal in origin. While generally accepted (Farrar¹⁶), this view has not been widely referred to in the literature; but the absence from all embryologies of any information concerning the development of the meninges is quite striking and it does not seem strange, therefore, that our information regarding the pia mater has not advanced in keeping with our knowledge of the embryology of other structures of the body. In the present section of this communication it is purposed to present merely a general consideration of the process by which the pia mater is formed and to point out some of its functional characteristics, especially in regard to the fluid channels.

The term pia mater is accepted throughout this article as designating solely the cellular membrane which adheres closely to the outer surface of the nervous system, but it is in direct connection with the arachnoidal trabeculae which traverse the subarachnoid space. Whether the two membranes should be considered together as the pia-arachnoid or as the leptomeninx is a question in regard to which there is some disagreement; it will suffice to consider the pia as a separate membrane.

THE GENERAL HISTOLOGY OF THE PIA MATER.

The findings in this investigation are wholly in accord with the conclusions of His²⁵, of Kolliker³¹, and of Farrar¹⁶, that the pia mater is derived from the middle germ-layer. In the earliest stages the mesenchymal elements may be made out adhering to the outer portion of the primitive nervous system. In the course of growth these cells are grouped about the mantle zone of the spinal cord in a rather dense layer, two or possibly three cells in thickness, with the typical oval nuclei of the mesenchymal elements. Certain stages of this process may be made out in the figures in this paper. Thus, in a human embryo of 4 mm. (No. 836 of the Carnegie collection) the mesenchymal elements form a definite layer around the neural axis (fig. 41). The nuclei are oval in shape, possessing a moderate amount of chromatin, and are found in a layer two cells in thickness. This membrane, with its fairly scant cytoplasm, is sharply differentiated by its existence between two layers, in one of which nuclei are wanting, and in the other somewhat widely separated—the mantle zone of the spinal cord and the periaxial mesenchyme.

This typical arrangement of the mesenchymal elements about the cerebro-spinal axis holds in almost unchanged form throughout the whole embryonic growth. Thus, about the nervous tissue in figures 48 and 52 (from human embryos of 7 and 9 mm., respectively) the same condensation of the mesenchymal elements to form the pia mater are made out. This appearance is so familiar that further description in the later stages seems needless, but certain characters of this embryonic arrangement seem to require comment.

The general appearance of the pial layer is greatly altered by the early formation of the capillary blood plexus about the nervous system. This plexus tends to render the pial tissue more cellular, on first microscopic examination, as the endothelial channels branch greatly outside of the nervous tissue in this mesenchymal pia. The general character of the pial layer, however, as a membrane with prominent nuclei and scanty protoplasm, is not altered at all by the vascular plexuses.

The ultimate fate of these undifferentiated mesenchymal elements forming this initial pial condensation is a gradual transformation of the cells into very low cuboidal mesothelial elements constituting the adult pia. The transformation concerns not only the differentiation of the cells but also a rearrangement so that the original layer of two or more cells in thickness becomes finally of but a single cell in thickness. The process, in a way similar to the development of the subarachnoid spaces, begins in the basilar portions and spreads upward; the process, hence, may often be studied in a single suitable specimen.

More important, for our consideration, is the peculiar relationship of the pia mater to the roof of the fourth ventricle, and in particular to the two area membranaceae. In this situation, in place of the slight mesenchymal condensation which characterizes the pia, and which Minot⁴⁹ pictures in his figure 114, the mesenchyme seems altered. The condensation to form the pia, which takes place in other situations about the true nervous tissue, has not here occurred. This absence of the typical pial arrangement may be noted even in very small embryos—those in which

the roof of the fourth ventricle is composed of the many-layered, epithelial-like cells. This is well shown in a photomicrograph (fig. 53) from an injected human embryo of 9 mm. (No. 721) of the Carnegie collection. Likewise, in this region in a pig embryo of 8 mm. (fig. 25), the same absence of a real pial condensation may be made out. But this peculiarity of the pia is most striking at the period of maximal differentiation of the superior membranous area in the rhombic roof. In figures 37 and 43, photomicrographs from pig embryos of this stage, the mesenchymal condensation, augmented by some vascular endothelium, is shown in adhesion to the ependyma on both sides of the membranous area; but directly behind the differentiated cells of the area membranacea evidence of a condensation of mesenchyme is wholly lacking, even though both specimens show vascular channels in close approximation. Similarly, in a human embryo of 14 mm. (No. 144, Carnegie collection) a total lack of the true pial thickening is to be observed (fig. 57).

Quite similar is the failure of a pial thickening about the inferior membranous area. This can be made out in figures 83 and 87, from human embryos in which the process of differentiation of the area is proceeding. In later stages of the formation of the area membranacea inferior, the marked absence of a true pial condensation in the mesenchyme in this region is noted in figure 75 (a specimen from a fetal pig of 23 mm.). But this apparent failure to form the typical mesenchymal condensation of the pia mater in certain areas in the roof of the fourth ventricle must not be construed as indicating an absence of pia mater. Such does not seem to be the case here, for in the later stages of the formation of the cisterna cerebello-medullaris the area membranacea inferior is found entirely unsupported, except for a layer of mesenchymal cells. This is shown in figures 77 and 79, both taken from fetal pigs of 32 mm. This mesenchymal layer must be considered as pia mater apparently modified for a specific purpose.

The general process, then, of formation of the pia mater concerns a condensation of mesenchymal elements to form an embryonic membrane about the central nervous system. From its earliest beginning very slight modification is needed to reduce it finally to the histological character of the adult membrane. The general process holds, except in the regions of the area membranacea in the roof of the fourth ventricle; here, apparently, a modification of the pia for a specific purpose, involving an absence of the primary pial condensation, takes place.

THE RELATION OF THE PIA MATER TO THE FLUID CHANNELS.

The cerebro-spinal fluid in its normal pathways comes everywhere into contact with the pia mater, which serves as the inner retainer for the subarachnoid space; therefore the functional relation of this membrane to the fluid which bathes it becomes of interest. To some degree the results of the experiments recorded in the earlier portions of this paper throw light upon the relation of the pia mater to the circulating fluid. The most important question in this connection is naturally that dealing with the possible penetration of the normal fluid through this embryonic membrane. In this regard the findings in replacement experiments with ferrocyanide solution serve to elucidate the problem. These observations give no evidence

of any penetration of the pia mater by the fluid. This is well brought out in figures 14 and 18. In every respect (as demonstrated by numerous experiments of this type in pig embryos of varying lengths) the pia mater is wholly impenetrable to true solutions of foreign salts when injected so that the normal tension is not altered. The whole subarachnoid space may, in such an experiment, be filled with the prussian-blue, but none of these granules are found within the cells of the pia mater or in any layer between these cells and the nervous system. Evidence that the fluid has bathed the outer pial cells is afforded by the adhesion of granules of prussian-blue to the outer cytoplasmic borders of the cells.

Likewise the cells comprising the embryonic pia have been found to be impenetrable to true solutions (ferrocyanide) when injected under varying pressures from a syringe. In these cases, rupture of the roof of the fourth ventricle or of the infundibulum may be produced by great pressure, without causing any of the fluid to penetrate the intact layer of the pia mater. The same result is obtained when india ink is substituted for the true solution.

The pia mater, then, even in its embryonic form, serves as an efficient fluid-barrier. This is demonstrated, in regard to the adult pia mater, in the report⁽⁵⁵⁾ of the observations made on adult cats, dogs, and monkeys. But the barrier which the pia offers to the entrance of fluid from without exists also for fluid coming in the reverse direction. This is shown by the well-known phenomenon of the so-called subpial extravasation, which occurs in blood vascular injections when the injections are continued for too long a time at too high a pressure. The perforating vessels in such cases rupture as they enter the nervous system, and the injection mass spreads extensively beneath the pia, stripping it away from the nervous tissue. Of interest in this discussion is the fact that the injection mass in these extravasations does not rupture the pia, which seemingly is an equally efficient fluid barrier to pressure exerted on it from within. Similar subpial spreads of the injection fluids have been observed in the course of this work. These extravasations resulted from the rupture of the whole nervous tissue from within, particularly in the region of the infundibulum, when the injection was made into the ventricular system under excessive pressure. In this respect, too, the pia seems to be wholly efficient as a retainer for true solutions or for granular suspensions. It is realized that the embryonic pia mater will not resist the passage of fluids through it under the highest pressures afforded by the syringe, but the membrane serves as an efficient barrier for all pressures such as are employed in careful anatomic injections.

With this conception of the impenetrability of the pia mater to fluids under ordinary pressures, it does not seem strange that there is a variation in the process of formation of the pia mater in the region of the roof of the fourth ventricle. It has been shown in the foregoing paragraphs that the phenomenon of mesenchymal condensation which results in the formation of pia elsewhere does not occur in the region of the two area membranacea. In view of the passage of cerebro-spinal fluid through these two membranous areas, the pia mater must necessarily be altered in these places. For were it not adapted to the purpose of affording fluid passage

the cerebro-spinal fluid would, in its course from the ventricle to the subarachnoid space, form a subpial extravasation. It would seem that this modification of the pia is designed to meet the particular need and function of this region.

THE ADHESION OF THE PIA MATER TO THE CEREBRAL TISSUE.

It is a well-known fact in embryology that the pia mater and the periaxial mesenchyme in poorly dehydrated specimens split away from nervous tissue, but in adult preparations, if the meninges and brain are dehydrated in a block, the separation of the tissues occurs between the dura and the arachnoid, or (in more exceptional instances) the dura and arachnoid come away, leaving the pial layer closely applied to the cortical tissue. It is quite difficult in any adult mammal to separate the pia from the brain tissue. Realization of these peculiarities in the degree of adhesion of the pia led to an attempt to ascertain what structures were involved in the attachment of this mesodermal layer to the epidermal nervous system. The results of this attempt add nothing to the ultimate solution of the problem, but are perhaps of sufficient interest to justify brief presentation.

Two theories in explanation of this adhesion of the pia immediately suggested themselves. One of these concerned a possible growth into the pia of neuroglial elements, causing an intimate association between the pia and the cerebral cortex. Our findings in reference to the neuroglial outgrowth in fetal pigs gave no reliable basis for the assumption. The second theory dealt with a diminution in the elasticity of the walls of the perforating blood-vessels which supply the nervous system. The early embryonic vessels, with walls composed solely of endothelium, when subjected to the distortions of poor dehydration, might possibly offer less resistance to the separation, so that the pia would come away from the nervous tissue. In the later stages, however, the thicker-walled perforating vessels would naturally oppose such a cleavage, so that the pia would remain adhering to the cortical tissue. This theory is also purely an hypothesis, although it does not seem unlikely, especially if one takes into account a possible connection of the pia with the perivascular system. In examining blocks of the meninges and brain tissue taken together it was found that the pia mater separated cleanly from the nervous tissue in fetal pigs 15 cm. in length. Beyond this stage the arachnoid might remain in adhesion to the dura, but in such cases there was always found a layer of cells on the outer side of the cortical tissue, constituting a true pia mater.

IX. THE DEVELOPMENT OF THE CRANIAL DURA MATER.

The dura mater, like the two other meninges, is derived from the mesenchyme about the central nervous system. The researches of Sterzi²³ on the comparative anatomy of the meninges furnish additional evidence for this conception in the higher mammals. The origin of the pachymeninx from the middle germ-layer is now well established. But there is lacking in the literature a comprehensive account of the formation of this fibrous envelope. The gross generalities of the process are given in part, but there is an almost total absence of the more intimate

details of the transformation. One of the most essential points in the process concerns the relationship of the dura to the bony coverings of the cerebro-spinal axis. Does the adult dura serve as the periosteum of the bony skull? In the standard text-books of anatomy the adult human dura is described as being composed of two layers. In the skull these layers split, to comprise the walls of the great venous sinuses. The outer layer of the dura serves as the periosteum for the bony skull, but below the foramen magnum the two layers separate to inclose the epidural space. The outer dural layer in this spinal region adheres to the inner surface of the bony vertebral column, where it functions as the periosteum; the inner layer here becomes the spinal dura.

In this account of the adult dura mater there is indicated a very suggestive periosteal relationship which implies an embryological basis for the disposition of the two layers of the membrane. It must be granted, however, that this division of the cranial pachymeninx into two layers is quite arbitrary; there is nothing in the general histology of the fibrous covering to suggest such a halving except its division about the sinuses and its spinal relationships.

THE GENERAL PROCESS OF THE FORMATION OF CRANIAL DURA.

The first evidence of the development of the pachymeninx is found in the basilar region of the skull, where the mesenchyme thickens, to form eventually the bony covering of the brain. This thickening of the mesenchymal elements results not only in the formation of the chondro-anium, but also in the final formation of the bony skull and possibly its internal periosteum and dura. In the process of differentiation the condensation of mesenchyme in the early stages gives no index of the varied character of the resultant tissues, so that, in the first place, the study of the process was necessarily related to the more adult stages. In this paper, however, the whole history of the dura will be detailed chronologically, beginning with the earliest stages.

Bardeen⁽²⁾ has given data on the first appearance of the mesenchymal condensations which go to form the blastemal phenomena in both the cranial and spinal regions. The blastemal vertebrae become fairly well differentiated in human embryos during the first month of intra-uterine growth. At the end of the first month, in the occipital region, three fairly well-marked occipital myotomes may be made out; these afterwards disappear. "During the early part of the second month the membranous anlage of the skull becomes extensively developed. The roof of the cranial cavity is formed by a dense membranous layer, which first becomes marked at the side of the head in embryos from 9 to 11 millimeters in length" (Bardeen).

These evidences of a primary mesenchymal condensation about the central nervous system are concerned in the problem of the differentiation of the dura only in so far as they indicate the onset of the process which will give rise to the bone and possibly the periosteum - a part of the dura about the cerebro-spinal axis. Gaupp⁽¹⁹⁾ has already pointed out that this cranial blastemal condensation gives rise to these adjacent but wholly different structures. These cranial mesenchymal condensa-

tions persist in simple form until after the cerebro-spinal fluid begins to fill its extra-ventricular bed; then, within a short time, the tissue becomes transformed by the development within it of cartilage, so that in the human embryo the caudal half of the chondro-eranium forms a ring of cartilage about the posterior portion of the brain. On the inner side of this ring of cartilage the mesenchyme later shows a marked condensation in the midst of the rarefied perimedullary tissues. In this layer the nuclei soon become fewer in number and the cytoplasmic structures fibrillar, the whole resulting ultimately in the formation of the fibrous adult dura. The mesenchymal condensations in the regions of the skull, where membranous bone formation holds, go directly into a membrane of fibrous tissue, in the outer portions of which bone is laid down. The details of these processes will now be taken up.

In figures 30 and 32, photomicrographs from pig embryos of 13 and 14 mm., respectively, the well-established vertebral differentiations and the now poorly differentiated base of the skull are shown. From this stage upward the mesenchymal condensation in the head region proceeds rapidly. Thus at a stage of 17 mm. in the human embryo (fig. 60) the ventral portion of the vertebral canal has become cartilaginous, while the base of the skull has also undergone the chondrogenous transformation in its more posterior portions. But of especial interest in our problem is the line of mesenchymal condensation, which may now be traced wholly around the brain-stem and hemispheres (fig. 60). The nature of this condensation is well shown in figure 61, an enlargement of the squared area of figure 60. The mesenchymal nuclei have become closely packed and rather sharply differentiated from the looser mesenchyme which in part goes to form the arachnoidea. Figure 59 similarly shows this condensation proceeding upward to the vault.

Examined in another plane, the process of mesenchymal condensation seems to proceed much more rapidly in the posterior than in the anterior region. This is brought out in a transverse section of a human embryo of 18 mm. (fig. 62). Here the condensation is much more extreme about the medulla and roof of the fourth ventricle than in the more anterior parts of the mesencephalon. The same general appearance, typical of this stage, may be made out in figures 56 and 57 from a human embryo of 14 mm.* (No. 144, Carnegie collection). In the slightly larger stages the process of mesenchymal condensation about the nervous system becomes rapidly more marked. This increase in the number of cells comprising the denser membrane is shown in figures 64 and 65, photomicrographs of embryo No. 460 (21 mm.).

The degree of condensation of the mesenchyme in the various stages of the human embryo is followed quite closely in the pig embryo. The comparative degree of differentiation coincides within a millimeter or two. Thus, in a section from a pig embryo of 19 mm. (fig. 38), the degree of condensation about the roof of the fourth ventricle is practically similar to that in human embryos of the same length.

The phenomena just commented upon represent the stages concerned in the formation merely of a cranial blastema and are related to the formation of the dura only so far as it is out of this mesenchymal condensation that the periosteal portion of the

*Measured on slide after sectioning.

pachymeninx may be derived. The degree of condensation referred to in the figures has been solely of the blastemal type, but in some of the specimens this simple condensation is seen only in the more cephalic portions of the cranium. Thus, in the figures (64 and 65) taken from embryo 460, the mesenchymal condensation is still of the simple undifferentiated type, whereas in this same embryo the more caudal sections show a chondro-eranium which is well developed. The process of formation of the cranial dura, then, is one which begins in the basilar portions of the cranium and proceeds from these points into the region of the calvarium. In general, all of the phases of this transformation into dura may be found in one specimen of sufficient and suitable size, the basilar differentiation representing the advanced stages, while the steps in the differentiation are found in the areas nearer the vertex.

It is quite difficult to decide exactly what importance the primary condensation of mesenchyme maintains in the formation of the dura, because, coincident with the chondrification of the blastema, there occurs another condensation which forms the line of division between the inner surface of the dura mater and the outer arachnoid membrane. The first evidence of this secondary perimedullary condensation is found in pig embryos of about 17 mm. In these specimens, in the narrow space formed by the mesencephalic flexure, mesenchymal cells collect together in the form of a fairly definite membrane. After its primary beginning in this area, the narrow line of its thickening may be traced to the basis cranii in embryos a little larger. In slightly older stages this secondary line of condensation is found to be fairly extensive throughout the area between the middle and posterior cranial chambers.

At a stage of 20 to 21 mm. the whole basilar portion of the cranium shows evidence of this secondary line of condensation lying between the pia mater and the cartilaginous skull. The condensation occurs in the outer portions of the loose tissue which, as shown in a foregoing section (No. VII) becomes the subarachnoid trabecule. The line of condensation is not broad on section; it comprises a cell-layer from three to six cells in thickness. Between this cellular border and the cartilaginous skull the tissue rapidly differentiates (a process seemingly synchronous with the development of this membrane). This tissue, which maintains dural relationships, is far more cellular and compact than the original perimedullary mesenchyme. Even without the rather dense line of division in the mesenchymal tissue, the dural structure can be easily outlined by its characteristic appearance.

The original dural condensation between the two wings of nervous tissue which unite in the mesencephalic flexure can be traced in slightly later stages around into the tentorium cerebelli. This structure develops as a wholly similar mesenchymal thickening in the midst of the perimedullary mesenchyme. The tentorium consists in these embryos of 20 to 25 mm. of two thin lateral plates which widen at their cranial attachments into prismatic areas. These areas, which finally lodge, in the two layers of dura, the sinus transversus, are characterized by the same dense type of mesenchyme. The peripheral edges of the prismatic portion of the tentorium spreads caudalwards as a definite line of condensation. In the earlier stages this line becomes indefinite as it extends from its tentorial attachment, but finally a similar

line of condensation about the whole posterior chamber may be made out. This lies within the area of the cartilaginous skull and bounds the subarachnoid spaces.

This same process of formation of dura holds for the formation of the basilar dura in the more anterior portions of the cranium. The appearance of the secondary zone, narrow and rather dense, may be made out inclosing the more cellular mesenchyme which extends to the cartilaginous skull. The same process also endures for the formation of the dura of the calvarium, but here the addition of tissue from the undifferentiated mesenchyme is undoubtedly very small in amount. This will be discussed in a later paragraph. The various stages in the formation of this secondary condensation which goes to form the major portion of the dura may be fairly well studied in any one embryo of suitable stage, because the process, as pointed out above, begins in the basilar portion of the cranium and extends upward. Likewise, the condensations directly beneath the region of the dorsal membrane are delayed as compared to those of the lateral regions.

Some of the phenomena shown in the formation of the dura mater are illustrated in figures 46, 76, 77, 78, 79, and 94. Throughout these figures the letters *dmc* refer to the line of the secondary mesenchymal condensation, which borders internally the dura and which goes to form the outer membrane of the arachnoidea.

In figure 46, a photomicrograph of a pig embryo of 32 mm., the dura mater (*dmc*) is shown as a somewhat condensed tissue separated a slight distance from the chondro-cranium. On the basilar surface, the inner line of dural tissue is quite remote from the inner surface of the basioccipital. Tracing this line of condensation forward, it is soon seen to merge more closely with the perichondrium* of the basioccipital. More anteriorly it again leaves the occipital plate and after a brief interval it fuses with the temporal perichondrium. Continuing slightly more anteriorly the dural process toward the mesencephalic angle may be made out; this appears as a doubled membrane at its basal attachment. In its further prolongations the dural surface is at times a distinct structure; at other times it is completely fused with the perichondrium.

Posteriorly, in this figure 46, the line of dural condensation (incorporated also with the outer arachnoidea) may be traced upward around the cisterna cerebellomedullaris. This line of condensation is seen to lose its definitive character as it curves inward toward the chorioid plexus of the fourth ventricle—a phenomenon shown particularly well in figures 77 and 79, taken from the same pig embryo of 32 mm. The dura in this termination may be said to be in its formative stage; but dorsally, over the mesencephalon, the inner surface of the dura again becomes a definite membrane, as shown in figures 76 and 78. In the latter figure it is shown inclosing a wide mesh of dural vessels, between the arachnoidal surface and the membranous skull. Anteriorly, again, it seems to lose its definite line of condensation.

*The term "perichondrium" is used throughout this paper to designate only the very dense cellular line delimiting the edge of the cartilage. This dense zone is composed of the nuclei of the cartilage, crowded together, and represents probably some phenomenon of the growth or resorption of the cartilage. In a much broader sense, the whole dural tissue, lying between the line of secondary condensation and the cartilaginous border, could be termed "perichondrium," as it probably represents the sole internal membrane which could be stripped from the cartilage.

Quite similar pictures are obtained regarding the dura mater in the human embryo. The relationships of the dura to the cisterna cerebello-medullaris are shown in figure 94, a photomicrograph of a human fetus of 35 mm. (No. 199 in the collection of the Carnegie Institution). In this reproduction the line of secondary mesenchymal condensation (representing the outer membrane of the arachnoidea and the inner surface of the dura) becomes widely separated from the occipitale superius in its superior portion.

In a fetal pig of 8 cm. the same general arrangements of the dura mater could be made out. The inner surface of the dura was in places still fused with the outer arachnoid membrane, but in other places the areas of attachment were lacking, so that a true separation of arachnoid from dura had taken place. Along the peripheral points of the tentorium the dura and arachnoid were still closely applied to each other. The dura itself was of the same cellular, rather loose tissue, with a dense inner surface. In places, as described in the younger stages, the dural tissue was incorporated with the definitive perichondrium over certain cartilages or even over parts of the same structure. In other places a definitive perichondrium may be wholly lacking; in these areas the indefinite cartilaginous border gradually merges into the dura. In still other situations an intermediate arrangement of dura and perichondrium exists, where the cartilage is bounded by a somewhat condensed but not fully developed perichondrium which is continuous with the dura. Everywhere in the membranous sutures between the cranial cartilages or bones, the dura bridges the gap as a loose, cellular tissue. Over the calvarium the dura appears solely as a dense, rather fibrous membrane which is incorporated with and serves as the inner periosteum. This dura over the hemispheres is continuous with the fibrous sutures of the cranial vault.

The findings in a fetal pig of 98 mm. were not dissimilar to those just recorded. The dura was everywhere quite well developed, a rather loose cellular tissue except over the hemispheres, where it showed a more fibrous character. In the region of the occipito-atlantoid ligament the dura was fused with the ligamentous tissue, while above (over the occipitale superius) the dura became a distinct, thick cellular layer. The structure of the tentorium was wholly similar to the occipital dura. In the basis cranii there are areas in which the dura is wholly fused with the periosteum or perichondrium; in other areas it bridges the sutures or exists as a definite membrane on the inner surface of a definite perichondrium.

The dura mater in a fetal pig of 15 cm. did not vary greatly from those larger stages already described. The tissue, however, had become somewhat more fibrous. The prismatic attachment of the tentorium was no longer as large proportionately, but the dura lining the occipitale superius remained a thick bulbous swelling on the dorsal surface. But most striking of all the features in the specimen was the very dense fusion of the dura of the calvarium with the fibrous sutures of the cranium. No line of demarcation between dura and fibrous suture could be made out; the two fibrous layers are anatomically one structure.

The falx cerebri forms in the pig and human embryo by a process similar to that of the inner portion of the dura mater. In the sulcus between the two cerebral hemispheres the mesenchyme remains undifferentiated until quite late; then there appears in the posterior portion a narrow zone of condensation which soon presents two lateral surfaces separated by a layer of rather loose cellular tissue, similar in all regards to the dural tissue already described. This zone of condensation spreads forward to comprise the whole falx. The double surfaces of this membrane finally separate into two parts, forming the outer surface of the arachnoidea and the inner surface of the falx. At the cranial attachment of the falx, the loose tissue forms a prismatic base, containing the superior sagittal sinus and spreading laterally over the denser dura of the calvarium. The whole appearance of this region, which will again be referred to, is that the falx has been added onto the dura of the vertex. Its time of initial appearance is later than that of the rest of the cranial dura and there is apparently no additional acceleration of development. Hence the dural tissue in the falx cerebri presents, in appropriate stages, a more immature type of differentiation than does the adjoining dura.

The process of the formation of the dura is not wholly a simple one due to the relation of the adult dura to, or its function as, the inner periosteum of the skull. In the figures already referred to, the almost complete fusion in some areas of the inner line of dural condensation with the perichondrium has been commented upon. In other situations definite separations of the inner dural surface from the perichondrium occurred; in still other regions no perichondrium could be made out as a definite membrane. These differences in relationships of the dural tissue to the line of the perichondrium can not at present be wholly explained, but some indication of the meaning of the process can be given.

Out of the original cranial blastema, as described by Gaupp¹⁹, there develops the cartilaginous and bony skull, the periosteum, and the dura. But the observations recorded above indicate that by far the major portion of the dura is formed by a secondary mesenchymal condensation, which was indicated by a thin zone of more condensed cells on its inner border. This inner zone ultimately separated to form the inner surface of the dura and the outer membrane of the arachnoidea. The tissue included between this inner line of condensation and the cranial wall gradually differentiated into a more condensed but still a loose cellular tissue and finally became a fibrous dura.

In all cases the dural tissue extends from the inner line of condensation to the cranial blastema, to the perichondrium, or to the cartilage of the skull. The presence of a definitive perichondrium can not at present be explained, but apparently the perichondrium is manufactured by the cells of the original cranial blastema and not by the dural tissue which lies in approximation to it. When a definite perichondrium is found, it seems quite uninfluenced by the dura; at other times a fusion of an indefinite perichondrium with the dura seems to occur. The fusion of the perichondrium with the dural tissue derived from the secondary mesenchymal condensation may occur, so that the small outer portion of the dura may be derived

from this layer. The findings, however, in this investigation, are against any addition of perichondrium to the dural tissue; histologically, a definitive perichondrium is a membrane entirely apart from the dural condensation.

Over the cerebral hemispheres the dura of the cranial vault offers more difficulties of study than does that of the basilar regions. With the formation of a blastemal condensation over the whole vertex—an extension of the dorsal membrane to form the membranous skull—there occurs very quickly a condensation to form the dura. This condensation may be first detected as a continuation anteriorly of the leaflet of the tentorium cerebelli, which stretches forward from the prismatic zone of the tentorial attachment. This zone of condensation is wholly similar to the narrow line of the mesenchymal thickening which was found in the more basilar regions of the skull. This zone of condensation occurs just within the cranial blastema and may be traced upward over the mesencephalon and laterally around the rapidly enlarging hemispheres. As the distance from the cerebellar attachment increases, the zone tends to approach the blastema, except in those regions in which the precursors of the dural veins occur. In such a situation this inner dural zone swings inward to encompass the vessels. Between this inner line of the dura (representing also the outer surface of the arachnoid) the same rather loose cellular tissue exists.

From the falx cerebri a zone of dural condensation in the mesenchyme spreads laterally also; this gradually may be traced anteriorly and laterally until fusion with the similar lines of condensation from the basis cranii and the prismatic zone of the tentorium are reached. The condensation connected with the falx cerebri, however, is not an extensive process, the greater part of the hemispheres being covered by the development from the basis cranii and from the tentorium. It must be understood, however, that there is no active migration of this line of condensation, for the whole process is a development *in situ*. The appearance of an active extension is derived solely from the study of various stages and the increased area of condensation appears as an increment which has developed at the terminal points of the previous condensation.

The amount of dural tissue delimited in the mesenchyme by the secondary zone of condensation is not great in the region of the vertex. It is a thin layer which fuses to the inner surface of the cranial blastema. At the stage of this fusion the blastema has become somewhat fibrous and it constitutes the membranous skull. In this fibrous tissue (the union of the blastema and the dura) bone is deposited, but only in the outer layers. The phenomenon is easily studied in any suitable stage, for the sutures between the flat cranial bones remain incorporated with the inner membrane—the dura which includes the periosteum. Hence, over the cranial vault, the dura and periosteum become incorporated as a single membrane; this serves as the membranous skull, into the outer layer of which bone is deposited.

In the basis cranii, as soon as ossification of the cartilaginous skull takes place, the dura becomes incorporated as the periosteum in a manner similar to that which takes place in the cranial vault. While no definite relationship of dura to the peri-

chondrium could be made out in the earlier stages, the later function of the dura as the inner cranial periosteum is quite obvious. Thus the adult relationships of the dura are obtained. But it is quite difficult to decide to what extent the dura (or internal cranial periosteum) is derived from the primary cranial blastema. It seems probable that this blastemal condensation, in its final resolution into bone, may contribute, in the form of a periosteal element, somewhat to the formation of the dura. Such an addition is very difficult of verification; certainly the greater part of the dura is derived by the secondary condensation from the perimedullary mesenchyme.

Before giving details of the fibrosis of the dura, it may perhaps be interesting to point out a peculiarity of the primary cranial blastema, which does not seem to be connected directly with the formation of the dura. This concerns the tendency of the membranous skull to form more than one layer in its original zone of condensation. In certain areas, as in figure 64, from a human embryo of 21 mm., the dorsal membrane is shown split into two layers. Somewhat similar to this is the occurrence of two zones in the cranial blastema of a pig embryo of 23 mm. (figs. 22 and 101). In this latter figure a less cellular outer layer and a more cellular inner layer are seen. Neither of these have particular significance in the formation of the meninges, although the inner layer in early stages actively functions as a fluid retainer.

The question of the development of fibrous tissue in the dura mater in the course of its development requires consideration here. This phase of the problem concerning the formation of the pachymeninx has been followed, in this study, in the dura of the vertex about the sinus sagittalis superior. The tissue was removed in blocks, including the meninges and cortex cerebri, and was then sectioned in the coronal plane. For the most part the deposition of fibrous tissue was studied in sections stained with hematoxylin and eosin; the findings were controlled by treating other sections from the same blocks with Mallory's connective-tissue stain. In this way the general histogenesis of the dural tissue could be well investigated.

Sections from such a block from a human fetus of 76 mm. (No. 1134, Carnegie collection) showed the dura to be composed of fibrous tissue everywhere except in the region of the great sagittal sinus. About this sinus an immature, almost embryonic, type of loose myxomatous tissue was observed. The fibrous tissue comprising the dura elsewhere is of a quite cellular, somewhat immature type of white connective tissue, with a considerable number of true fibrils. A wholly similar picture is found in a section, stained by Mallory's method, of a block from a fetal pig of 80 mm. (fig. 104). Unfortunately the cellular character of the fibrous dura is not brought out, but the photomicrograph shows well the avoidance of the lateral walls of the sinus by the process of fibrosis. The more embryonic type of tissue in the region between the hemispheres is also well presented.

The dura mater of a human fetus of 100 mm. (No. 928-E, Carnegie collection) possesses fewer nuclei in a given area than does the dura from the specimen of 76 mm. (No. 1134). The tissue is fibrous, except in the immediate region of the sinus sagittalis superior; but interspersed among the connective-tissue fibrils are many

stellate or spindle-like nuclei, greatly exceeding in number the nuclei found in the dense dura of the adult. Bone is being laid down in the outer portion of this dura where it merges with the membranous skull. The lateral walls of the great sinus are still free from fibrillar depositions. A somewhat analogous picture is afforded by a photomicrograph of a specimen stained after Mallory's method, from a fetal pig of the same length (fig. 105). In this specimen the outer portion of the dura, incorporated into a part of the membranous skull, is quite dense with the fibrous tissue; about the superior sinus, however, the decrease in the amount of fibrous tissue is very striking. The falx is beginning to exhibit a fair degree of fibrillar structure; it forms a definite division between the two hemispheres.

In the larger fetuses, above 100 mm. in length, the process of formation in the dura of denser and denser connective tissue proceeds rather slowly. It is realized, however, that this fibrous transformation in fetuses of 10 cm. is very extensive, the region about the sinuses alone remaining comparatively free from the development of the fibrils. The chief difference between the dura of this stage and the dura of the adult is a greater number of cell-nuclei in the fetal membrane. It is well, then, to consider the cellular character of the fibrous membrane and the region about the sinuses in the larger stages.

In a human fetus of 125 mm. (No. 900-H) the dura is quite fibrous, but still contains an increased number of the stellate and spindle forms of nuclei; likewise, about the superior sinus the tissue is an immature myxomatous structure, fairly free from connective-tissue fibrils. This increased number of nuclei in the dural tissue holds also for human fetuses of 165 mm. (as in No. 745), but seems slightly decreased as compared with the smaller specimens. The lateral wall of the great sagittal sinus in this stage possesses distinct bands of white fibrils, but the tissue is much looser and more cellular than the fibrous dura over the hemispheres. These phenomena may be made out in similar stages of the fetal pig, as shown in figure 106, a photomicrograph from a specimen of 17 cm. In this specimen, treated by Mallory's stain, the superior longitudinal sinus is shown surrounded by a clear zone in which the deeply staining fibrils are comparatively few in number. On each lateral wall of the venous channels distinct fibrous bands may be made out, lying in the looser, more immature tissue. The lower portion of the falx has assumed quite an adult character.

Gradually the conversion of the tissue about the cerebral sinuses into the adult structure progresses. Thus, in both human and pig fetuses of 20 cm. length, the dura mater has acquired practically all of its adult features. Everywhere over the cerebral cortex the dura is characterized by dense layers of interlacing strands of white fibrous tissue, but the number of nuclei in these bundles may still be slightly greater than in the adult structure. In the more posterior regions, at this stage of 20 cm., the lateral walls of the sinus sagittalis superior are found to be completely occupied by the white fibrous tissue; in the anterior portion of the sinus much thinner tissue, resembling myxomatous structure, appears, as shown in figure 107. But in this specimen the invasion of the area about the great venous channel by

fibrils has begun; isolated bundles may be made out everywhere in the lateral walls of the sinus. This freedom from connective-tissue formation does not persist, however, and the area is gradually invaded by the continued growth of the fibrils. The avoidance of the region about the sinuses by the connective-tissue resolution will be further commented on in the following subdivision of this paper.

The dura, then, develops probably first in connection with the mesenchymal condensation which ultimately forms the bony skull and a portion of the dura (the cranial periosteum). It first becomes apparent, as a structural unit, as a more cellular layer differentiated, by a secondary condensation, out of the periaxial mesenchyme. As the chondrogenous stage is approached it becomes differentiated as a distinct layer, maintaining varying relationships with the inner perichondrium of certain of the cranial bones. At a stage of 40 mm. in the fetal pig, the dura of the vertex may be dissected out as a distinct, somewhat fibrous layer. The process of fibrous-tissue transformation, however, is slow; the dura until late in fetal life shows an increased number of nuclei, as does any young connective tissue. The invasion of the region about the superior longitudinal sinus by connective-tissue fibrils is much more tardy than is the transformation over the hemispheres.

THE SUBDURAL SPACE AND THE MESOTHELIAL LINING OF THE DURA.

The subdural space (*cavum subdurale*) has been the subject of controversy in regard to its rôle in the pathway of the cerebro-spinal fluid. Before the work of Key and Retzius²⁹ the view was held that the cerebro-spinal fluid occupied the subarachnoid space in the spinal cord, but that in the cranium the subdural space afforded an analogous pathway. This conception was largely due to the fact that, in dissection on fresh material, the dura and arachnoid in the spinal region are found to be in approximation; in the cranium the greater adhesion, by trabeculae, of the arachnoidea to the pia renders the freeing of the dura from the leptomeninges the simplest line of cleavage. This view was entirely disproved by the beautiful injections of Key and Retzius, who demonstrated the anatomical and physiological continuity of the subarachnoid spaces.

With the introduction of this latter view by Key and Retzius the conception of the subdural space naturally changed. These Swedish investigators demonstrated a typical mesothelial cell-lining on the inner surface of the dura, as shown by the method of silver reductions. Without an intimate connection with the true cerebro-spinal fluid, the subdural space has come to be looked upon as somewhat analogous to the serous cavities of the body. Quinke⁴⁶, after a subdural injection of cinnabar granules, ascertained that communications existed between the subdural and subarachnoid spaces, but only in the direction from subdural to subarachnoid. Leonard Hill²⁴, from the results of physiological experiments, assumed that fluid passed from the subdural to the subarachnoid space, and in the reverse direction, with great ease. The more recent investigations, however, lend evidence to the view that in the normal animal with undisturbed intracranial pressure relations the two spaces are physiologically as well as anatomically separate. The current

impression that the subdural space is in many respects a serous cavity will probably finally have greatest support; intimate connections with the lymphatic system are, however, entirely lacking in the dura.

The development of the subdural space must necessarily follow the development of the dura. It has been mentioned that in fetal pigs of 50 mm. the dura can be freed from the arachnoid by gross dissection, but that at this stage many areas of adhesion between the two membranes exist. Such an observation has considerable bearing on the subdural space. For in the development of this space two processes must proceed far enough to permit the separation of the dura and arachnoidea by the capillary layer of fluid. The first of these processes, in order of probable importance, concerns the condensation of mesenchymal cells to form the outer membrane of the arachnoidea. This thickening and resolution into a true membrane takes place in close approximation to the inner surface of the dura. The second factor concerns the covering of this inner surface of the dura with mesothelial cells.

The lining of the subdural space by mesothelial cells can be readily demonstrated on the inner surface of the dura by silver reductions, but the outer membrane of the arachnoid does not permit of a similar technique. This technical failure in regard to the outer arachnoid surface is probably to be accounted for by the dissimilarity in cell-structure in the two situations. Similar difficulties have been encountered by other observers.

In order, then, to ascertain, if possible, at what stage a really adult subdural space could be demonstrated, the inner surface of the dura from fetal pigs of various lengths was subjected to treatment with silver nitrate. After the reduction had taken place to a sufficient degree, the whole dura was washed with distilled water, stained with hematoxylin, and cleared in glycerin. The pictures afforded by this method were quite satisfactory, and the technical procedure was so simple and reliable that considerable faith could be placed in the absence of the intercellular reduction lines.

The smallest fetal pig in which a typical mesothelial cell-pattern could be demonstrated on the inner surface of the cranial dura was one of 50 mm. In this specimen the inner surface of the dura was not uniformly covered with the mesothelial cells; certain ragged areas seemed to represent the points of adhesion of the arachnoid to the dura. Figure 108 is a reproduction of a drawing made from one of the areas in this specimen where a mesothelial cell pattern could be seen. The drawing shows many of the characteristics of mesothelial cell-patterns of other parts of the body. The irregularities in the cell-borders, the frequent accumulations of the reduced silver in the cellular angles, and the general cellular pattern are quite typical; but the variation in the size of the cells, as shown in figure 108, is also somewhat different from the usual finding in the adult, where there is considerable constancy in the size of the cells. About half the cells in this fetal pig of 50 mm. are diminutive in size; the smallest are hardly a third the size of the largest. Transitions between the smallest and largest cells are also shown in this figure. It is difficult to ascertain whether these smaller cells represent young elements which have not yet reached

their maximal growth; no evidence of cellular division, as evidenced by mitotic figures, has been observed, although in this connection it must be granted that the cleared specimens are hardly the most favorable. Undoubtedly this explanation of the smaller cells would seem to be the true one, but there is little proof for the view, except their absence from the adult dura and their disappearance in larger specimens.

This disappearance of the smaller mesothelial cells is not rapid, but is seemingly delayed over into the larger fetuses; thus, in figure 109, a similar preparation from a fetal pig of 75 mm., corresponding smaller cells are outlined. On account of the absence from the field of the drawing of the larger elements, these cells do not appear relatively as great in number as in the preceding figure. Likewise, in figure 110 every gradation in cell-size is shown, in a specimen made in the same manner from a dura of a fetal pig of 90 mm.

Very slowly in the course of growth of the fetus the cells lining the inner surface of the dura reach their standard size and compose the mesothelial surface, with very little variation in size. The process, however, is apparently very tardy, even though the fetus at 16 cm. shows an inner surface to the dura which is largely composed of standard cells (fig. 111); but even in this figure, from a relatively large fetus, the standard size of the cells has not been attained, for a few cells of small size appear in the drawing. In other respects the whole pattern, in general appearance, resembles closely the adult.

It seems most fair to assume that the occurrence of a true mesothelial cell-pattern on the inner surface of the dura represents the initial establishment of a subdural space. On this basis the subdural space may be said to occur in fetal pigs 50 mm. in length; in the present investigation it has been found impossible to demonstrate the existence of the mesothelial cell-pattern in fetuses smaller than 50 mm. The separation of the dura, possible by gross dissection in pig fetuses of 40 mm., suggests that the space may be found at a slightly earlier stage than that in which the mesothelial cells have been demonstrated.

Anatomically the subdural space in pig fetuses resembles in every particular the adult space in cats and dogs; this was described in a paper⁵⁵ published in 1914. In the large pig fetuses injections of solutions of potassium ferrocyanide and iron-ammonium citrate were made into the spinal subarachnoid space. After precipitating the foreign salts as prussian-blue, the injection is found to be wholly within the subarachnoid spaces, both in the spinal and cranial regions: the subdural space is absolutely free from any evidence of connection with the subarachnoid space. These findings wholly accord with the opinion concerning the adult subdural space which has been repeatedly expressed.

THE COMPETENCY OF THE EARLY DURA AS A CELLULAR MEMBRANE.

During the stage when the condensation of mesenchyme to form the cranial blastema is pronounced the spread of the cerebro-spinal fluid becomes more and more extensive. In these stages, when the pig embryo measures from 16 to 25 mm. approximately, the outer membrane of the arachnoid is not yet formed, the arach-

noid spaces extending from pial to blastemal condensation. When in these embryos the cerebro-spinal fluid is replaced by the ferrocyanide solution and the embryo kept alive for some time, the course of the injection may be traced to varying extents throughout the periaxial tissue. To this spread of the injection fluid (a true solution, during the progress of the experiment), however, the blastemal condensation of mesenchyme opposes an absolute barrier. This peculiarity of the early condensation may be readily seen in figures 16 and 18. At this stage in development the blastemal thickening may be said to play the rôle of the outer membrane of the arachnoidea or of the inner surface of the dura.

This feature of the blastema as an impenetrable membrane—an absolute barrier to the passage of fluid—is found also to endure during injections of the ferrocyanide solution under pressures sufficient to rupture other parts of the central nervous system. Similarly, the early blastemal condensation resists the inflow of the other injections used (india ink and silver nitrate) under similar pressure conditions. In later stages the injection solutions, from ventricular or subarachnoid introduction, do not reach the dura. This is due to the development of an outer membrane of the arachnoidea and the formation of the subdural space. The arachnoid membrane when formed does not permit fluid to pass outward into the subdural space; but the competency of the early blastemal condensation in the mesenchyme affords a very good example of the perfect function of a tissue as a fluid-barrier.

An interesting phase of the competency of the secondary mesenchymal condensation (forming dura and outer membrane of arachnoid) may be seen in the region of the cisterna cerebello-medullaris. Here, as shown in figure 77, the zone of secondary condensation, while complete below, does not remain definitive above as the mesenchyme sweeps inward to the chorioid plexuses. At such a stage of 32 mm. in the pig, a replacement experiment would show no penetration of this secondary dural condensation by the foreign solution, where the condensation made a definitive membrane; above, however, in the region of the plexuses, a limited penetration by the introduced fluid could be made out.

X. THE RETURN OF CEREBRO SPINAL FLUID TO THE VENOUS SYSTEM.

The question of the exact mode of return of the cerebro-spinal fluid to the general circulation has interested many investigators. It has occasioned a large amount of work, with the presentation of several hypotheses. Key and Retzius²⁹, from the results of injections of colored gelatin into the spinal subarachnoid space, held that the cerebro-spinal fluid returned through Pacchionian granulations into the great dural sinuses. Other workers, following Key and Retzius, were dissatisfied with this theory, because of the apparent lack of these granulations in infants and in the lower animals. Cathelin⁶¹, with but little evidence, hypothecated an absorption of the fluid by way of the perineural sheaths into the lymphatic system, although the physiological findings of Ziegler⁵⁷, Reiner and Schnitzler⁴⁸, Leonard Hill²⁴, and others made it necessary to consider a direct absorption into the blood system. Cushing⁹

premised the drainage of fluid into the great sinuses through a valve-like mechanism. Dandy and Blackfan⁴⁰ suggested its absorption by the capillaries of the pia-arachnoid—an untenable hypothesis in view of the work of Kadyi²⁸, Shroeder van der Kolk⁵¹, Ekker¹⁴, Adamkiewicz¹, and others. Still another conception of the process has been advanced by Mott⁴¹, namely, that the absorption of cerebro-spinal fluid is one of the functions of the cerebral capillaries. In a previous investigation⁵⁵, making use of a method similar to the one here employed in the replacement experiments, evidence was presented indicating the drainage of cerebro-spinal directly into the great dural sinuses through arachnoid villi. These structures represent an invasion of arachnoid tissue through the lateral wall of the sinuses.

In view of the findings in adult laboratory animals, interest naturally turned, during the course of this work, to the process of drainage of the embryonic cerebro-spinal fluid. The evidence afforded by the replacement experiments with the ferrocyanide solution indicated that in pig embryos of over 20 mm. cerebro-spinal fluid circulated throughout most of the periaxial tissue, and that in embryos of about 26 mm. the periaxial distribution was complete, the relations of the fluid at this stage becoming adult. With this evidence before us, the question of the drainage of the fluid became important. Was the absorption process similar to the normal adult procedure, or was it entirely lacking, the production of the fluid being balanced by the growth of the nervous system and its meningeal spaces?

The question of the absorption of cerebro-spinal fluid was approached in the embryo in a similar manner to that employed in the adult animal. The problems incurred by the use of abnormal intracranial pressures were eliminated by the method of replacing, without disturbing the normal tension, the embryonic cerebro-spinal fluid with the ferrocyanide solution. The embryo was then kept alive and was finally fixed in a preservative which would precipitate the replaced fluid as prussian-blue. This procedure was carried out in many embryos of varying lengths and the specimens were subsequently stained in serial sections.

The smallest embryo in which any evidence of absorption of the fluid from the periaxial tissue was obtained was a pig embryo, 23 mm. in length. In this specimen granules of prussian-blue could be traced through the mesenchymal spaces (arachnoidal) to the inner wall of the sinus transversus. The sinus is well differentiated at this stage in the human embryo of 21 mm., as demonstrated by Streeter⁵⁴. The wall of the sinus in this pig embryo was quite thin, the mesenchyme lending the endothelium but little support. The prussian-blue granules could be traced directly through the endothelial wall of the sinus, and a few were identified lying free in the lumen. The conditions of the observations, permitting a flow of venous blood through the sinus, undoubtedly accounted for the fact that but few of the granules were found lying free in the sinus. This passage of the replaced fluid into the lateral sinus is portrayed in figure 21, taken from the pig embryo of 27 mm.

The same process of drainage of cerebro-spinal fluid may be observed in pig embryos more than 23 mm. in length. In all but one particular it corresponds exactly to the process observed in adult laboratory animals. There is the same lack

of absorption on the part of the cerebral veins and embryonic capillary plexuses. In the adult, however, the process is not diffuse, but is confined to the arachnoidal villi, while in the embryo a considerable extent of the inner wall of the sinus lying in the mesenchymal tissue, which is breaking down to form the arachnoidal spaces, serves as a site for the fluid passage. In these earlier stages the sinus transversus functions as the chief sinus of absorption. This is probably to be explained by the primary basilar spread of the replaced cerebro-spinal fluid and also by the fact that the true sinus sagittalis superior is a much later addendum. In the human embryo, according to Streeter⁽⁵⁴⁾, it is found in stages of over 50 mm.

The absorption of cerebro-spinal fluid in the embryo seems to follow the direct-*ing* agencies which operate in the adult. Increase in the pressure employed in the injection of true solutions results in the drainage of more of the fluid, as determined by subsequent microscopical examination. This suggests that the process is determined by factors other than that of diffusion; it seems most likely that here, too, the process is one of filtration, with a possible distension of the cellular membrane, so that intercellular spaces are opened. The histological picture of the sinus wall, however, undoubtedly gives the impression that the fluid has passed almost solely through the cytoplasm of the endothelial cells and likewise through the layer of supporting mesenchyme. These findings are in accord with observations on the adult.

With dilute suspensions of india ink as the injection mass, the results are quite different in regard to the passage of the material into the sinus. Replacement experiments making use of this suspension of particulate matter yield no evidence, as the carbon granules do not leave the ventricular system. Likewise, simple injections of the suspension into either the central canal of the spinal cord or into the perispinal spaces furnish no information unless the syringe-pressure be high. In this case the carbon granules may be traced into the sinus transversus, which is apparently the point of least resistance. Because of the obscuring of the picture by the carbon it can not be determined histologically whether the granules pass into the sinus in the same manner as does a true solution, or whether the passage is effected by numerous small ruptures of the tissue. The impression gained from our study would incline one toward the latter view.

If the injection of india ink be made under very great pressure from a syringe, the segmental veins may be filled with the carbon. This filling is always subsequent to its flow into the sinus transversus. But in no case was an evidence of a flow into lymphatic channels observed.

The process of drainage of the cerebro-spinal fluid into the venous system of fetuses will not be detailed here. This undoubtedly concerns a study of the formation of arachnoidal villi and of the differentiation of the lateral walls of the superior sagittal sinus, the best site for this study. The material at hand is not suited for this investigation, so that postponement is necessary.

XI. THE CHORIOID PLEXUSES AND THE ELABORATION OF CEREBRO-SPINAL FLUID.

With the realization that at a definite period in embryonic life, cerebro-spinal fluid passes from the cerebral ventricles into the periaxial spaces, it seemed desirable to ascertain what relationship existed between the developing chorioid plexuses and the elaboration of the fluid; for with the extension of the fluid into the periaxial tissue it becomes obvious that the balance between the development of the intra-ventricular fluid and the volume of the ventricles is destroyed and that more fluid is being elaborated than can be contained within the medullary-canal system. This relationship between the ventricular volume and the production of cerebro-spinal fluid has been described at some length in a preceding section of this communication.

The determination, then, of the exact rôle played by the chorioid plexuses in the further extension of the fluid into the periaxial tissue appeared to be of importance, for it could be conceived that the embryonic ependymal cells might be capable of elaborating the excess of fluid. With this purpose in mind the chorioid plexuses were investigated from morphological and cytological standpoints, in the hope that some index might be afforded as to the assumption of function on the part of the developing chorioid plexuses. These methods of study were applied solely to the chorioid plexuses of pig embryos, for it is from them alone that evidence of the period of extraventricular extension of the cerebro-spinal fluid has been obtained.

THE DEVELOPMENT OF THE CHORIOID PLEXUSES.

The development of the chorioid plexuses is so well understood that only a very brief outline will be given here. The general scheme of origin of these glandular structures concerns a gradual histological differentiation in certain localities of the ventricular ependyma. The ependyma of the roof of the fourth ventricle thickens along the transverse invagination (plica chorioidea) and then gradually becomes tufted in villous projections into the ventricle, following the ingrowth of a capillary plexus and supporting mesenchyme. This general process of differentiation occurs at first along the lateral portions of the plica; the central portion of the ependyma remains unaffected by the villi even when the tufts have become quite well differentiated (fig. 23).

Quite similar to this process of development of the plexus chorioideus of the fourth ventricle is the differentiation of the other plexuses. The plexus of the third ventricle develops as an infolding of the tela chorioidea of the roof. In every case the process holds of ependymal invagination and subsequent vascularization and suspension by mesenchymal ingrowth.

The histological differentiation of the ependymal cells into the glandular type of plexus, as first determined by Luschka³⁴ and Faivre¹⁵, is hardly satisfactory as an index of the production of fluid, as the secretory phenomena of the adult cells have not as yet been completely established. The researches of Pettit and Girard⁴³, dealing with the correlation of histological changes in the chorioidal cells and their functional state, first furnished reliable evidence that these cells give rise to cerebro-

spinal fluid. Since the publication of their investigations in 1900 many workers—Meek⁽³⁷⁾, Findlay⁽¹⁷⁾, Pellizzi⁽⁴²⁾, Mott⁽⁴¹⁾, Hworostuchin⁽²⁶⁾, Engel⁽¹²⁾, and others—have been concerned with this problem and have established on fairly definite bases the relationship of the plexuses to the production of the fluid. The histological appearances of the secretory cells, however, does not rest on incontrovertible ground, as has been stated in a previous paper⁽⁵⁵⁾.

The process of differentiation of the ependymal cells which form the glandular elements of the chorioid plexuses occurs with the invagination and tufting of these structures. The various stages of transformation from the low type of cubical epithelium constituting the ependymal layer are shown in various figures in this paper. The nuclei of these cells assume basilar positions and the outer zones of the cytoplasm become granular with their greater height. The process is rather a slow one, as might be expected from the fact that the whole villus is gradually enlarging and becoming more and more tufted.

The histological differentiation of the plexuses need hardly concern us here, except as an index of the assumption of function. The final completion of this change into the adult morphology occurs at a much later stage of development than our evidence indicates for the establishment of a cerebro-spinal circulation. It becomes obvious, then, that the final histological changes are not necessary for the process of elaboration of the fluid. This assumption seems warranted also by the fact that the embryonic fluid contains much more albuminous material than does the adult fluid.

The time of appearance of the chorioid plexuses in relation to the extraventricular spread of the fluid would surely seem to offer undoubted evidence in regard to the first elaboration of the fluid by the plexuses. It has been shown that in pig embryos over 14 mm. in length the replaced solution in the cerebro-spinal system spreads from the roof of the fourth ventricle into the periaxial tissues. This extraventricular extension occurs practically simultaneously with the first indications, in the pig embryo, of the formation of the chorioid plexuses of the fourth ventricle. Thus, in a pig embryo of 14 mm., the primitive thickening and tufting of the ependyma of the roof of the fourth ventricle may be observed (fig. 32). In earlier stages no definite evidence of this developmental process is found.

From the first indication of a developing chorioid plexus in a pig embryo of 14 mm., the growth of the tufts progresses rapidly, so that at 18 mm. the process is well advanced. In embryos of 20 mm. and over the tufts of the plexuses in the fourth ventricle are quite marked, as shown in figures 22, 44, 46, and 92.

The chorioid plexuses of the third and lateral ventricles appear at a somewhat later stage than do those in the more caudal ventricle. Thus the first indication of their appearance in pig embryos is found in specimens measuring 19 mm. in length. This coincides well with the further extension of the replaced fluid in specimens of 19 mm. and over. The definite differentiation of these plexuses, however, does not actually take place until the embryo reaches a length of 23 mm.—a fact suggestive of some relationship to the complete periaxial spread found in embryos of this measurement.

Considered, then, as a whole, there seems to be a very definite relationship between the developing chorioid plexuses and the periaxial spread of the embryonic cerebro-spinal fluid; for immediately after the first appearance of chorioidal tufting in the roof of the fourth ventricle (at 14 mm.) the replaced injection spread appears in the periaxial tissue (fig. 3). This extraventricular spread does not become marked until a length of 19 mm. is attained (fig. 5)—a factor in accord with the elaboration of the villi in the chorioid plexus of the fourth ventricle. The periaxial spread remains localized in the rhombencephalic region until the 20 mm. stage is attained, when it rapidly becomes pericerebral and perispinal (figs. 6 and 7). This coincides with the first indications of the chorioid plexuses in the more cephalic ventricles. But the further spread is here delayed (as in the stages between 14 and 19 mm.) until a length of at least 24 mm. is reached—which is perhaps of importance in the further development of the cerebral plexuses and the greater elaboration of the cerebro-spinal fluid. Thus it seems possible to conclude that coincident with the first appearance of the chorioid plexuses a more rapid production of cerebro-spinal fluid occurs, necessitating the passage of the fluid into the periaxial tissues.

THE GLYCOGEN CONTENT OF THE CHORIOID PLEXUSES.

In the hope that some cytological method might afford direct and incontrovertible evidence of the time of the assumption of function by the chorioid plexuses, stains demonstrating the intracellular presence of glycogen were applied to these structures. The quantity of the starch in the chorioid plexuses of rat and mouse embryos, as shown by Goldmann, suggested that this substance might be associated with the early elaboration of the cerebro-spinal fluid. Furthermore, the presence in the adult fluid of a definite reducing body, demonstrated by Nawratschi to be dextrose, added some weight to the hope that a definite conclusion might thus be afforded.

Several important studies concerning the presence of glycogen in the cells of the embryonic and fetal chorioid plexuses have been made. Creighton⁸ found that the glycogen of the chorioid plexus was very abundant about the middle of embryonic life, while von Loeper concluded that the great content in the cells of the fetal plexus was characteristic. Goldmann²⁰ found large quantities of glycogen in the plexus in rats and mice, not only in embryonic life but also in animals from two to three weeks old. In the adult plexuses the cells contained no trace of glycogen.

The observations here included were made after fixing the chorioid plexuses of various pig embryos in absolute alcohol and staining the sections (cut either from eeloidin or paraffin blocks) by Best's carmine method. This technique is similar to that employed by Goldmann. The staining reaction is such that a very striking differentiation of the glycogen occurs, but the shrinkage of the embryonic tissue in the fixation in absolute alcohol is a disadvantage. In these observations the plexuses from the fourth and lateral ventricles were used.

As shown in the table on page 94, glycogen could be identified in the cells of the chorioid plexuses in pig embryos varying in length from 28 to 155 mm.

Below the first measurement no glycogen was demonstrated by the method employed: above the higher limit in only one instance (series No. 41) was glycogen found. This finding of a limited period in the embryonic life of a pig during which glycogen occurs in the cells of the chorioid plexuses does not coincide with Goldman's observations on the rat and mouse. Furthermore, it was found here that in stages up to 100 mm. the glycogen was practically generally distributed throughout all the cells of the chorioid plexus, occurring with great regularity in every villus and cell. This general distribution was not found in the plexuses of embryos over 110 mm. in length; in these more advanced stages the cells containing starch occurred in clumps, giving a localized distribution. In the stages under 100 mm. the glycogen was present in very large amount, as estimated histologically. As the stages advanced the quantity of glycogen decreased rapidly. This great amount of starch was present in the same stages in which the general distribution of the cells occurred.

Occurrence of glycogen in the chorioid plexuses of embryo pigs.

C. P. series, No.	C. R. measure, mm.	Glycogen.	Globular forms of glycogen.	Plaques of glycogen.	Amount of glycogen.	Distribution of glycogen throughout plexus.	Intracellular distribution of glycogen.
16	18	Absent.....					
3	23	Absent.....					
13	28	Present.....	Present.....	Present.....	Great.....	General.....	Basilar.
12	33	Present.....	Present.....	Present.....	Great.....	Localized.....	Basilar.
14	36	Present.....	Present.....	Present.....	Great.....	General.....	Basilar.
6	39	Present.....	Absent.....	Present.....	Great.....	General.....	Basilar.
9	40	Present.....	Present.....	Present.....	Great.....	General.....	Basilar.
8	55	Present.....	Present.....	Present.....	Great.....	General.....	Basilar.
4	66	Present.....	Present.....	Absent.....	Great.....	General.....	General.
1	80	Present.....	Present.....	Present.....	Great.....	General.....	General.
42	90	Present.....	Present.....	Present.....	Great.....	General.....	General.
17	100	Present.....	Present.....	Absent.....	Great.....	General.....	General.
15	105	Present.....	Present.....	Present.....	Small.....	General.....	Basilar.
20	118	Present.....	Present.....	Absent.....	Small.....	Localized.....	General.
10	132	Present.....	Present.....	Absent.....	Small.....	General.....	General.
18	155	Present.....	Present.....	Absent.....	Small.....	Localized.....	General.
27	155	Present.....	Present.....	Absent.....	Small.....	Localized.....	General.
39	155	Present.....	Present.....	Absent.....	Small.....	Localized.....	General.
25	158	Absent.....					
32	160	Absent.....					
40	163	Absent.....					
41	170	Present.....	Present.....	Absent.....	Small.....	Localized.....	General.
24	173	Absent.....					
19	185	Absent.....					
23	195	Absent.....					
11	209	Absent.....					
21	213	Absent.....					
22	223	Absent.....					
26	244	Absent.....					
5	260	Absent.....					

Goldmann⁽²⁰⁾ pictures the glycogen as occurring throughout the cells of the chorioid plexus in the form of globules of larger or smaller size. Some of these globules may be seen even in the surrounding cerebro-spinal fluid. This general intracellular disposition was observed in this series in specimens measuring 66 mm. and over (fig. 95). Below this measurement the glycogen occurred practically

entirely in the basilar portion of the cell, central to the nucleus. Furthermore, in the stages between 30 and 60 mm. the glycogen globules were present in but small numbers and the glycogen was found in crescentic plaques (fig. 96). This formation of definite plaques is apparently to be ascribed to the fusion of the globules when the amount of glycogen becomes extreme. As far as is known this plaque formation with glycogen has not previously been noted; in one of Goldmann's figures the fusion of some of the globules has apparently taken place.

The table on page 94 records the findings in these observations.

The occurrence of glycogen in the cells of the chorioid plexus only during a certain portion of embryonic life is, as shown by the foregoing table, a fairly definite phenomenon, but there is surely no indication that this temporary presence of the animal starch bears any relation to the assumption of function on the part of the chorioid plexuses. The evidence afforded by the extraventricular flow of the replaced fluid, with the apparent relationship of the developing chorioid plexuses to the periaxial extension of the fluid, argues strongly against such an assumption.

XII. PERIVASCULAR SPACES IN THE EMBRYO.

In 1865 His⁽²⁵⁾, using a puncture injection, found that each nerve-cell existed in a so-called space. These pericellular spaces connected, as demonstrated by the flow of the injection mass, with an extensive perivascular network, more complex in its gray matter than in the white. In all of His's cases continuation of the injection led to a peripheral spread toward the pia, both in the spinal medulla and in the brain.

Mott⁽⁴¹⁾, working on the brains of animals in which an experimental cerebral anemia had been produced by ligation of the head arteries, found the perivascular spaces enormously dilated and the perineuronal spaces likewise very evident. Direct connections between the perivascular and perineuronal spaces are pictured in Mott's communication.

The deduction which Mott made from his findings, regarding the possible absorption of cerebro-spinal fluid by the cerebral capillary bed from this perivascular and perineuronal system, was discussed by the present author in a paper two years ago⁽⁵⁵⁾. It was there shown that, with the use of true solutions as the injection (potassium ferrocyanide and iron-ammonium citrate), the whole perivascular system could be filled. This injection of the spaces, however, occurred only when the pressure conditions within the cranial cavity were such that the subarachnoid pressure exceeded the vascular tension. This reversion of the pressure relations was accomplished by maintaining at normal the subarachnoid pressure with the injection fluid, and occasioning a simultaneous and complete vascular anemia. Under the routine conditions of injection (with undisturbed pressure relations) no injection of the perivascular system from the subarachnoid space resulted. It was found impossible to inject the perivascular system, using granular suspensions as the injection mass, without employing pressures far above the normal.

From these results here recorded briefly, the belief was expressed in this former paper that each nerve-cell was surrounded by a capillary space which drained along

the perivascular channels into the subarachnoid spaces. Probably this system represents a mechanism for accessory tissue drainage comparable physiologically to the lymphatic channels of the other parts of the body.

In view of these findings in the adult mammal it seemed desirable to ascertain at what period of intra-uterine life such function was acquired. It also seemed not unlikely that information of interest might be acquired from the embryonic intramedullary circulation which would amplify our knowledge of this system in the adult. It was thought that there might be a correlation between the production of the perivascular fluid and the enlargement of the subarachnoid channels, similar to the evident connection between the chorioidal invagination and the extraventricular spread of the fluid.

Experiments to demonstrate possible perivascular and perineuronal spaces were first attempted on rather large fetuses (pig), as follows: The spinal meninges were exposed in a fetus in which the heart was still beating vigorously. Into the spinal subarachnoid space was introduced a needle connected with a small reservoir, containing the injection solution (potassium ferrocyanide, 0.5 gm.; iron-ammonium citrate, 0.5 gm.; water, 100 c.c.). The reservoir was then adjusted so that a pressure of 160 mm. of water was maintained in the subarachnoid space. The arteries and veins in the neck of the fetus were then severed, and the subarachnoid pressure maintained at its former level. At the end of 20 minutes the head was placed in a fixative containing 1 per cent hydrochloric acid.

This procedure, as outlined above, in the adult laboratory mammal, usually resulted in a complete injection of the perivascular system. In the embryo, however, the procedure was uniformly unsuccessful. The injection solution, as shown subsequently by the precipitated prussian-blue, rarely ascended over a centimeter above the point of injection. This indicated that the existent cerebro-spinal fluid was not replaced by the injection solution, and that the failure to demonstrate the perivascular system was to be explained on this basis, if the system were functional at this stage. Attempts were then made to replace the subarachnoid fluid with the injection solution before the cerebral anemia occurred. These attempts likewise met with failure, because of the impossibility of keeping the heart beating for any length of time in the larger pig fetuses. Other attempts were also made to demonstrate these channels, in larger pig embryos, by means of a procedure which in the adult gave at times good injections of these intracortical canals. This method differed from the method first employed only in the maintenance of a high pressure (100 mm. Hg) in the spinal subarachnoid spaces. It likewise met with failure, due apparently to the same causes which occasioned its failure in the adult: the high subarachnoid pressure operated chiefly to compress the cerebral and spinal tissues, rendering the injection of the perivascular spaces impossible.

The same procedures were attempted in smaller pig embryos (15 to 60 mm.). The method usually successful in demonstrating the spaces (subarachnoid pressure slightly above normal, with subsequent cerebral anemia) failed, apparently because the cranial cavity at these stages is in no sense a rigid closed box, as in the adult.

Any method of service in the adult — which must have in consideration the physical character of the skull as a closed box — was here necessarily doomed to failure.

Together with these technical failures to demonstrate a perivascular system, it must be borne in mind that these are merely failures to demonstrate the existence of the perivascular system in the pig embryo. The system will probably be demonstrated as soon as a suitable technique is devised. The spaces are very likely present soon after the capillary plexus invades the nervous system, but the observation in many histological preparations of the spaces around the cerebral vessels must not be considered as offering proof of their existence, because of the likelihood of shrinkage influencing the picture. It is interesting, however, to note that elasticity of the cerebral tissues seems greatest along the course of the blood-vessels, for here the phenomenon of shrinkage is most frequently observed. The existence of the perivascular and perineuronal spaces, probably of only capillary thickness, must remain—in the embryo as in the adult—a subject of physiological demonstration; histological evidence, except with proper physiological regard, is of no value.

The early development and function of such a system as the perivascular and perineuronal canals afford seems most likely from the standpoint of pure speculation. It is not improbable that fluid is poured from this system into the embryonic subarachnoid space at a period soon after the capillary plexus invades the cerebrum. There is no evidence, however, from the observations recorded in foregoing paragraphs, that adequate subarachnoid channels are afforded until the pig embryo reaches a length of about 25 mm. The hypothesis of Essick¹³ regarding the damming of the perivascular fluid as the cause of the two cava corporis striati is of extreme interest in this connection. It remains, however, for future work to afford real evidence in regard to the embryonic perivascular system.

XIII. THE PERINEURAL SPACES IN THE PIG EMBRYO.

The question of the existence of potential or actively functional spaces around the peripheral nerves is of great interest, partly because of the possible relation of these spaces to the developing lymphatic system, and also on account of the anatomical evidence of the possible existence of such spaces.

It is realized that before much dependence can be placed on any theory regarding these potential spaces around the cerebro-spinal nerves, the possibility of their being purely artifacts must be dealt with. The methods of demonstration, in the adult, in the hands of the earliest workers were such as to favor the production of artifacts. As far as can be ascertained, Cotugno⁷, dealing with the nervus ischiadicus, was the first to conceive of these possible spaces. His method of demonstration consisted in filling the spinal subarachnoid space with mercury (in a cadaver placed in the erect posture). Globules of the mercury were subsequently found about the sciatic nerve in what then became the perineural spaces.

Modern anatomical interest in these spaces was aroused by the remarkable injections of Key and Retzius⁽²⁹⁾. These investigators, by means of gelatin injections into the spinal subarachnoid space, were able to demonstrate perineural

spaces around the cranial nerves, especially around the optic pair. Their results, however, are open to criticism, because of the excessive pressures employed ("not over 60 millimeters of mercury") and because the injections were made in fresh cadavers kept warm for periods of 10 or more hours.

Some of the difficulties concerned in the problems of the perineural spaces were cleared up in a study⁽⁵⁵⁾ of the cerebro-spinal circulation published in 1914. In this work injections of true solutions (similar to those used in the present study) were introduced into the spinal subarachnoid space in living cats and dogs, under pressures but slightly exceeding the normal intraspinal tension. These injections were continued for several hours, and the course of the injection fluid was then established by precipitating the solution *in situ*. By means of this procedure, which it was believed approached the physiological, the perineural spaces around the cranial nerves could be demonstrated. In these adult laboratory mammals the cerebral nerves without exception showed prussian-blue granules in a perineural relation, extending outward along the nerves beyond the termination of the dural cuff. This extension of the injection mass outward was more striking around the first two cranial nerves than about any of the others. Thus, the olfactory nerves uniformly showed perineural deposits beyond the cribriform plate, extending downwards into the nasal epithelium, while the optic nerves were surrounded by the granules in the infravaginal sheath, which spreads out over the posterior surface of the eyeball. The caudal cranial nerves were likewise characterized by extensive perineural injections.

These findings were interpreted as evidencing a true perineural space, probably of only capillary thickness, which could be injected by filling the cerebro-spinal spaces with a demonstrable true solution. As far as could be made out under the microscope, they had no appreciable existence except when filled with the precipitated true solution. These spaces were not filled in the early moments of the injections under low pressures, and could be demonstrated only when the injection had been continued for several hours.

The perineural spaces are quite different from the spaces surrounding the spinal ganglia and the ganglia of the cranial nerves. These ganglia lie in the true subarachnoid space, with the dura investing the arachnoid membrane. Distal to the ganglion the dura ends upon each nerve. In the injection under low pressure with the ferrocyanide the cranial and spinal ganglia were all surrounded by the precipitated salts; the cranial nerves showed extensive perineural injections, whereas the spinal nerves rarely showed a true perineural injection, and then only of limited extent.

The existence of perineural spaces in the embryo, however, has been under dispute. The larger nerves in sectioned embryos almost invariably show spaces about them, either a complete separation of the surrounding mesenchyme or a partial dilatation of the mesenchymal interstices. Sabin⁽⁴⁹⁾, in 1902, noted that in perispinal injections with india ink the spinal nerves could be outlined by the carbon granules, but in no case did such an injection run into true lymphatic channels. No evidence was afforded by her work of any lymphatic channels arising from these apparent perineural channels.

In the course of this investigation of the cerebro-spinal spaces interest naturally turned to the perineural spaces. In the typical experiments (a replacement of the embryonic cerebro-spinal fluid with a demonstrable true solution in the living embryo), there was evidence of a spread of the replaced solution around the cranial nerves. Because of the procedure used (merely a filling of the ventricles and central canal of the spinal cord) no evidence of a perineural spread occurred until the foreign solution passed into the periaxial tissues. Here the spread chiefly involved the caudal cranial nerves curving around the lateral surface of the modulla in fan-shaped processes (figs. 5, 6, 8, and 9). The spread, however, was not extensive. In figure 8 a similar slight spread along the spinal nerves is to be made out. Closer study of these cleared specimens, and examination of the same and of similarly injected embryos after serial sectioning, convinces one that the apparent perineural spread in these cases extends around the sensory ganglia and not further toward the periphery. In no case, either in the caudal portion of the cranial or in the spinal region, has the replaced injection fluid passed the blastemal condensation of mesenchyme. This finding is well shown by the distribution of the injection fluid in figures 9, 16, and 18.

The optic nerves, however, possessing ganglia in the retina, usually show, in the typical replacements in the living embryo, a partial or complete surrounding of the nerves by the precipitated prussian-blue. An incomplete example of this—more typical, according to these observations, than a total circumvention—is given in figures 19 and 20. The higher-power reproduction of this field is very interesting. It shows in the central portion the fiber bundles comprising the optic nerve, surrounded by mesenchyme and the developing ocular muscles. In the region between the nerve and the muscles is an undifferentiated mesenchyme which is characterized by a crescent of the precipitated granules of prussian-blue. The non-penetration of the surrounding tissue by the ferrocyanide is very well brought out in this drawing. The prussian-blue has reached its position about the nerve by extension from the pericerebral spaces; actually it has still the same distribution as noted in figure 8 above. The adult dura will completely surround the optic nerve in its whole extent; the subarachnoid space will likewise extend unbroken to the posterior surface of the eyeball. Hence it must be assumed that in this case the perineural space does not extend beyond the peripheral ganglion. With regard to the olfactory nerves, no evidence of a perineural spread was obtained in specimens of pig embryos up to 45 mm. in length.

It seems obvious, then, that in the embryo pig true solutions, when substituted for the cerebro-spinal fluid, do not extend peripherally along the nerves any further than does the dura in the adult. The replaced fluid (if, as appears most likely, it indicates the true circulation of the cerebro-spinal fluid) extends only through the future subarachnoid space. Such a conclusion is best supported by the observations. The only discrepancy between the findings in the pig embryo and those in the adult with the same method lies in the fact that in the adult the cranial nerves showed a much more extensive perineural injection. This seeming discrepancy may be

accounted for in two ways. In the first place, the experimental replacement in the embryo pigs lasted at most one hour (due to the fact that the embryo's heart frequently ceased beating at the end of this time), while in the adult cat or dog they were continued for several hours; and it was only in the long-continued experiments in the adult that the extensive perineural injections were obtained. On this basis it seems more than likely that the communications between peripheral perineural spaces and the subarachnoid space are very small and that diffusion must account for the slow filling of the peripheral system. The second explanation seems undoubtedly to concern the time of development of these perineural spaces in the embryo. It may be that the spaces are morphologically non-existent until late in fetal life; in that case, of course, it is not strange that they have not been filled with the injection fluid.

From the observations recorded above it is quite apparent that in the typical experiment in which the normal cerebro-spinal tension is not increased no evidence of the perineural space, as injected by Miss Sabin, has been adduced. However, the possibility of injecting these spinal spaces as was done by Miss Sabin is easily demonstrated. The injections may be made with ease, either with granular suspensions or with true solutions. Success invariably attends such an injection into the perispinal tissues. The injection solutions easily run out around each nerve, more readily, apparently, in the younger embryo than in the older. It is not clear whether this difference is due to the fact that in younger embryos the resistance is greater to the perispinal flow and less peripherally, or merely to the fact that a greater amount of fluid must be introduced in order to attain the same result. Careful repetition of these observations has led to the conclusion that such a demonstration of the spinal perineural spaces results from excessive pressures of injection. Whenever the pressure exerted by the injection is but slightly above the normal, or does not exceed the normal (as in replacements), the perineural spaces are not injected around the spinal nerves. Miss Sabin's conclusions from her results, that no connection exists between the spaces and the lymphatic system, seem to be wholly substantiated by these observations.

The apparent perineural spaces around the embryonic nerves must be looked upon as artifacts. In tissue carefully fixed, dehydrated, and embedded, there is no real evidence of these spaces. Their size apparently varies with the care observed in the histological technique.

XIV. GENERAL SUMMARY.

In the foregoing sections of this communication some of the problems concerned with the embryology of the cerebro-spinal spaces have been discussed and observations have been presented in the hope that a better conception of the processes might obtain. It is purposed to present here briefly the results of these observations and to attempt to correlate the findings so far as is possible; and in this, as in the detailed reports in the preceding pages, the relationship of the physiological processes concerned will be referred to the morphological changes in the developing embryo.

As a means of studying the physiological extent of the embryonic cerebro-spinal spaces, a method of replacing the medullary fluid with a foreign solution was devised. The procedure consisted in substituting, in the living embryo, a solution of potassium ferrocyanide and iron-ammonium citrate for the cerebro-spinal fluid. The embryos were then kept alive, for periods of about an hour, by placing them with the attached placenta in an incubator at 38°. At the end of this time, which varied in the many experiments, the whole embryo was fixed in a medium containing hydrochloric acid, thereby precipitating an insoluble prussian-blue. Specimens prepared in this manner were studied after sectioning or after clearing by the Spalteholz method.

Pig embryos, subjected to such experimental replacements, exhibited only an intraventricular retention of the foreign solution until after a stage of 14 mm. was attained. In the earliest specimens, embryos of about 9 mm., there was no characteristic distribution of the foreign solution, except that it remained within the medullary-canal system. In stages of about 13 mm. the replaced fluid also was retained within the cerebral ventricles, but in these specimens a dense accumulation of the precipitated prussian-blue may be made out in a distinct oval in the superior portion of the rhombic roof. This granular aggregation occurs against a histological differentiated area in the roof of the fourth ventricle—an area which represents apparently the more epithelial-like elements of the earlier roof-plate. This area must be considered solely as a differentiation of the epidermal lining of the medullary-canal system.

In living pig embryos of 14 mm. and over, the result of the routine replacement of the ventricular cerebro-spinal fluid was a slight extraventricular spread into the tissues posterior to the rhombic roof. The passage of this foreign solution outward occurred through the same area of ependymal differentiation, outlined by the collection of granules against its inner surface in the previous stage. The extraventricular spread remains definitely localized to a very small conical area which does not rapidly increase in size.

The factors which cause this initial flow into the pericerebral spaces are of interest. It follows that in the growth of the embryo the production of the intraventricular and intraspinal cerebro-spinal fluid must necessarily keep pace with the increasing size of the cerebral ventricles. It is also necessary for the occurrence of an extraventricular spread of the fluid that the production of the fluid within the ventricles must exceed the amount required to keep the medullary-canal system filled. From our knowledge of the elaboration of the adult cerebro-spinal fluid, it

is impossible to conceive of the production of a true cerebro-spinal fluid in the perimedullary mesenchyme. Such a view would be a reversion to the old hypothesis of Haller, who regarded the leptomeninges as the elaborators of the fluid. Likewise, the passage of the replaced foreign solution into the extraventricular spaces would render such a hypothesis untenable.

Hence, it becomes incumbent to regard such an extraventricular spread of the experimental solution as an indication that the production of the cerebro-spinal fluid within the cerebral ventricles exceeds the capacity of the ventricles to care for the fluid. This argues strongly that the process of elaboration of the fluid in these pig embryos of 14 mm. is no longer sluggish, but that an active production, sufficient to cause a slight extraventricular flow during the observation, is now taking place. This acceleration of the flow is not great, but it represents a marked change in the relationship of the process of fluid elaboration to the increasing volume of the ventricles.

It seemed desirable to endeavor to correlate this extraventricular spread of the experimental fluid with the morphology of some intraventricular structure at this critical stage of 14 mm. in the pig embryo. The first evidences of villous tufting in the chorioid plexus of the fourth ventricles were found to occur at this stage in the pig. Other studies of this plexus, particularly those which concerned the occurrence of glycogen in these glandular cells, were found to offer no additional evidence of value in regard to the onset of function in these structures. The correspondence between the initial tufting of the ependyma to form the rhombic chorioid plexuses and the initial extraventricular spread must be regarded as of the utmost importance. It would appear most likely that as soon as the chorioid tufts occurred an increased production of cerebro-spinal fluid took place, necessitating an extraventricular expulsion of the excess of fluid. Such a view receives the utmost support from these recorded observations; it is in keeping with the best conceptions of the processes of production of cerebro-spinal fluid in adult mammals.

With the initial pericerebral extension of the experimental fluid occurring in pig embryos of about 14 mm., the further extension of this spread did not occur until after a length of 18 mm. was attained. At this stage the replaced foreign solution passed from the fourth ventricle through two areas in the roof-plate. The chorioid plexuses now have divided the roof into two portions; from each, fluid escaped. The superior area of fluid passage is the same which was concerned in the initial outpouring of the ventricular fluid. The inferior area, like the superior, is an area of ependymal differentiation, of which the first evidence may be made out in pig and human embryos of 15 mm. This differentiation consists in the transformation of the densely staining ependymal elements into cells with larger nuclei, poor in chromatin, and with more abundant cytoplasm.

After the functional employment of the two membranous areas is established at about 18 mm. in the pig, the further pericerebral spread of the replaced solution occurs very rapidly. The peribulbar tissues are filled with the fluid and from this region extensions occur downward into perispinal spaces and upward into the more

basilar pericerebral spaces. Thus, the spinal spaces must be considered as developing physiologically from above, and not from below upward, as Reford found. The complete filling of these perispinal spaces is found in pig embryos of 21 mm. At this stage the pericerebral spaces are filled, with the exception of those around the superior portion of the midbrain and about the cerebral hemispheres.

The final filling of all the periaxial spaces occurred in pig embryos of about 26 mm. This phenomenon may be taken to indicate the establishment of the true cerebro-spinal relationships of the adult, for in this case there is an intraventricular production of the fluid and an extraventricular spread. Likewise, the fluid returns to the venous system in embryos of over 23 mm., and this escape of the fluid from its periaxial bed is, as in the adult, directly into the venous sinuses of the dura mater.

The rapidity of the further extension of the replaced solution after the stage of 18 mm. is passed is apparently due to a second marked acceleration in the rate of production of the ventricular cerebro-spinal fluid. As in the first instance, this increased elaboration seems connected intimately with the formation of the chorioid plexuses of the third and lateral ventricles. As soon as these tufts develop, the cerebro-spinal fluid is produced in amounts which far exceed the quantities for which the more slowly enlarging ventricles can provide.

The histories of the two *area membranacea* of the fourth ventricle are dissimilar. Both are areas apparently differentiated from the normal lining ependyma for a specific functional purpose—the passage of fluid from the ventricles into the future subarachnoid spaces. The superior membranous area reaches its maximum functional importance in the stages of 18 to 20 mm. in the pig and also in the human embryo and from these stages on it slowly regresses. The final obliteration of the area, if it do not persist as an occasional small remnant, is due to the increasing growth of the cerebellum and the enlargement of the chorioid plexuses of the fourth ventricle. On the other hand, the inferior membranous area continues to increase both in size and functional importance after its initial differentiation from the ependyma; it finally occupies the greater portion of the *velum chorioidea inferior*. These observations can not solve the interesting question of a perforation of the *inferior velum* to form the *foramen of Magendie*.

Of the factors which influence the passage of fluid outward into the periaxial spaces, it must be realized that probably there is difference in this regard between the true solutions of the salts and the colloidal suspensions. For the true solutions (as in the experimental replacements) diffusion probably plays some rôle; but that this is not the sole factor is shown by the failure of the fluid to pass through the membrane in the stages under 14 mm. The findings of the granules of prussian-blue within the cytoplasm of the cells of this membrane indicates that the fluid passage is similar in every way to that through a true membrane. There is also a possible site of fluid passage between the cells of this membrane. But, surely, the most important factor in this process is one of filtration of the fluid from the point of higher pressure to one of lower. This is indicated by all of the findings: that the increased production of the fluid or the increased intraventricular pressure (whether

due to normal or experimental agencies) causes a marked extraventricular spread seems firmly established. For the colloidal suspensions (particularly the protein of the normal ventricular fluid) a slower process of diffusion and filtration seems the probable agency for passing the ventricular colloids into the subarachnoid spaces.

That the results obtained by the method of replacement were not solely due to diffusion, but represent a filling of the physiological extent of the cerebro-spinal spaces, has been shown in many ways, but probably the chief argument against such a view is that wholly similar extensions of the foreign solution may be obtained by injections under mild pressures from a syringe; with increasing pressures these injections show the same type of spread, but always in a smaller embryo than the replacement method demonstrates as the standard for a given stage of the extension. The results recorded in the foregoing pages indicate also that suspensions (india ink) and true solutions (when powerful precipitants) are valuable only for affording comparisons in problems concerning the normal processes of absorption.

Of interest in any discussion of the results of injections into the perispinal spaces or into the spinal central canal are the findings in regard to the perineural spaces. It is possible to inject such spaces around each of the segmental nerves, but only when the pressures of injections are extreme. In no case, however, were such injections found to enter the lymphatic system—a finding in accord with the observations of Reford and Sabin. The physiological importance of these spaces in the adult is probably great, but the same methods of demonstration (with carefully controlled pressures) which suffice in the adult are unavailing in the embryo.

The origin of the three meninges from the perimedullary mesenchyme is well established. His, Kölliker, Sterzi, Farrar, and others have placed this conception on a very firm basis. Most of the investigators have been concerned with the differentiation of the spinal meninges, while the observations here reported have been concerned solely with the cranial portion of these membranes. In general, the same phenomena in the transformation of the primitive periaxial mesenchyme as recorded by these earlier workers may be found in the cranium. The division of the primitive mesenchyme by a secondary condensation, a view advanced chiefly by Salvi, seems well supported. The findings in the cranium are in accord with this conception; the outer portion of this primitive meninx becomes the dura mater, the inner forms both the pia and arachnoid. The processes in the formation of the arachnoid are, however, quite diversified and concern both the formation of the subarachnoid spaces and the outer membrane of the arachnoid.

Out of the rather loose-meshed periaxial mesenchyme, the subarachnoid spaces develop. The process concerns the transformation of the small "tissue spaces" of this mesenchyme into the larger subarachnoid channels, which are interrupted by the well-known arachnoid trabeculae. Well-marked stages in this metamorphosis, which begins in the basis cranii, can be made out. The first appearance of a differentiation is seen in the gradual increase in the size of the mesenchymal mesh. This is closely associated with an increased amount of an albuminous coagulum which in a measure fills the larger interstices. Following this initial dilatation of the spaces

occurs a breaking-down of some of the syncytial strands; these ruptured mesenchymal processes then retract and adhere to the persisting trabeculae. The process continues with the formation of larger channels in this mesodermal tissue, with also the formation of the permanent arachnoidal trabeculae. Throughout these larger spaces, in the smaller fetuses, the coagula of protein material are everywhere found, the remains apparently of the albuminous portion of the circumambient fluid.

In the formation of the various cisterne, particularly the great cisterna cerebello-medullaris, the process of the dilatation and confluence of the original mesenchymal spaces reaches its maximum. Here the breaking-down of the original syncytial strands proceeds to such an extent that very few of the strands remain to persist through life.

Such a process of the enlargement of mesenchymal spaces to form the larger subarachnoid spaces, as described in some measure by His for the spinal meninges, is apparently intimately connected with the circulation through these spaces of the embryonic cerebro-spinal fluid. The fluid flows everywhere through the spaces, as evidenced by the replacement experiments and by the increased content in albumen, before the process of enlargement of the mesenchymal spaces begins. It seems most likely that this circulation of the fluid acts as the causative agent in initiating and probably also in completing the enlargement of the "tissue spaces." The great content of albumen in the embryonic cerebro-spinal fluid has greatly facilitated the investigation, as the presence of the coagula from this protein has permitted the absolute exclusion of artifacts in the process of the tissue-dilatation.

This mechanism of enlargement of the tissue spaces finds its analogue in the formation of the anterior chamber of the eye and in the perilymphatic spaces of the ear (Streeter). In both these situations, as in the meningeal spaces, there are special body-fluids, more or less characteristic in their physical and chemical characters, obviously subserving specialized functions. In both the eye and cranium, the absorption of the fluids is by way of special organs, directly into venous sinuses; in both, the origin of the specialized fluid is from epidermal organs; this fluid is at first poured into epidermal spaces and then subsequently into mesodermal spaces (subarachnoid space and anterior chamber of the eye). Thus, in these situations, the characteristic fluids have certain definite channels through rather larger spaces, connected finally with the venous system, and only indirectly with the lymphatic system.

In no sense must the cerebro-spinal circulation be taken as a portion of the lymphatic system. Increasing knowledge of the cerebro-spinal fluid, of its physiology and chemistry, and of its pathway, have separated it permanently from any connection with the lymph of the lymphatic system, variable though that be. No longer may the meningeal spaces be compared to serous cavities, except possibly in the case of the subdural space, and this space is really a space apart from the true cerebro-spinal or subarachnoid spaces. Quite similarly, in place of the many varying channels in the dura and to a lesser extent in the leptomeninges, which older writers considered lymphatic in nature, our increasing knowledge has caused

the introduction of specialized arachnoidal cell-chains running throughout the pachymeninx. Unquestionably, the cerebro-spinal fluid possesses its own peculiar and characteristic pathway, analogous in no way to the lymphatic vessels of other tissues.

The outer continuous membrane of the arachnoidea forms as a mesenchymal condensation, at first in common with the inner surface of the dura mater, but soon separated from it by the subdural space. The very low cubical mesothelium which covers the arachnoid membrane on both surfaces and also invests the arachnoid trabeculae differentiates apparently from the original mesenchymal elements in the periaxial tissues.

One of the most interesting features of this study has been the relation of the various mesenchymal condensations to the foreign true solution which was introduced into the medullary-canal system. This fluid circulated throughout the periaxial spaces which enlarge to form the subarachnoid channels, but it never penetrated the primary blastema which served as a primitive dura, nor did it ever invade the pial cells which so closely adhere to the nervous tissue; likewise, as soon as the secondary mesenchymal condensation dividing the dura from the arachnoid spaces appeared, this condensation was impervious to the true solution. No evidence of any penetration, as might be expected as due to diffusion, could be made out.

This summary has been included in order that some correlation between the topics discussed separately in the foregoing sections might be made. No attempt has been made here to present the findings in abstract form; these have been summarized at the end of each division of this communication.

XV. CONCLUSIONS.

Based on the observations recorded in the foregoing sections, the following conclusions seem warranted:

(1) During the early part of the growth of the pig embryo there is no extra-ventricular spread of the cerebro-spinal fluid. The first extension of the ventricular fluid into the periaxial tissues occurs in pig embryos of 14 mm.; the adult relationship of the ventricular and meningeal cerebro-spinal fluid is established in pig embryos of about 26 mm.

(2) The ventricular cerebro-spinal fluid escapes into the periaxial tissues through two areas of ependymal differentiation in the roof of the fourth ventricle. Both of these areas differentiate at a slightly earlier period than that at which they function actively. The area membranacea superior undergoes a gradual regression and obliteration due to the changing form of the rhombic roof; the area membranacea inferior gradually occupies the major portion of the velum chorioidea inferior.

(3) The embryonic cerebro-spinal fluid, as evidenced by the replacement with a true solution, spreads from the ventricles into the mesenchymal tissue about the central nervous system. It does not penetrate the cranial or vertebral blastemal condensations, nor does it invade the pial cellular layer.

(4) The subarachnoid spaces arise by a process of breaking-down of the perimedullary mesenchymal syncytium and a dilatation of the existent mesenchymal spaces. This phenomenon of the enlargement of the mesenchymal spaces is associated with the presence in the spaces of an increased amount of albumen. The process occurs at a period slightly later than that at which the initial flow of the cerebro-spinal fluid into the spaces is recorded.

(5) The dura mater, arachnoid, and pia mater develop out of the perimedullary mesenchyme. The arachnoid trabeculae are left by the breaking-down of the original mesenchymal strands, while the outer arachnoid membrane is formed, together with the inner surface of the dura, by a separate mesenchymal condensation. The dura develops between this secondary line of condensation and the embryonic skull.

(6) There is indicated a very close relationship between the tufting of the chorioid plexuses of the fourth ventricle and the first extraventricular spread of the cerebro-spinal fluid.

(7) By means of the method of replacement it is possible to demonstrate perineural spaces as far out along the nerve trunks as the peripheral ganglia. The extensive injections of the perineural spaces along the segmental nerves are not obtained by the method of replacement.

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EXPLANATION OF PLATES.

KEY FOR FIGURE-LEGENDS.

ami, area membranacea inferior.
ams, area membranacea superior.
cbi, cranial blastema.
cm, cisterns cerebelli-medullaris.
chp, plexus chorioideus.

dine, dura mater cerebri (inner surface, in approximation with arachnoid).
epc, epithelial-like cells lining ventricle.
epc, ependyma.
4re, ventriculus quartus.

pme, pia mater cerebri.
ppb, precipitated prussian-blue.
psn, reduced silver nitrate.
sas, subarachnoid spaces.
str, sinus transversus.

PLATE I.

- FIG. 1. Drawing of a pig embryo of 9 mm., into the spinal central canal of which an injection of 0.5 per cent solution of potassium ferrocyanide and iron-ammonium citrate was made under very mild syringe-pressure. The embryo was fixed in Carnoy's fluid to which 1 per cent hydrochloric acid had been added. The specimen was carefully dehydrated and cleared by the Spalteholz method. The resultant precipitate of prussian-blue is found wholly within the central canal of the spinal cord and within the cerebral ventricles. Enlargement, 11 diameters.
- FIG. 2. Drawing of a pig embryo of 13 mm., in which the cerebro-spinal fluid was replaced by a 1 per cent solution of potassium ferrocyanide and iron-ammonium citrate. The embryo was kept alive for 90 minutes after this replacement and was then fixed in 10 per cent formol containing 1 per cent hydrochloric acid. After dehydration the specimen was cleared by the Spalteholz method. The occurrence of a definite oval, outlined by the denser mass of the granules, in the roof of the fourth ventricle, is characteristic of this stage. Enlargement, 9 diameters.
- FIG. 3. Drawing of a pig embryo of 14.5 mm. in which the cerebro-spinal fluid was likewise replaced by the ferrocyanide solution. After the replacement, the embryo was kept alive for 60 minutes; it was fixed in Carnoy's fluid (with 1 per cent hydrochloric acid added) and after dehydration it was cleared by the Spalteholz method. The earliest indications of a periaxial spread of the replaced fluid from the roof of the fourth ventricle is here shown. Enlargement, 8 diameters.

PLATE II.

- FIG. 4. Drawing of a pig embryo of 18 mm., in which a typical replacement of the spinal fluid had been made. The animal was kept alive for 45 minutes and was then fixed, dehydrated, and cleared in the usual manner. The extraventricular spread of the replaced fluid from two areas in the roof of the fourth ventricle is well illustrated. Enlargement, 9 diameters.
- FIG. 5. Drawing of a pig embryo of 19 mm., in which likewise a typical replacement of the cerebro-spinal fluid by the ferrocyanide solution had been made. After this procedure, the embryo was kept alive for 55 minutes and was then carried through the routine technique for the Spalteholz method. The further pericerebral spread of the replaced fluid is recorded. Enlargement, 8 diameters.

PLATE III.

- FIG. 6. A frank lateral drawing of a pig embryo of 21 mm. The typical replacement of the embryonic cerebro-spinal fluid by the ferrocyanide solution was effected in this embryo and it was then kept alive for 45 minutes. At the end of this time the embryo was fixed in an acid fluid, dehydrated, and cleared. The almost complete periaxial spread of the replaced fluid is indicated by the precipitated granules. Enlargement, 7.6 diameters.
- FIG. 7. A dorsal view of the embryo illustrated in fig. 6. The perispinal spread of the replaced fluid is well shown. Enlargement, 7.8 diameters.

PLATE IV.

- FIG. 8. Drawing of a pig embryo of 26 mm. in which the typical replacement of the cerebro-spinal fluid has been made. After the introduction of the ferrocyanide solution the embryo was kept alive for one hour; at the end of this time it was fixed in an acid solution, subsequently dehydrated, and cleared in oil of wintergreen. The specimen shows a complete periaxial spread of the replaced fluid, as evidenced by the precipitated granules, in addition to a total filling of the intramedullary system. Enlargement, 6.5 diameters.
- FIG. 9. Drawing of a pig embryo of 16 mm., in which the central canal of the spinal cord was injected with the ferrocyanide solution under moderate syringe-pressure. After fixation in an acid medium the embryo was dehydrated and cleared by the Spalteholz method. The extraventricular spread in the peribulbar region is easily made out. Enlargement, 9 diameters.

PLATE V.

- FIG. 10. Drawing of a pig embryo of 21 mm., in which an injection of diluted india ink was made into the central canal of the spinal cord. The pressure employed was the highest obtainable from the syringe, yet below the tension causing rupture. The specimen, after injection, was fixed, dehydrated, and cleared. The slight extent of the periaxial spread of the carbon granules can be easily seen. Enlargement, 7 diameters.
- FIG. 11. Drawing of a pig embryo of 16 mm., in which an injection (under moderate syringe-pressure) of 0.5 per cent solution of silver nitrate was made into the central canal of the spinal cord. The silver was reduced in the sunlight, the embryo then fixed. After dehydration, the embryo was cleared in benzol and oil of wintergreen. Enlargement, 7.5 diameters.
- FIG. 12. Drawing of a pig embryo of 13 mm.; into the central canal of the spinal cord a dilute solution of nitrate of silver was injected under strong syringe-pressure. Reduction of the silver was accomplished by exposure to sunlight; the embryo was then fixed, dehydrated, and cleared. Enlargement, 9 diameters.

PLATE VI.

- FIG. 13. Photomicrograph of transverse section of a pig embryo of 18 mm. Specimen obtained from an embryo in which the cerebro-spinal fluid was replaced by a 1 per cent solution of potassium ferrocyanide and iron-ammonium citrate. After this replacement the embryo was kept alive for 65 minutes. The resultant prussian-blue precipitate is not included in this photomicrograph. Enlargement, 13 diameters.
- FIG. 14. Drawing of blocked area in fig. 13, under higher magnification and including the resultant precipitate of prussian-blue. The typical ependymal cells (*epe*) lining the fourth ventricle are shown on either side; between them occurs the area membranacea superior (*ams*). The transit of the replacement fluid through the membranous area and the spread through the adjacent mesenchyme are illustrated. Enlargement, 245 diameters.
- FIG. 15. Photomicrograph of transverse section from embryo pig illustrated in fig. 13. Section taken from more caudal plane than that given in the former figure. The prussian-blue spread is not illustrated. Enlargement, 10 diameters.
- FIG. 16. Drawing, under higher magnification, of the rectangular area in fig. 15. The passage of the replaced solution, as shown by the resultant precipitate of prussian-blue, through the area membranacea inferior (*ami*) is here illustrated. The extension of the replaced fluid through the adjacent mesenchyme and the non-penetration of the solution into the condensed mesenchyme are shown. Enlargement, 140 diameters.
- FIG. 17. Photomicrograph of sagittal section of a pig embryo of 18 mm. Specimen obtained from an embryo in which the cerebro-spinal fluid was replaced by a 1 per cent solution of potassium ferrocyanide and iron-ammonium citrate. After this replacement the animal was kept alive for 45 minutes. Fixed for 5 minutes in 10 per cent formol containing 1 per cent hydrochloric acid; then over night in modified Bouin's solution (saturated aqueous solution of picric acid 75, formaldehyde 10, glacial acetic acid 10). Dehydrated by 2 and 4 per cent grades of alcohol; embedded in xylol-paraffin. Serial sections, stained by hematoxylin and eosin. The resultant precipitate of prussian-blue has not been reproduced in the photomicrograph. Enlargement, 8 diameters.
- FIG. 18. Drawing of blocked area in fig. 17 under higher magnification. The granules of prussian-blue are here represented by the blue stenciling. The transit of the fluid, as shown by the granules, into the periaxial mesenchyme through the two membranous areas (*ams* and *ami*) in the roof of the fourth ventricle are well shown. Enlargement, 35 diameters.

PLATE VII.

- FIG. 19. Photomicrograph from a sagittal section of a fetal pig of 27 mm. The cerebro-spinal fluid in this specimen was replaced by a 1 per cent solution of potassium ferrocyanide and iron-ammonium citrate; the fetus was kept alive for 40 minutes; fixed in 10 per cent formol containing 1 per cent hydrochloric acid for 15 minutes; then over night in modified Bouin's solution; dehydrated by 2 and 4 per cent grades of alcohol; embedded in xylol-paraffin. The prussian-blue granules are not represented in this photomicrograph. Enlargement, 8 diameters.
- FIG. 20. Drawing of squared area in fig. 19. The center of the field is occupied by the optic nerve; around it the developing extrinsic optic muscles are shown. The precipitate of prussian-blue occurs in the perineural mesenchyme. Enlargement, 190 diameters.
- FIG. 21. Photomicrograph of rectangular area in fig. 19. The passage of the ferrocyanide solution into the sinus transversus (*str*) is represented by the precipitated blue granules. Enlargement, 133 diameters.
- FIG. 22. Photomicrograph of a transverse section of a pig embryo of 23 mm. The cerebro-spinal fluid was replaced in this embryo with a 1 per cent solution of potassium ferrocyanide and iron-ammonium citrate. The embryo was kept alive for 50 minutes and was then fixed over night in 10 per cent formol containing 1 per cent hydrochloric acid. The granules of prussian-blue are not shown in this reproduction. Enlargement, 13 diameters.
- FIG. 23. Drawing of squared area in fig. 22. The area membranacea superior (*ams*) is shown, surrounded on either side by tufts of the chorioid plexus (*chp*) and the typical ventricular ependyma. The transit of the solution is shown, as represented by the resultant granules, through the area, with the subsequent spread into the periaxial mesenchyme. Enlargement, 125 diameters.

PLATE VIII.

- FIG. 24. Photomicrograph of a transverse section of a pig embryo of 8 mm. Fixed in modified Bouin's solution over night, dehydrated by 2 and 4 per cent grades of alcohol, embedded in xylol-paraffin. Enlargement, 30 diameters.
- FIG. 25. Photomicrograph, retouched, of the blocked area in fig. 24. The character of the cells (*cpc*) composing the roof of the fourth ventricle (*4ve*) is shown in this reproduction. Enlargement, 165 diameters.
- FIG. 26. Photomicrograph of a sagittal section from a pig embryo of 11 mm. Fixed in modified Bouin's solution over night, dehydrated by 2 and 4 per cent grades of alcohol, embedded in xylol-paraffin. Enlargement, 11 diameters.
- FIG. 27. Photomicrograph of the blocked area in fig. 26. The area membranacea superior (*ams*) in the roof of the fourth ventricle is shown sharply delimited from the two processes of typical ependyma (*epe*). Enlargement, 67 diameters.
- FIG. 28. Photomicrograph of a more lateral section of the pig embryo of 11 mm. given in fig. 26. Enlargement, 11 diameters.
- FIG. 29. Photomicrograph, under higher magnification, of the blocked area in fig. 28. The lateral border of the area membranacea superior (*ams*) of the roof of the fourth ventricle is given. Enlargement, 50 diameters.
- FIG. 30. Photomicrograph of a sagittal section from a pig embryo of 13 mm. Fixed in modified Bouin's solution, dehydrated by 2 and 4 per cent grades of alcohol, and embedded in xylol-paraffin. Enlargement, 8 diameters.
- FIG. 31. Photomicrograph, under higher magnification, of the squared area in fig. 30. The reproduction comprises a sagittal section of the area membranacea superior (*ams*) of the roof of the fourth ventricle. Enlargement, 67 diameters.
- FIG. 32. Photomicrograph of a sagittal section of a pig embryo of 14 mm. Fixed in modified Bouin's solution, dehydrated by 2 and 4 per cent grades, and embedded in xylol-paraffin. Enlargement, 11 diameters.
- FIG. 33. Photomicrograph of the blocked area in fig. 32 under higher magnification. The area membranacea superior (*ams*) in the roof of the fourth ventricle is reproduced. Enlargement, 75 diameters.

PLATE IX.

- FIG. 34. Photomicrograph of a transverse section of a pig embryo of 18 mm. Fixed in Carnoy's fluid (6 : 3 : 1), dehydrated by 2 and 4 per cent changes of alcohol, and embedded in xylol-paraffin. Enlargement, 13 diameters.
- FIG. 35. Photomicrograph, under higher magnification, of the blocked area in fig. 34. The area membranacea superior (*ams*) is here given, flanked on either side by typical ependyma (*epe*). Enlargement, 170 diameters.
- FIG. 36. Photomicrograph of a transverse section of a pig embryo of 18 mm. Fixed in modified Bouin's fluid, dehydrated by 2 and 4 per cent changes, and embedded in xylol-paraffin. Enlargement, 13 diameters.
- FIG. 37. Photomicrograph of rectangular area outlined in fig. 36. The extent of the area membranacea superior (*ams*), with its adherent coagulum of albuminous material, is well differentiated from the adjacent typical ventricular ependyma (*epe*). Enlargement, 100 diameters.
- FIG. 38. Photomicrograph of a transverse section of a pig embryo of 19 mm. Fixed in modified Bouin's solution, dehydrated by 2 and 4 per cent grades, and embedded in xylol-paraffin. Enlargement, 13 diameters.
- FIG. 39. Photomicrograph, under higher power, of the rectangular area in fig. 38. A small break in the integrity of the lining ependyma of the roof of the fourth ventricle, representing the irregular boundary of the area membranacea superior (*ams*), is given. Enlargement, 290 diameters.
- FIG. 40. Photomicrograph of a transverse section of a human embryo of 4 mm. (No. 836 of collection of Carnegie Institution of Washington). Enlargement, 33 diameters.
- FIG. 41. Photomicrograph, retouched, of the blocked area in fig. 40. The epithelial-like cells (*epc*) composing the roof of the fourth ventricle (*4ve*) are here shown separated from the denser nervous tissue. Enlargement, 100 diameters.

PLATE X.

- FIG. 42. Photomicrograph of transverse section of pig embryo of 19 mm. Fixed over night in modified Bouin's solution, dehydrated by 2 and 4 per cent changes of alcohol, and embedded in xylol-paraffin. Enlargement, 13 diameters.
- FIG. 43. Photomicrograph of squared area in figure 42, under higher magnification. The area membranacea superior (*ams*) with the attached coagulum of albumen is reproduced. Enlargement, 115 diameters.
- FIG. 44. Photomicrograph of sagittal section of pig embryo of 23 mm. Fixed in modified Bouin's fluid, dehydrated by 2 and 4 per cent changes, and embedded in xylol-paraffin. Enlargement, 5 diameters.
- FIG. 45. Photomicrograph, under higher magnification, of squared area in fig. 44. The area membranacea superior (*ams*) is here shown, delimited by the cells of the chorioid plexus (*chp*) on one side and by the further ependymal prolongation (*epe*) of the cerebellar lip. Enlargement, 88 diameters.
- FIG. 46. Photomicrograph of sagittal section of pig embryo of 32 mm. Fixed in modified Bouin's solution, dehydrated by 2 and 4 per cent changes, and embedded in xylol-paraffin. Certain portions of the dura mater (*dme*) are indicated. Enlargement, 5 diameters.
- FIG. 47. Photomicrograph of blocked area in fig. 46, under higher magnification. The small remaining area membranacea superior (*ams*) is quite surrounded by encroaching ependyma in the chorioid folds. Enlargement, 88 diameters.

PLATE X—Continued.

- FIG. 48. Photomicrograph of transverse section of human embryo of 7 mm. (No. 617 of the collection of the Carnegie Institution of Washington). Enlargement, 10 diameters.
- FIG. 49. Photomicrograph of squared area in fig. 48, under higher magnification. The epithelial-like cells (*epc*) composing the roof of the fourth ventricle at this stage are well shown. Enlargement, 100 diameters.
- FIG. 50. Photomicrograph of transverse section of human embryo of 7 mm. (No. 617 in the Carnegie Institution of Washington). Enlargement, 10 diameters.
- FIG. 51. Photomicrograph of blocked area in fig. 50. The marked invagination of the roof of the fourth ventricle (*4ve*) with the lining of epithelial-like cells (*epc*) is given. Enlargement, 33 diameters.
- FIG. 52. Photomicrograph of transverse section of human embryo of 9 mm. (No. 721 in the collection of the Carnegie Institution of Washington). Enlargement, 10 diameters.
- FIG. 53. Photomicrograph of squared area outlined in fig. 52. The pale, large cells (*epc*) comprising the roof of the fourth ventricle characterize the reproduction. Enlargement, 50 diameters.
- FIG. 54. Photomicrograph of blocked area in fig. 54. The apparent break in the continuity of the roof of the fourth ventricle with exudation of the ventricular albumen into the mesenchyme is brought out. Enlargement, 50 diameters.

PLATE XI.

- FIG. 56. Photomicrograph of sagittal section of human embryo of 14 mm. measured on the slide (No. 144 of the collection of the Carnegie Institution of Washington). Enlargement, 8 diameters.
- FIG. 57. Photomicrograph, under higher magnification, of blocked area in fig. 56. The greater part of the ventricular wall shown is composed of the area membranacea superior (*ams*), bounded below by typical ventricular ependyma (*epe*). Enlargement, 67 diameters.
- FIG. 58. Photomicrograph of sagittal section of human embryo of 17 mm. (No. 576 of the collection of the Carnegie Institution of Washington). Enlargement, 10 diameters.
- FIG. 59. Photomicrograph of rectangular area in fig. 58, showing the area membranacea superior (*ams*) of the roof of the fourth ventricle. Enlargement, 50 diameters.
- FIG. 60. Photomicrograph of sagittal section of human embryo of 17 mm. (No. 576 of the collection of the Carnegie Institution of Washington). Enlargement, 7 diameters.
- FIG. 61. Photomicrograph of the blocked area in fig. 60 under higher magnification. The aggregation of epithelial-like cells (*epc*) on the lateral border of the area membranacea superior is here portrayed. Enlargement, 67 diameters.
- FIG. 62. Photomicrograph of transverse section of human embryo of 18 mm. (No. 409 of the collection of the Carnegie Institution of Washington). Enlargement, 7 diameters.
- FIG. 63. Photomicrograph, under higher power, of squared field in fig. 62. The peculiar inversion of the roof of the fourth ventricle (*4ve*) indicated in fig. 62, has resulted in a marked dislocation of the area membranacea superior (*ams*), shown in this figure. Enlargement, 75 diameters.

PLATE XII.

- FIG. 64. Photomicrograph, retouched, of a transverse section of a human embryo of 21 mm. (No. 460 of the collection of the Carnegie Institution of Washington). The field taken consists of a portion of the fourth ventricle with the lining of typical ependyma (*epe*) on either side. The area membranacea superior (*ams*) is shown between the two lips of ependyma. Enlargement, 33 diameters.
- FIG. 65. Photomicrograph, retouched, of a similar section to that given in fig. 64, but taken from a more anterior plane from the same embryo. The field shown is analogous in every way to that in the preceding figure.
- FIG. 66. Photomicrograph of a transverse section of an embryo chick of 121 hours' incubation. Fixed in Bouin's solution. Enlargement, 15 diameters.
- FIG. 67. Retouched photomicrograph, under higher magnification, of the blocked area in fig. 66. The area membranacea superior (*ams*) is here given, delimited sharply from the lips of ependyma (*epe*) which line the roof of the fourth ventricle. Enlargement, 133 diameters.
- FIG. 68. Photomicrograph of a more caudal section from the same embryo as portrayed in fig. 66. Enlargement, 15 diameters.
- FIG. 69. Retouched photomicrograph, under higher magnification, of the blocked area in fig. 68. The area membranacea superior (*ams*) is shown at the point of its greatest transverse diameter. Enlargement, 88 diameters.
- FIG. 70. Photomicrograph of a sagittal section of a pig embryo of 15 mm. Fixed in modified Bouin's solution, dehydrated by 2 and 4 per cent grades of alcohol, and embedded in xylol-paraffin. Enlargement, 8 diameters.
- FIG. 71. Photomicrograph, under higher magnification, of blocked area in fig. 70. The earliest evidence of the area membranacea inferior (*ami*) in the roof of the fourth ventricle is here shown. Enlargement, 125 diameters.

PLATE XIII.

- FIG. 72. Photomicrograph of sagittal section of a pig embryo of 18 mm. Fixed in modified Bouin's solution, dehydrated by 2 and 4 per cent grades of alcohol, and embedded in xylyl-paraffin. Enlargement, 11 diameters.
- FIG. 73. Photomicrograph, under higher power, of the rectangular area outlined in fig. 72. The enlarging area membranacea inferior (*ami*) is shown in the midst of the typical lining ependyma of the roof. Enlargement, 100 diameters.
- FIG. 74. Photomicrograph of sagittal section of a pig embryo of 23 mm. Fixed in modified Bouin's solution, dehydrated by 2 and 4 per cent grades of alcohol, and embedded in xylyl-paraffin. Enlargement, 6 diameters.
- FIG. 75. Photomicrograph of blocked area in fig. 74. The area membranacea inferior (*ami*) is, at this stage, quite extensive, as shown in the reproduction; the early stages in the development of the cisterna cerebello-medullaris may also be seen. Enlargement, 75 diameters.
- FIG. 76. Photomicrograph of sagittal section of a pig embryo of 32 mm. Fixed in modified Bouin's solution, dehydrated by 2 and 4 per cent grades of alcohol, and embedded in xylyl-paraffin. Enlargement, 7 diameters.
- FIG. 77. Photomicrograph, under higher magnification, of blocked area in fig. 76. The unsupported character of the area membranacea inferior and the formation of the cisterna cerebello-medullaris is here reproduced. Enlargement, 67 diameters.

PLATE XIV.

- FIG. 78. Photomicrograph of a sagittal section of a pig embryo of 32 mm. Fixed in modified Bouin's solution, dehydrated by 2 and 4 per cent grades of alcohol, and embedded in xylyl-paraffin. Enlargement, 7 diameters.
- FIG. 79. Photomicrograph of the blocked area in fig. 78, under higher magnification. The intact area membranacea inferior (*ami*), unsupported by any mass of tissue, is shown separating the ventricular cavity from the developing cisterna cerebello-medullaris. Enlargement, 67 diameters.
- FIG. 80. Photomicrograph of a sagittal section of a human embryo of 16 mm. (No. 406 in the collection of the Carnegie Institution of Washington). Enlargement, 7 diameters.
- FIG. 81. Photomicrograph of the area outlined in fig. 80, but under higher magnification. An early stage in the differentiation of the area membranacea inferior (*ami*) is given. Enlargement, 50 diameters.
- FIG. 82. Photomicrograph of a sagittal section of a human embryo of 17 mm. (No. 576 in the collection of the Carnegie Institution of Washington). Enlargement, 6 diameters.
- FIG. 83. Photomicrograph, under higher power, of the area blocked in fig. 82. The chorioid plexuses of the fourth ventricle lie in the central portion of the field; above is the thick cell-layer on the lateral side of the area membranacea superior (*ams*), while below the upper limit of the area membranacea inferior (*ami*) appears. Enlargement, 67 diameters.
- FIG. 84. Photomicrograph of a transverse section of a human embryo of 18 mm. (No. 409 in the collection of the Carnegie Institution of Washington). Enlargement, 5 diameters.
- FIG. 85. Photomicrograph of the blocked area in fig. 84. The cellular character, and especially the clumping of cells, of the area membranacea inferior (*ami*) is shown. Enlargement, 25 diameters.
- FIG. 86. Photomicrograph of a sagittal section of a human embryo of 19 mm. (No. 431 in the collection of the Carnegie Institution of Washington). Enlargement, 5 diameters.
- FIG. 87. Photomicrograph of the blocked area outlined in fig. 86. The area membranacea inferior (*ami*) appears separating the fourth ventricle from the developing cisterna cerebello-medullaris. Enlargement, 25 diameters.

PLATE XV.

- FIG. 88. Photomicrograph from a sagittal section of a human embryo of 17 mm. (No. 576 of the collection of the Carnegie Institution of Washington), representing an enlargement of the second blocked area in fig. 58. The area membranacea inferior (*ami*) appears sharply delimited from the adjoining typical ependyma. Enlargement, 67 diameters.
- FIG. 89. Photomicrograph of a sagittal section of a human embryo of 23 mm. (No. 453 of the collection of the Carnegie Institution of Washington). Enlargement, 6 diameters.
- FIG. 90. Photomicrograph of the blocked area in fig. 89. The area membranacea superior (*ams*) appears in the stage of closure, while the area membranacea inferior (*ami*) is becoming well differentiated from the typical ependyma lining the other portions of the fourth ventricle. Enlargement, 26 diameters.
- FIG. 91. Photomicrograph of a sagittal section of a human embryo of 20 mm. (No. 1008 of the collection of the Carnegie Institution of Washington). Enlargement, 4.5 diameters.
- FIG. 92. Photomicrograph, under higher magnification, of the blocked area in fig. 91. The area membranacea superior has been almost completely closed by the dense ependyma of the superior half of the roof of the fourth ventricle, while the inferior area (*ami*) has become a membrane lacking wholly the character of ependyma. Enlargement, 23 diameters.
- FIG. 93. Photomicrograph of a sagittal section of a human embryo of 35 mm. (No. 199 of the collection of the Carnegie Institution of Washington). Enlargement, 3 diameters.
- FIG. 94. Photomicrograph, under higher powers, of the blocked areas in fig. 93. The formation of the cisterna cerebello-medullaris is shown in relation to the ventricular roof. Enlargement, 23 diameters.
- FIG. 95. Drawing of cells of the chorioid plexus from the lateral ventricles of a fetal pig of 132 mm. The specimen was fixed in absolute alcohol, and stained by Best's carmine stain for glycogen. The glycogen occurs in the form of globules within the epithelial cells. Enlargement, 950 diameters.
- FIG. 96. Drawings of the cells of the chorioid plexus from the lateral ventricles of a fetal pig of 36 mm. The specimen was fixed in absolute alcohol and stained by Best's carmine method. The glycogen appears in the epithelial cells in the form of basilar plaques. Enlargement, 950 diameters.

PLATE XVI.

- FIG. 97. Photomicrograph of a transverse section of a pig embryo of 19 mm. Fixed in modified Bouin's fluid, dehydrated by 2 and 4 per cent grades of alcohol, and embedded in xylol-paraffin. Enlargement, 10 diameters.
- FIG. 98. Photomicrograph, under higher magnification, of the blocked area in fig. 97. The double condensations of mesenchyme to form pia mater (*pmc*) and cerebral blastema (*chl*) appear separated by a region of mesenchyme which is breaking down. This central area of mesenchyme, with the marked albumen-content, is to become the arachnoid spaces. Enlargement, 133 diameters.
- FIG. 99. Photomicrograph of a transverse section of a pig embryo of 20 mm. Fixed in modified Bouin's fluid, dehydrated by 2 and 4 per cent changes of alcohol, and embedded in xylol-paraffin. Enlargement, 10 diameters.
- FIG. 100. Photomicrograph, under higher powers, of the blocked areas in fig. 99. The relations of the pial condensation (*pmc*) of mesenchyme to the nervous system, as well as the infiltration of the arachnoid mesenchyme (*sas*) with albumen, is reproduced. Enlargement, 133 diameters.
- FIG. 101. Photomicrograph, under higher magnification, of the blocked area in fig. 22. The reproduction is included here to show the double condensation (*chl*) of mesenchyme which goes to form ultimately bone and possibly a portion of the dura. Enlargement, 132 diameters.
- FIG. 102. Photomicrograph of a transverse section of a pig embryo of 18 mm. The embryo was one in which the cerebro-spinal fluid was replaced by the ferrocyanide solution. Subsequently the embryo was fixed in 10 per cent formol containing 1 per cent hydrochloric acid for a few minutes to precipitate the prussian-blue. It was then transferred to modified Bouin's solution, dehydrated by 2 and 4 per cent grades of alcohol, and embedded in xylol-paraffin. The granules of prussian-blue are not represented in this figure. Enlargement, 10 diameters.
- FIG. 103. Photomicrograph of the squared area in fig. 102. The relation of the thinning mesenchyme in the arachnoid areas to the caudal cranial nerves is shown. The granules of prussian-blue, scattered through the area of thin mesenchyme (*sas*), are not reproduced. Enlargement, 40 diameters.
- FIG. 104. Photomicrograph of a coronal section of a tissue block which includes the meninges and cerebral cortex in the region of the sinus sagittalis superior. The block was obtained from a fetal pig of 80 mm., fixed in Zenker's fluid, and stained, after embedding in celloidin, by Mallory's technique for connective tissue. Enlargement, 27 diameters.

PLATE XVII.

- FIG. 105. Photomicrograph of a coronal section of a tissue block including cerebral cortex and meninges in the region of the sinus sagittalis superior. The block was obtained from a fetal pig of 10 cm., fixed in Zenker's fluid, and stained by Mallory's technique for connective tissue. Enlargement, 13 diameters.
- FIG. 106. Photomicrograph of a coronal section, similar to that in figs. 104 and 105, except in that it was obtained from a fetal pig of 17 cm. The same technical procedures employed in the other specimens were used in this. Enlargement, 27 diameters.
- FIG. 107. Photomicrograph of a similar section to those of the foregoing figures. The specimen was obtained from a fetal pig of 20 cm. and was treated in the manner outlined above. Enlargement, 20 diameters.
- FIG. 108. Drawing of the cell pattern from the inner surface of the dura mater of a fetal pig of 5 cm. The specimen was prepared by the reduction of a dilute solution of silver nitrate in sunlight. The preparation was subsequently stained by hematoxylin. Enlargement, 190 diameters.
- FIG. 109. Drawing of a preparation, similar to that of fig. 108, but obtained from the inner surface of the dura mater of a fetal pig of 75 mm. Enlargement, 285 diameters.
- FIG. 110. Drawing of a preparation, similar to those of figs. 108 and 109, obtained from the inner surface of the dura mater of a fetal pig of 90 mm. Enlargement, 285 diameters.
- FIG. 111. Drawing of a preparation from the inner surface of the dura mater of a fetal pig of 16 cm. The specimen was made in the same manner as outlined in fig. 108. Enlargement, 285 diameters.
- FIG. 112. Photomicrograph of a sagittal section of a pig embryo of 17 mm. An injection of an 0.5 per cent solution of nitrate of silver was made into the central canal of the spinal cord; the silver was reduced in sunlight and the embryo fixed in formalin. Enlargement, 13 diameters.
- FIG. 113. Photomicrograph, under higher powers, of the blocked areas in fig. 112. The accumulation of the reduced silver (*psn*) against the area membranacea superior is represented in black. Enlargement, 117 diameters.
- FIG. 114. Photomicrograph of a transverse section of a pig embryo of 19 mm. An injection of 0.5 per cent solution of silver nitrate was made into the central canal of this embryo and the silver immediately reduced in sunlight. The embryo was fixed in formalin, carefully dehydrated, and embedded in xylol-paraffin. Enlargement, 10 diameters.
- FIG. 115. Photomicrograph, under higher magnification, of the blocked area in fig. 114. The collection of reduced silver (*psn*) against the cells at the inferior end of the area membranacea superior is illustrated. Enlargement, 160 diameters.
- FIG. 116. Photomicrograph of a transverse section of a pig embryo of 16 mm. The central canal of the spinal cord of this embryo was injected with a 1 per cent ferrocyanide and citrate solution under mild syringe-pressure; the embryo was then fixed in 10 per cent formol containing 1 per cent hydrochloric acid. Enlargement, 10 diameters.
- FIG. 117. Photomicrograph of the blocked area in fig. 116, under higher magnification. The accumulation of the precipitated injection fluid against the area membranacea superior is represented in black. A slight extraventricular spread of the fluid, which is found in this as in all embryos of this stage, can not be made out in the reproduction. Enlargement, 67 diameters.



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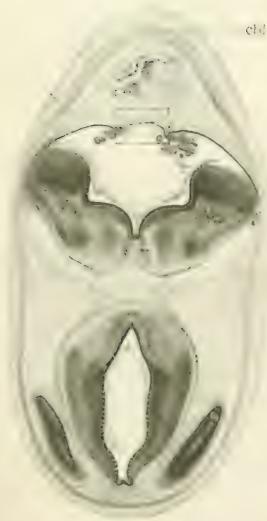
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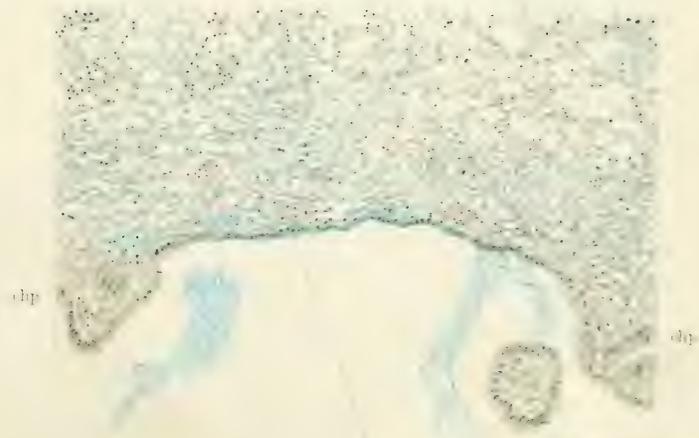
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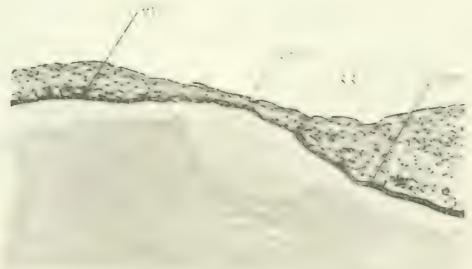
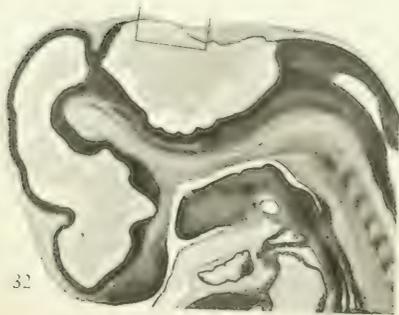
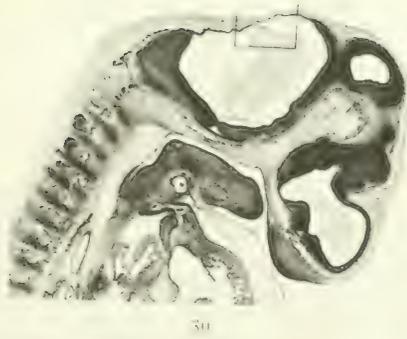
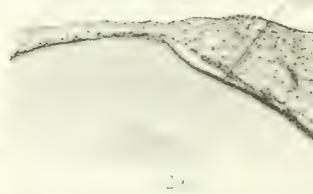
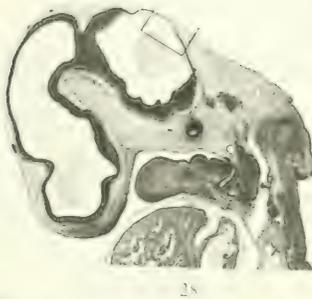
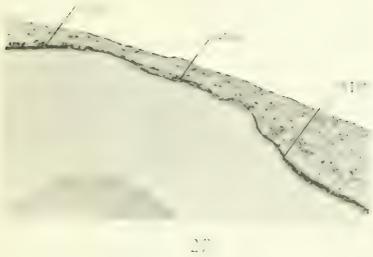
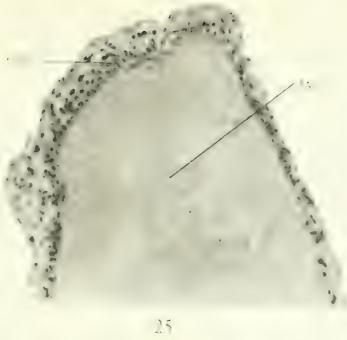
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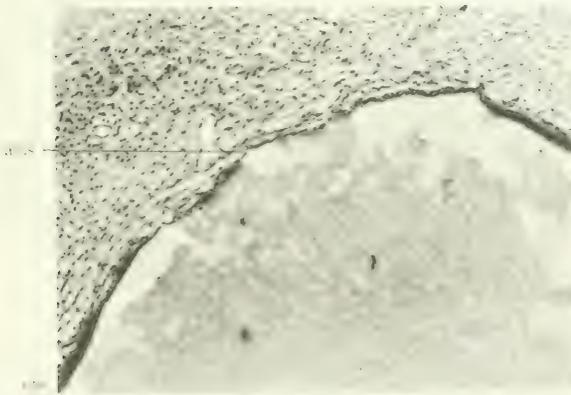


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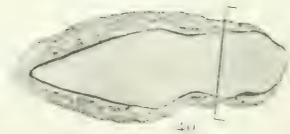
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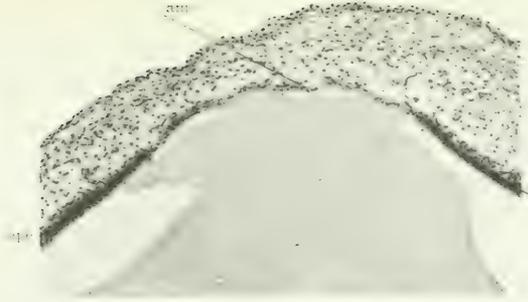


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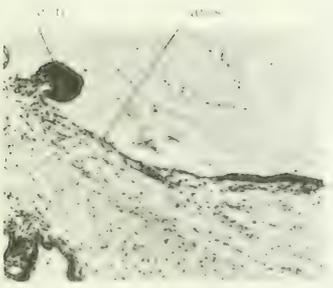
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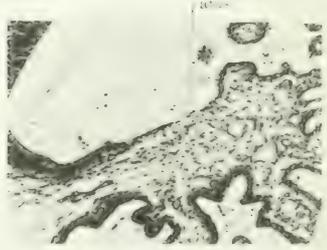
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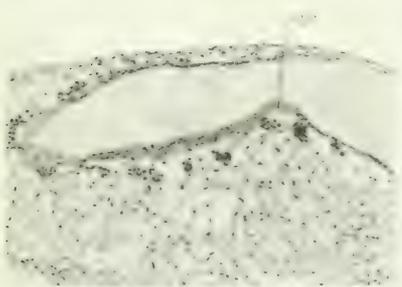
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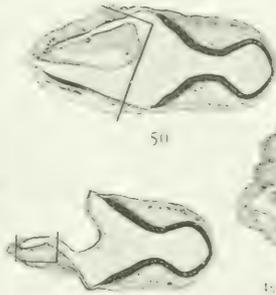
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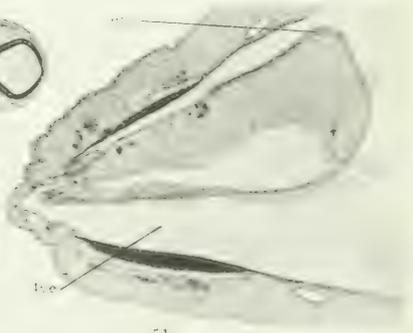
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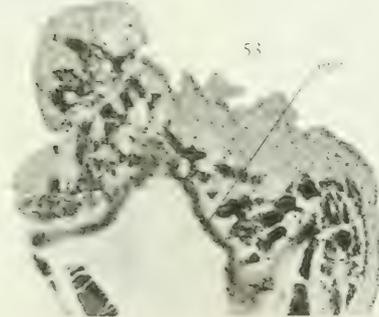
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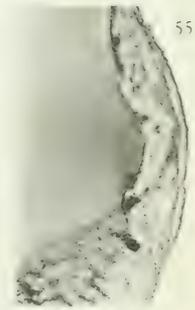
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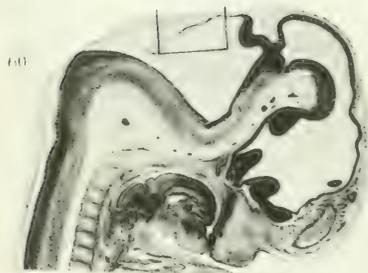
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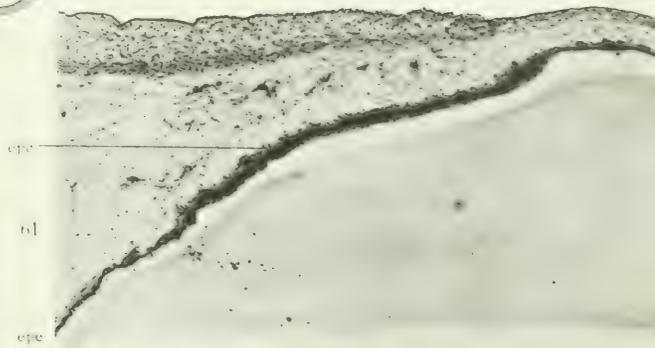
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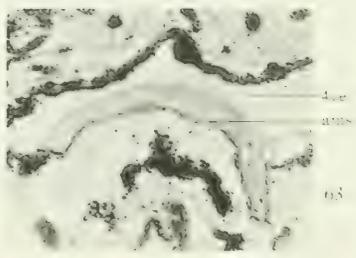


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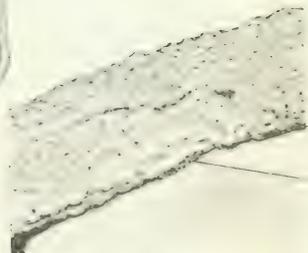
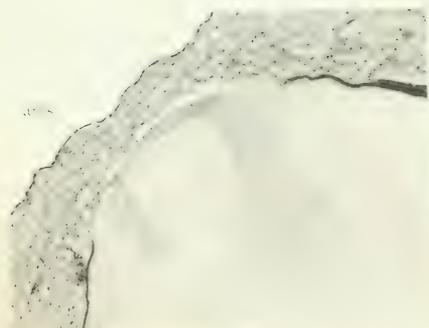
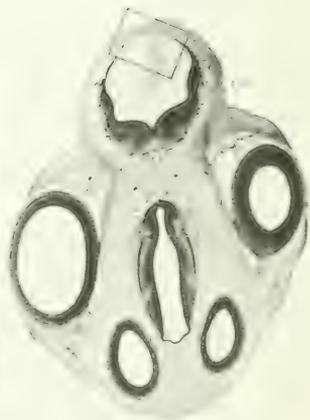
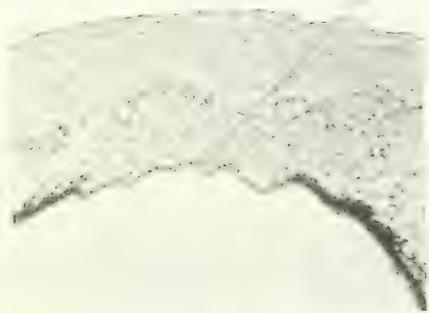
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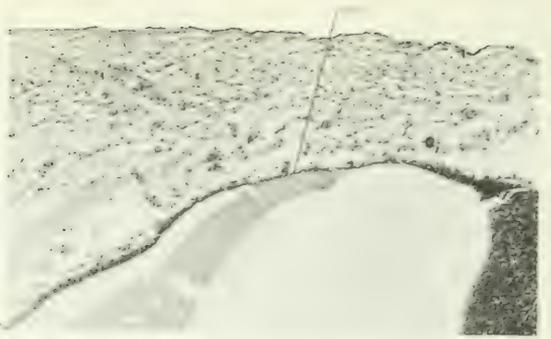


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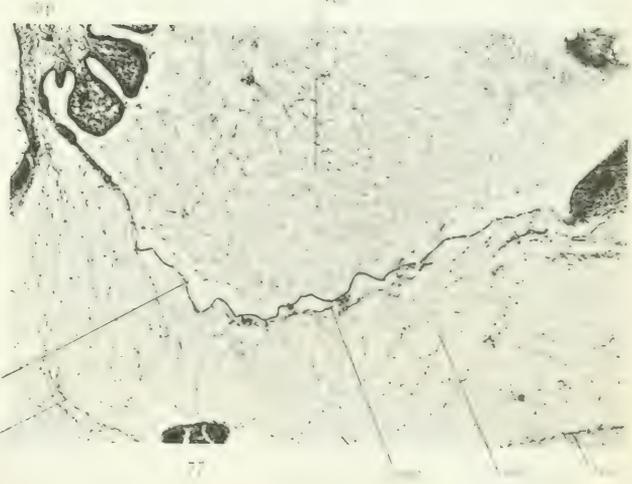


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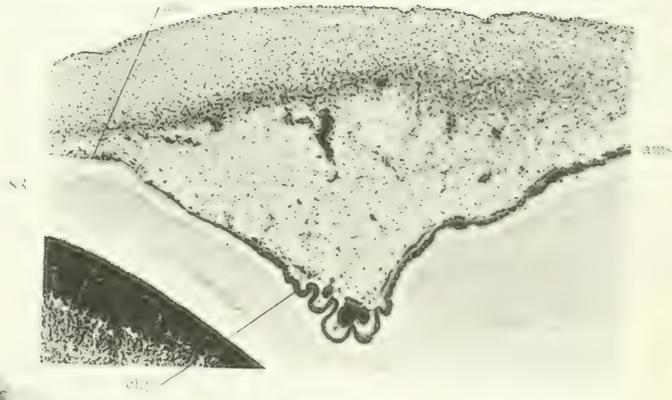
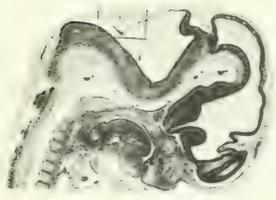
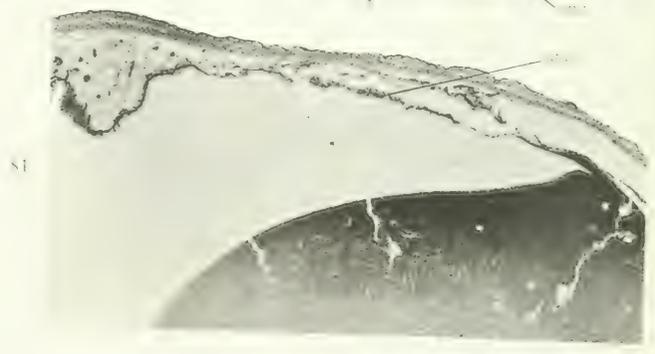
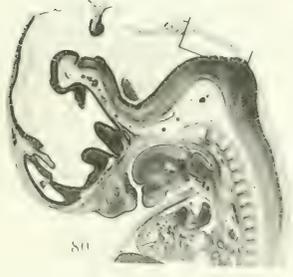
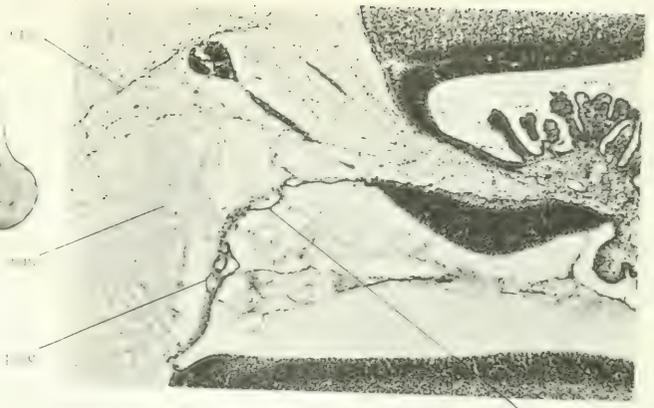
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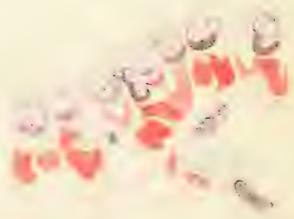
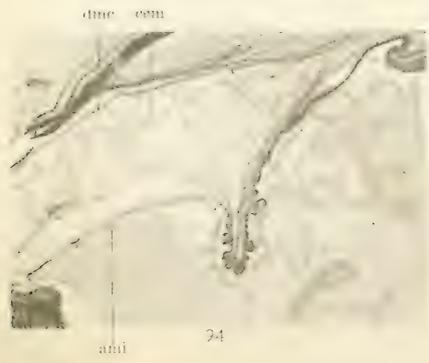
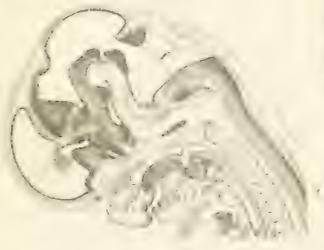
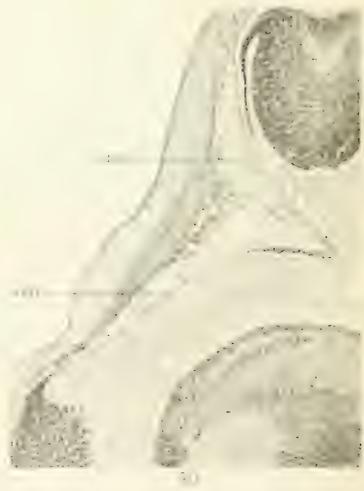
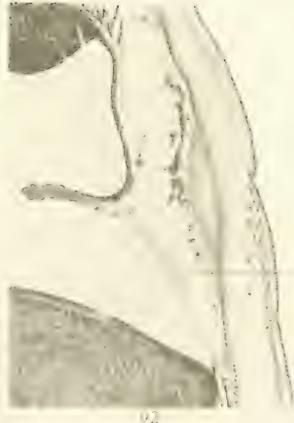
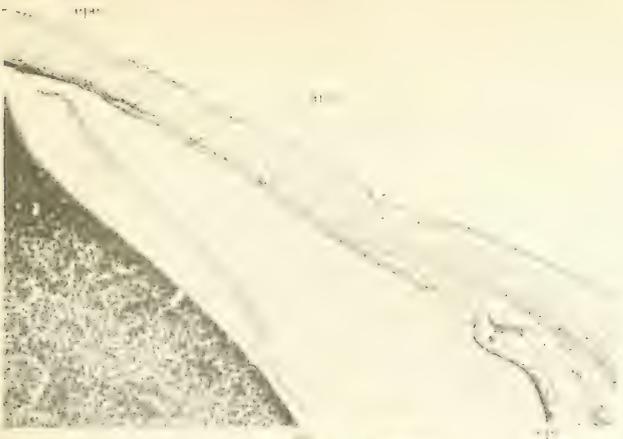


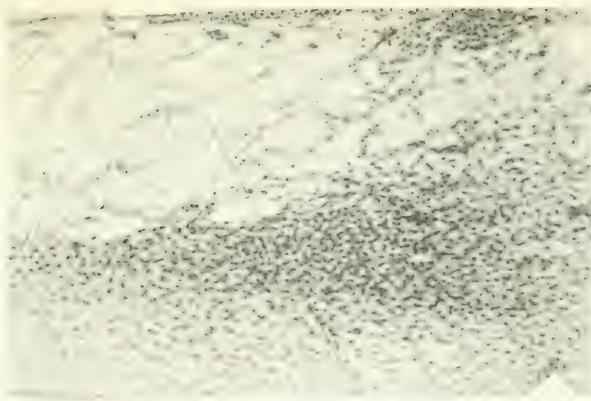
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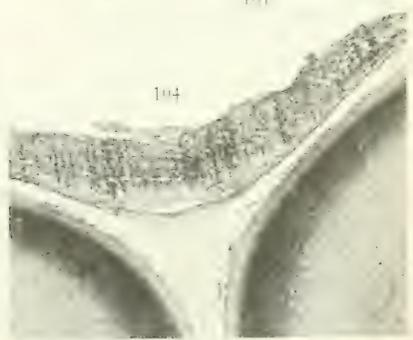
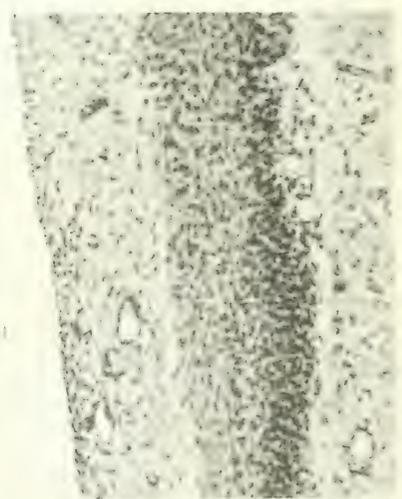
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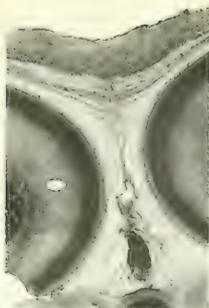




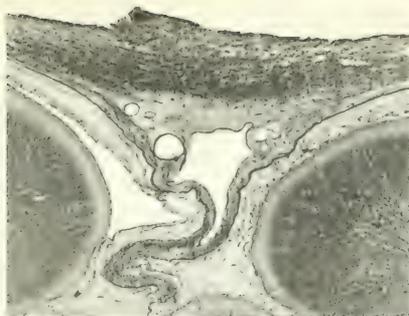


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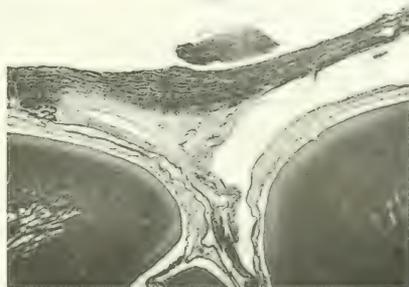
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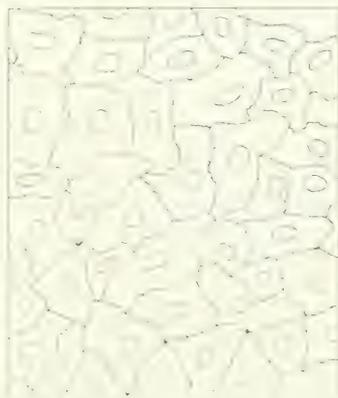
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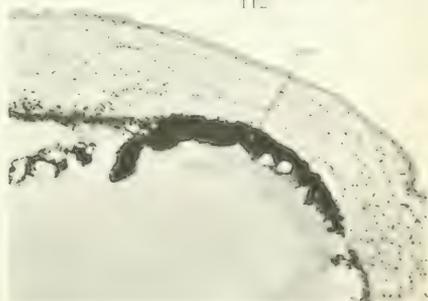
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CONTRIBUTIONS TO EMBRYOLOGY

VOLUME VI, Nos. 15, 16, 17, 18, 19



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CONTRIBUTIONS TO EMBRYOLOGY, No. 15.

CYCLOPIA IN THE HUMAN EMBRYO.

BY FRANKLIN P. MALL.

With three plates and seven figures.

CYCLOPIA IN THE HUMAN EMBRYO.

BY FRANKLIN P. MALL.

The progress made in recent years on the study of teratology has been so marked that it is now possible to reconsider the whole subject and to place it upon a permanent scientific basis. For this progress we are indebted almost exclusively to the experimental embryologists. Problems which formerly seemed impossible of solution—for example, the formation of the double monsters—have yielded as by magic to the embryologist, who made experimental studies upon the living egg. Perhaps the best example that can be brought forward to illustrate this point is the question of the cause of cyclopia. As soon as it was possible to experiment on eggs in such a way that practically all of them developed into cyclopean monsters the explanation of this condition was at hand. For this work we are indebted entirely to Stockard.

Before reviewing the four specimens which I have to report it may be well to give an account of the theories regarding the origin of the cyclopean condition. There are two chief theories, both resting upon an embryological basis. The first of these is that the eggs begin to develop normally and that subsequently, on account of an imperfect development of the head, the eyes coalesce to form a single eye. This theory can be traced back to Meckel. The second is that the eyes arise normally from the midventral line of the brain as a single structure, which in the course of development divides into two eyes. This view was first advanced by Huschke, who believed cyclopia to be due to an arrest of the development of the brain at the time the eyes are forming. Although Huschke's opinion seemed to be quite sound at the time it was advanced, it did not attach itself firmly to literature, nor could we well accept it at present as resting upon a sound embryological basis. The figures which he gives in illustration shows first an early stage of development of the brain, with a marked forebrain, and then an embryo with two eye-vesicles hanging to the forebrain. He apparently confounded the whole forebrain with the eye primordium.

Meckel's studies rest upon much sounder embryological and anatomical evidence, and his views gradually made their way into the literature of teratology. Until a decade ago it was practically impossible to find any description of cyclopia in which Meckel's studies were not reflected in the background. According to Ahlfeld, Meckel states that cyclopia is characterized by a coalescence of the eyeballs as well as of the orbital cavities. In case the orbital cavities unite very early in development they distend evenly in a lateral direction. The tissues which normally separate these cavities are absent or are pushed aside. In fact, the structures which give the frame to the nose are most rudimentary, or absent, while the nose itself is represented as a membranous snout, varying in form and located above the confluent eyes. The mouth is frequently involved in this type

of monster and is usually rudimentary, while in some instances it, as well as the snout, is missing altogether. Since the eyeballs are developed from pouches which arise from the forebrain, it follows that the primary cause of this anomaly is not to be sought in the development of the skull, but in the development of the brain itself. We find in these cases that the width of the forebrain and midbrain diminishes in the course of their development, corresponding to the union of the orbital cavities and the eyeballs, making the brain appear at term much like that of an embryo of the twelfth week. In addition to the atrophy of these parts, the formation of the hemispheres as a single body is especially noticeable—that is, they have not been divided into two lobes. This division often is only slightly indicated. The ventricles have united to form a single large cavity. In most of the cases at birth the quantity of fluid within the ventricle is increased, so that as a rule we have a large, bladder-like body in place of the forebrain. In this way is the fact explained that in spite of the rudimentary development of the brain there is no diminution of the size of the fore part of the head, as most cases of cyclopia are accompanied with hydrocephalus. There are cases, however, in which there is no hydrocephalus, which naturally result in a small head. This is most pronounced in cases of cyclopia in double monsters in which the head contains two brains. In these cases a symmetrical development of the brain is very rarely found. The rudimentary brain can no doubt be held responsible for the most pronounced specimens of cyclopean faces. It may be taken for granted that the nerves which are to supply the deformed eye and face are simple in their development, corresponding in amount with the degree of the anomaly.

This general description of the anatomy of the eye and face in cases of cyclopia is one which will be found in most teratologies, and in all of these accounts it would appear as though the authors mean to say that the eyes must arise from the forebrain and that they subsequently unite into a single compound eye, more or less hourglass-shaped, due to an arrest of the growth of the brain which in some way interfered with the development of the forehead and eventually left the nose above the cyclopean eye. Teratologists are inclined to believe that the accompanying hydrocephalus is to be viewed as the primary cause of the anomaly, although in many instances they try to trace this back to amniotic bands, which, however, are not found in human specimens of cyclopia, and of course such bands could play no rôle in the formation of this anomaly in animals which develop without an amnion. Furthermore, the explanation of the formation of monsters by means of amniotic bands is alluded to in recent teratologies only as one of the myths of teratology.

In my paper on monsters some ten years ago I gave a review of the experimental work upon cyclopia as it appeared at that time. These statements I shall recapitulate in part in order to bring out more clearly the recent progress made in the study of cyclopia.

In numerous experiments upon frog's eggs, Born, in 1897, occasionally produced monsters by splitting the head through its sagittal midplane after the medullary plate was formed, and then readjusting the two halves. The pieces

united at once, but in a few instances a double eye was formed. Later Spemann, making similar experiments, also produced cyclopean embryos. In some of Spemann's experiments triton eggs were ligated in the sagittal plane during segmentation, and frequently embryos with double heads resulted, one or both being cyclopean. Spemann believes this experiment proves that in its differentiation the cyclopean eye is defective from its beginning and is not produced by concrescence of two eyes which started to develop normally. Levy also produced cyclopean monsters by cutting off the front of the head of triton larvae. In the course of two weeks the two eyes approached each other and formed a double eye, but they did not fuse. However, the pigment layer was destroyed, or absent, at the point of contact. The two optic cups touched each other, but did not unite.

In 1906 Harrison produced a new variety of cyclopia by removing the entire brain from frog embryos. In these specimens the eye moved to the back of the head and appeared to unite in a single vesicle in the region usually occupied by the pineal eye. These specimens are still unpublished.

By pricking the extreme anterior end of the embryonic shield in *Fundulus* eggs, Lewis found that many of the eggs developed into cyclopean monsters. All grades of defective eye were formed—from a double eye and hourglass-shaped eye with two lenses to oblong eyes with two lenses or with but a single lens. The optic cups blended absolutely, thus apparently showing the mode of development of these eyes. Lewis also found that in many of the embryos the brain had not been injured at all, but that the prick had destroyed the nose only. This experiment seems to show conclusively that it is the absence of tissues between the eye primordia which allows them to come together and unite, and that a rudimentary brain is unnecessary.

In his remarkable experiments on the artificial production of a single median eye in the fish embryo by means of sea-water solutions of magnesium chloride, Stockard found that 50 per cent of the embryos developed cyclopia. In these embryos the optic cups were fused at an early developmental stage, much as was the case in Lewis's specimens, in which the embryonic shield had first been pricked. The union of the two cups formed a large compound eye, which in turn derived its lens from the epidermis immediately overlying it in the midline of the embryo. How the magnesium acts upon the embryo is not clear from Stockard's description. No doubt it will be found that it retards the growth of the frontal process, much as in Lewis's experiments. The salt, however, acted upon the whole body of the embryos, for their development was retarded, thus making them smaller than usual, and their circulation was feeble, but they did not die. In these embryos, as in Lewis's specimens, the growth of the brain was normal. The remarkable experiments of Stockard set at rest all germinal theories of cyclopia and prove that every egg has in it the power to develop cyclopean monsters.

These experiments, as well as the numerous pathological embryos with deformed heads and faces which I have studied, prove at any rate that in the formation of many monsters there is an extensive destruction and shifting of tissues. This is also well illustrated in the production of club-foot in the human

embryo. It has frequently been noticed that tadpoles whose growth has been arrested develop stubby or club tails and fins—a condition which corresponds well with club-shaped extremities in man. Our collection contains 18 embryos with deformed legs and feet, with catalogue numbers less than 400, ranging from the very earliest period until the fetus is well formed. The leg-buds are irregular in shape and are filled with condensed mesenchyme; sometimes they are stubby on one side of the body and normal on the other. The study of the larger embryos shows that there is a variety of inflammation of the tissues which is especially well marked in the tendons and around the cartilages. In general this condition may be accounted for by an arrest of development due to impaired nutrition. At any rate, embryos that are not developing well—experimental larvæ and human embryos with other malformations—often have club-shaped arms, legs, fins, or tails.

The inference to be drawn from the above summary is that after the eyes have become well formed they do not pass out to the side of the head as in normal development, but approach each other and more or less unite, and thus form cyclopia. Recent embryological studies of Stockard and of Spemann show conclusively that this view can not be correct, for it is found that the cyclopean condition can be followed back through earlier and earlier embryos, and that all varieties of cyclopia are present while the eyes are still firmly attached to the brain. It is now maintained by Stockard that, from its very beginning, the eye primordium is in the midventral line of the brain, and that in cyclopean embryos there is an arrest of its development, the eye remaining median or dividing in part, forming the hourglass-shaped cyclopean eye with two lenses, etc. This view is combated more or less by Spemann; but I must confess that it is difficult for me clearly to understand his view as given in his various papers.

Through his well-known magnesium experiments, Stockard has been able to procure an abundance of material for the study of the early development of cyclopia. He proves first of all that the condition of cyclopia is present in the earliest stages in which it would be possible to recognize it. At no stage are there two normal eyes which subsequently blend to form a single eye. The cyclopean condition is present in the eyes while they are still closely attached to the brain. Stockard observes, secondly, that the cyclopean eye is rarely equal in extent and size to the sum of two normal eyes combined. A cyclopean eye is, as a rule, very slightly if any larger than one normal lateral eye, and in fact it is often much reduced or actually minute in size as compared with a normal eye. According to Stockard, this fact indicates most decidedly that the eye material, as such, has been injured or arrested in development and differentiation. He believes that we are scarcely warranted in assuming, as have various authors at different times, that cyclopia is due to a fusion of the eyes after they have arisen from the brain and that the earlier in development the fusion occurs the more intimately associated the two eye components become. This view, according to Stockard, has been proved incorrect by actual observation on cyclopean monsters, where it is found that the cyclopean condition of the eye—whether large and hourglass-shaped or of small size resembling a normal eye—is present from the earliest

appearance of the optic vesicle from the brain. In other words, the several degrees of the cyclopean eye come off from the brain in their final condition.

The idea of the fusion of the eye parts, Stockard continues, was deep-rooted, however, and exists in the recent views of Spemann in a refined form. Spemann believes, as others have previously suggested, that cyclopia is due to an absence of non-ophthalmic tissue in the median region of the medullary plate or groove. This lack of median tissue allows the eye primordia, which he holds to be lateral in position, near the borders of the medullary plate, to come together and fuse in the median plane and later give rise to a cyclopean eye. Cyclopia, according to this view, occurs in a more or less passive manner, and is actually a fusion of the eye primordia of the two sides during development. Stockard adds that he is certain that this fusion explanation, which has now been forced entirely back into the medullary plate, is as false as its bolder predecessor, which assumed the fusion to take place outside of the brain-tissues after the optic vesicles or cups had arisen. He says that Spemann did not at first advocate this late-fusion view, but claimed (from his experiment on *Triton*) that the cyclopean eye arose out of the medullary tissues in its final condition; subsequently, however, he assumed the rôle of a most ardent supporter of the view that the fusion of the optic primordia takes place within the medullary plate.

It may be added that there is no known instance of the formation of cyclopia by experimental methods after the eyes are fairly well formed in normal development. All the experiments in which cyclopia has been produced were made upon the embryo at a stage before the eyes could be recognized under the microscope. One must recall that Stockard's magnesium experiment is effective only when it is done before the embryo is 15 hours old. In fact, Stockard found that the best results were obtained if the eggs were placed in magnesium-chloride solution immediately after fertilization. If the eggs are not placed in the solution until 15 hours after fertilization, before the germ-ring forms and begins its downward growth from the yolk-mass, no cyclopia occurs. Cyclopia is less frequent in eggs which are treated at later stages than in eggs immersed in magnesium-chloride solution during the fourth and eighth cell stage. It appears, then, that the critical stage at which cyclopia is best produced with magnesium is shortly before the germ-ring is formed. According to Stockard, a 15-hour embryo has the germ-ring beginning to form and descend over the yolk-sphere; the embryonic shield is scarcely indicated, but appears soon afterward. Embryos of later stages subjected to the same treatment develop normally, or at least do not show cyclopia, while embryos younger than 15 hours, and at as early a stage as the first cleavage, are much more readily affected in such a manner as to cause the cyclopean defect. The optic vesicles appear at about 30 hours after fertilization, but the stimulus must be applied at a time sufficiently long before this process occurs, since a number of important steps in eye formation are doubtless taking place before the visible signs of optic vesicles are present.

It is interesting to note that the Lewis pricking experiment is made at a stage in which cyclopia can no longer be produced by placing the eggs in a magnesium

solution. According to Lewis, the experiment should be made on the second day. Although he does not give his experiment in hours, his illustrations show the stage of development. According to these the embryonic shield is well formed. The experiments of Lewis were first described in my monograph on monsters, but they have since been reported in detail by their author. As has been stated, Lewis produced cyclopia in *Fundulus* by pricking the middle of the anterior end of the embryonic shield two days after fertilization. In the course of a few days it became apparent that in some of the eggs operated upon the eyes had developed normally, while in others they had become cyclopean. Most of the specimens were killed after 15 days. Pricking of the embryonic shield was accompanied by the escape of a slight amount of tissue, and as there is little or no regeneration of the central nervous system in *Fundulus*, the defect caused at the time of pricking subsequently became more and more apparent as development proceeded. Both Lewis and Stockard have found that cyclopean *Fundulus* embryos usually develop with a normal brain, thus no doubt accounting for the vitality of this special cyclops. Furthermore, it appears that the eye primordium in *Fundulus* is more circumscribed than in many other animals.

In a number of his experiments Lewis found that the material withdrawn with the needle-point came from one side of the anterior end of the embryonic shield, with a resulting abnormality of the eye on that side. In a specific case, at the time of hatching, the right eye of the specimen consisted of a small bit of retina connecting with the otherwise almost normal brain-wall. The left eye was apparently normal, as were also the brain and the nasal pit. In other specimens, in which the operation was about medial and was done at the time the embryonic shield was beginning to form, the embryos developed with the two eyes in contact, with two optic nerves and two lenses. Among other specimens there is one with a cyclopean eye, having a layer of pigment narrowing between the two eyeballs. In specimens operated upon at a little later stage there is a median cyclopean eye with two lenses, one pupil, and one cup cavity. Using Lewis's language, the large optic cup shows in sections a very beautiful median eye with complete continuity of the layers of the retina of two components about a single large cup cavity of a single lens.

According to Lewis, the explanation of these various abnormalities is in a way comparatively simple, if we assume that in the early embryonic-shield stage the various parts of the central nervous system and the eyes are probably already predetermined, and, secondly, that there is very little or no power of regeneration in this tissue. Numerous experiments on regeneration indicate very clearly that there is little or no regeneration of the tissue (at least of that of the central nervous system) extruded at the time of the operation. The repair which takes place after the operation consists merely of a rapid closing together of the parts left remaining, and thus a healing of the wound occurs without regeneration of lost parts. This closing of the wound is accomplished in a few minutes, and primordia are thus brought into contact which normally are quite widely separated—those of the two eyes, for example. The subsequent differentiation adjusts itself to the

new relations of these primordia with the resulting abnormal forms. Thus, as one examines these developing embryos, from the time the eye primordia are first visible in the living specimens under the binocular microscope, they appear to have the same amount of fusion or loss of eye that is clearly to be found in the same individual at later stages and at the time of hatching. So we can explain these cyclopean forms by a fusion of the primordia of the two eyes immediately after the operation, even though at this time no primordia are visible. Differentiation of the eye-tissues evidently occurs some time before it is visible by our crude microscopic methods.

Briefly summarizing the experiments of Stockard and Lewis, it may be said that Stockard produced cyclopia by immersing *Fundulus* eggs in a magnesium solution before the formation of the germ-ring, while Lewis operated upon the embryonic shield after it had arisen from the germ-ring. According to Stockard, the magnesium solution possesses a decidedly anesthetic effect and inhibits the growth of the optic out-pocketings; and therefore the condition of cyclopia must be present before the formation of the optic cup—which he believes to be median—the anesthetic effect preventing the medial cup from dividing, thus bringing about the cyclopean condition. According to Lewis, the optic primordia are brought together through the removal of a small amount of tissue which normally lies between them. The primordia then unite and produce all degrees of the cyclopean condition. For practical purposes either theory will suffice to explain the condition as found in man, and there is at present no evidence which can decide which of the two is correct, for I may add that (as Dr. Lewis informs me) the optic primordia arise very close to the ventral midline of the brain, being separated by only a few cells.

Stockard has recently attempted to define more accurately the eye primordia in *Amblystoma* by operating upon the medullary plate. First of all, he found that pricking the medullary plate, as Lewis pricked the germ-shield in *Fundulus*, had no effect whatever upon the growth of the eyes. They invariably grew in a normal way. He then removed various parts of the medullary plate and found that the removal of a median strip about one-fourth to one-third the width of the medullary plate resulted in eyeless embryos. The entire eye primordium apparently lies within this median strip. When a narrower strip was removed the embryos developed with one eye, with defective eyes, or with no eyes at all. From these experiments he concludes that the primordia of *Amblystoma* arise in the antero-median portion of the medullary plate, and not from two independent primordia, as is believed by Lewis.

It may be added that the earlier papers of Lewis and of Stockard were written partly to demonstrate that cyclopia is not an hereditary but an acquired quality. This opinion is much at variance with that of Wilder, who upholds the hereditary theory. In this relation may be stated that there are two records of cyclopia in twins. One, by Ellis, is referred to by Ashfeld on page 283 and is also illustrated in figures 11 and 12, plate 47, of his Atlas. The other is by Van Duyse, and is referred to by Schwalbe and Josephy on page 210. The Van Duyse case is inter-

esting, as both parents and grandparents were perfectly healthy and monsters were not known to have occurred in the family. The mother had been pregnant eight times, four of the pregnancies ending in abortions. The first child had harelip, the second had cleft palate, the third was normal, and the fourth pregnancy resulted in the cyclopean twins. In my own experience I can report an even more remarkable case. In 1900 a pig uterus was brought to me which contained a number of normal embryos and three cyclopean embryos, all of about the same stage of development. The cyclopean pigs measured about 40 mm. in length, and each of them had a marked depression in the front of the head and a single pigmented eye with a snout over it. Unfortunately I did not keep the uterus of this specimen, so that it was impossible for us to examine it with care.

The somewhat lengthy discussion on the differentiation of the eye from the medullary plate is justifiable, because at least one of the specimens I have to report is practically a perfect one, which enables me to discuss the origin of the cyclopean eye in a somewhat connected way, from the condition found in normal embryos. After a description of this specimen I purpose to compare the eyes and brain with the same structures in several younger embryos in the Carnegie collection, as well as with those found in the literature. I shall begin with the smallest specimen to be described, namely, No. 559.

EMBRYO, CR 6.5, NORMAL IN FORM, WITH CYCLOPIA, CARNEGIE COLLECTION, No. 559.

This interesting specimen was sent to me by Dr. Merrill, of Stillwater, Minnesota, on December 21, 1911. Dr. Merrill writes that the specimen came from a white American, who is the mother of one child, 9 years old. The patient gives a subsequent history of three of four abortions which took place early in pregnancy. The last menstrual period before the present abortion occurred on October 27, 1911. The abortion followed on December 20. No particulars are obtainable to account for the abortion and there is no evidence of its having been produced by mechanical means. The patient has a history of irregular menstruation and has been treated for metritis and endometritis. It is impossible to obtain any history of venereal disease.

The unopened ovum, measuring 20 by 15 by 12, came to us fixed in formalin. It is almost entirely covered with villi which branch three or four times and are about 3 mm. long. On one side there is a small area without villi, covered only by the transparent chorionic membrane. Through this can be seen a well-formed embryo, apparently normal, measuring about 8 or 9 mm. in length and filling about one-half of the ovum. The remaining half is filled with dense reticular magma. The umbilical vesicle is spherical, measuring about 3.5 mm. in diameter. The appearance of the ovum before and after opening is shown on plate 2, figures 2 and 5. The embryo was removed by cutting the umbilical cord near its attachment to the chorion. Photographs were then made at both sides of the embryo, at one diameter enlargement, care being taken to get the exact profile pictured. Numerous other photographs were taken, and it then became apparent that we were dealing with an embryo with a very curious deformity of the head. We

also made profile outlines of the two sides of the specimen, being careful to have them in geometrical projection. The branchial region of the two sides of the head were then very carefully drawn. These drawings are reproduced in plate 1 figures 2 and 4. The specimen was dehydrated by placing the entire specimen in several grades of alcohol. It was opened a year later, January 1913, at which time the photographs were made. The specimens were prepared for embedding in February 1914. At this time the embryo measured in absolute alcohol GL 8.6 mm., CR 6.5 mm. In xylol the GL measurement was reduced to 8.2 mm. It remained in several changes of xylol for 30 minutes and then was rapidly transferred through several dishes of paraffin, the entire time of this operation being one hour. The aim was to embed the specimen as quickly as possible in order to avoid any undue shrinkage. It was then cut into transverse sections 20μ thick. These were stained upon glass slides in hematoxylin and Congo red. It was now found that we had an unusually good series of practically perfect sections, none of them being distorted. Many cell divisions were found in the tissues of different parts of the embryo. We now readily realized the condition of the head, as we found in the sections a perfect series of an exquisite cyclopean embryo. A month later the half of the chorion which was removed to expose the embryo was also cut into serial sections 20μ thick and stained with hematoxylin and eosin. Further examination of the part of the chorion from which the embryo was peeled showed the amnion close to the chorionic wall, at the point where the cord had been cut. At the point of juncture there is no complete cord, but in its place are numerous single blood-vessels, making the chorionic attachment of the cord appear like the hairs of a camel's-hair brush. This part of the chorion was now stained *in toto* with alum cochineal and cut into serial sections, in order to determine the exact nature of the tissue of the cord as it spreads out into the chorion; for the anomaly here seen had not been encountered by us before.

A superficial survey of the villi of the chorion shows it to be apparently normal, but the interior of the ovum, on account of the great amount of magma encircling the embryo, indicates that the ovum is pathological. Furthermore, the chorion is much too small for a normal embryo of this size. As a rule, pathological embryos are contained in ova which are larger than normal. The attachment of the cord to the chorion is also anomalous and a superficial glance at the head of the embryo shows that it is atrophic. The whole front of the head is occupied by the mid-brain. There is no lateral bulging to correspond with the cerebral vesicle. The small pigmented eyes are buried deeply in the head and there is a pronounced frontal process in front of them. On the right side of the head, just in front of the eye, was a small protruding villus-like body which subsequently proved to be the snout (plate 1, fig. 2; plate 2, fig. 7; and plate 3, fig. 5). A more detailed description of the anatomy of the eye region will be given in considering the sections of this specimen. In order to interpret the sections properly a plaster-of-paris model of the brain and head was made at a scale of 50 diameters. Later it was found that this model was on too small a scale to include the muscles of the eye, and a second model of the eye region was made at a scale of 100 diameters.

The two halves of the chorion having been cut into serial sections, it was possible to ascertain with greater precision the attachment of the umbilical cord as well as the amniotic adhesions spoken of above. It was found that the cord was attached to one half and that there were delicate amniotic adhesions in the other half of the specimen. The attachment of the cord was by means of blood-vessels passing directly from the embryo to the chorion, while the adhesions in the second half were by means of loose strands of mesenchyme cells binding the amnion to the chorion.

The tissue of the chorion appears much like that in normal ova. It is of about the same quantity and is possibly a little more fibrous. The mesenchyme of the villa is well infiltrated with embryonal blood-cells, and their trophoblast is quite scanty. At points the villi are stuck together with maternal blood, in which are found islands of syncytial cells, showing that the infiltration of blood took place before the time of the abortion. The mesenchyme of a few of the villi takes on an intense hematoxylin stain, which indicates that it is degenerating. In fact, the cells of the mesenchyme in many of the villi have mostly disintegrated.

The reticular magma stains intensely with eosin. Scattered through the dense network composing it are numerous large protoplasmic cells containing nuclei. In certain places the cells accumulate in large masses, forming colonies. No doubt these are the so-called Hofbauer cells, so well described by Essick.

The embryo had been cut into a very perfect series of transverse sections, which show that very little unequal shrinkage took place while it was being embedded. Nearly the entire wall of the central nervous system is in apposition with the surrounding mesenchyme. However, there are occasional separations along the dorsal midline in the head region, the most pronounced one being around the midbrain, but this is not marked. The thin roof-plate of the hindbrain has collapsed only to a slight extent. All in all, the preservation, embedding, mounting, and staining of this specimen is quite perfect.

There are numerous cell divisions in the brain-tube around the central canal as well as in the retina of the cyclopean eye. Furthermore, these cell divisions are found also in the atrophic cerebral vesicle, showing that at the time of the abortion the atrophic cerebral vesicle, as well as the cyclopean eye, was growing actively.

The form of the head is well shown in the figures. In order to study the head with greater care a model was made of the external form at a scale of 50 diameters. This model shows the shrinkage of the head while the specimen was being embedded. It was made so that the entire head could be removed from the body in order to give a face view of the embryo, which could not be obtained from the specimen before it was cut into sections (plate 1, fig. 3). The model also shows the mid-brain to be very prominent, the frontal process being pronounced but small. The eyes are shown deep in the head, and the snout protrudes above the mouth from a point immediately below the process and just in front of the eye. The body otherwise appears to be normal in form, and a microscopic survey of the sections shows that the tissues are also normal in structure.

The lateral view of the head as given in the illustrations on plate 1 may be compared with a normal embryo of the same size; for this purpose I will take the Huber embryo No. 3, pictured by Streeter in figure 86, Volume II, of the Manual of Human Embryology. In the Huber specimen the floor of the forebrain is unusually large, as may be observed by comparing the above-named figure (86) with Streeter's figure 83, which is taken from His's embryo Br₃. This region is also somewhat smaller in the Huber embryo than in a model of the brain of our No. 163 made by Dr. Lewis. No. 163 is 9 mm. long, and a profile drawing of its brain is shown in figure 428 in the second volume of the Manual. Careful comparison of the model of the brain of the cyclopean embryo No. 559 with models of the brain of normal embryos shows clearly that there is a decided ventral median defect of the brain of this embryo, from the mammillary bodies to the front end of the brain. This defect naturally takes away the tissues between the eyes and between the cerebral vesicles. In other words, the floor-plate, as shown in figure 55 in the Manual, reaching from the mammillary bodies to the neuropore, has been cut out. In the cyclopean specimen the hypophysis is also absent. The eye-stems have been taken out, the olfactory lobes are absent, and the brain is reduced to a single vesicle, as is the case in older specimens of cyclopia. Such an extreme destruction of the base of the brain rarely occurs in cyclopean *Familialis* embryos, as in this animal, according to Stockard and Lewis, the brain is frequently entirely normal, the eyes alone being deformed; but in man marked brain defects have always been found to accompany cases of cyclopia.

The optic vesicles in No. 559 form an hourglass-shaped body with two lenses, as shown in plate 1, figure 1, and plate 3, figure 4. The tissues are beautifully preserved and apparently normal in structure. The primary chambers of the eyes communicate freely with each other (plate 3, fig. 4), and through a common eye-stalk, which in turn communicates with the ventricle of the brain (plate 3, fig. 2). The tapetum nigrum covers the optic vesicles and crosses the midline on the dorsal side of the eyes—that is, between the eyes and the common cerebral vesicle. The tapetum stops abruptly at the optic stem and passes down slightly along the anterior wall which joins the two retinas. The choroidal fissures reach clear through the front of the eyes, running almost together as they approach the common eye-stem, as shown in plate 1, figure 1. The topography of the optic stem, ganglion layer of the retina, and tapetum is in the order given, starting from the midline, in the normal embryo, as is probably the relation of their primordia in the normal neural plate, judging by the work of Eyeleshymer and of Lewis. Eyeleshymer showed that very early in development the eye appears, in amphibia, as two pigmented spots lying quite close to the midline in the anterior end of the medullary plate. If these groups of pigmented cells are destined to form the tapetum, then the ganglion layer of the retinas would form nearer the midline, while the cells which cross the midline would probably form the optic stem. That Eyeleshymer's view is correct is indicated by the work of Loey in his studies upon the shark and by Keibel in his studies upon the pig. According to these authors, the eye primordia arise from small depressions near the midline of the

anterior end of the medullary plate. According to L ewis, certain groups of cells in the medullary plate are predetermined to form the tapetum, the retina, and the optic stem. Lewis's theory has been objected to by Bell, but it has been amply confirmed by Spemann. At any rate, the arrangement of the structures in our cyclopean embryo indicates that the optic stems have been cut out and that the primordia of the retina and tapetum of the two sides have united, blending absolutely with each other across the midventral line.

The tissues of the midbrain and most of those of the interbrain appear to be

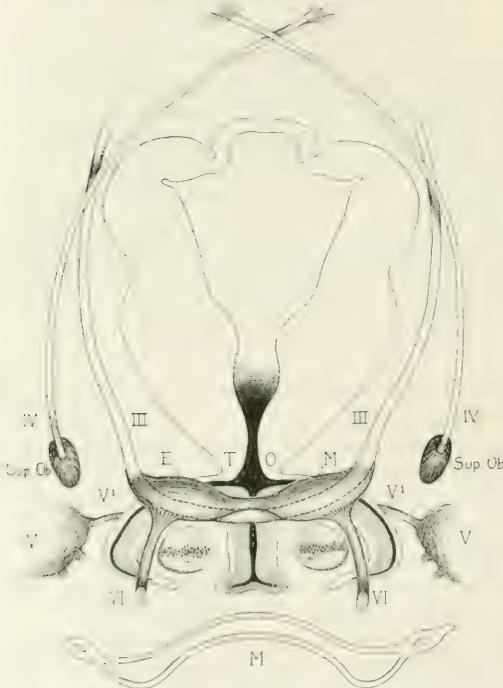


FIG. 1.—Semi-diagrammatic section through the interbrain and cyclopean eye of embryo No. 559.  50. The cranial nerves are marked with Roman numerals. *Sup. Ob.*, superior oblique; *E. M.*, eye-muscle; *M.*, mouth.

fig. 31. This cap is composed of pale cells of uniform size, undoubtedly belonging to the neural tube. It is located on the part of the brain which gives rise to the olfactory lobes in normal development; and it may represent these lobes in a degenerated form.

It was necessary to make a model (enlarged 100 diameters) of the eye region in order to study carefully the anatomy of the structures of the orbit. In this model the nerves, from the third to the seventh, were worked out to their terminations. The muscle masses, as far as they could be determined, are also included in this model.

normal, but our knowledge of the normal brain at this period of development is so scanty that it is dangerous to make any definite statement. Single groups of cells may be wanting or may be blending without our noticing the change. Such a blending is clear only when it involves a sharply circumscribed structure like the eye. However, the tissues of the hypothalamus seem to be disarranged (plate 3, fig. 2), and those of the single united cerebral vesicle (plate 3, fig. 5) are certainly dissociated. In the cerebral vesicle the cells form a uniform layer, which is not beautifully stratified, as is the case in normal development. Over the most anterior part of the brain (plate 3, fig. 5), crossing the midline, is a crescent-shaped cap covering the outside of the brain and reaching back to the hypothalamus just below the point of attachment of the common optic stem (plate 3,

The first branch of the fifth nerve is much thinner than the second and third, and passes directly from the Gasserian ganglion back of the eye on either side of the optic stalk; the branches anastomose with each other through several delicate filaments back of the eyes, and then a larger bundle and several very small ones enter the snout, to be lost there. The nerves are shown in section in the figures on plate 3 and in reconstruction in plate 1 and figure 1.

The fourth nerve takes its usual route and finally ends in a very large pre-muscle mass lateral to the eye and to the first branch of the fifth nerve. It may be noted that this arrangement of the fourth and first branches of the fifth appears to be the reverse of the normal distribution according to Lewis's reconstruction of our embryo No. 163 (fig. 368 of the Manual). In the Lewis reconstruction all of the muscle of the primordium of the eye is blended into a single muscle mass, while in the cyclopean embryo the pre-muscle mass of the superior oblique muscle is entirely separated from the remaining pre-muscle mass of the orbit.

The sixth nerve takes its usual course and ends independently in the hour-glass-shaped pre-muscle mass which crosses the midline between the cyclopean eye and the hindbrain (fig. 1). It is interesting to know that the transverse median muscle mass, as well as the median anastomosis of the third nerve, occurs at the point through which the pharynx gives rise to the hypophysis. In this embryo the chorda ends in the pharynx behind this muscle mass and the pontine flexure of the hindbrain. It appears as though, on account of the great amount of kinking, the region of the infundibulum of the interbrain were pushed away from the pharynx, thus making it impossible for the hypophysis to reach it. As a result of this the third nerve and its muscle masses cross the midline. It may be that the curious cytological changes in the muscle masses (plate 3, fig. 1, *Oc.*) indicate that destructive changes are taking place in them, and that these small round nuclei correspond with the Hofbauer cells as described by Essick in his studies of the transitory cavities in the corpus striatum of the human embryo. The primordium of the eye-muscles show some very remarkable cytological changes. As the sixth nerve approaches the muscle mass of the lateral rectus it is at once observed that this muscle falls into two sharply defined groups of cells, namely, a median group which appears to be normal, and a lateral mass of smaller round cells, the nuclei of which stain intensely. The same grouping is present in the muscle mass of the third nerve. Near the midline the cells appear to be normal, and laterally they are again composed of small round cells. The pre-muscle mass at the end of the fourth nerve—that is, the superior oblique muscle—can be outlined only with difficulty.

The third nerve shows most remarkable changes in this specimen. It passes along its usual course until it reaches the common eye-stem, over which it circles, for the nerves from the two sides anastomose here, without any diminution in size, within the common median primordium of the eye-muscle (fig. 1). This pronounced anastomosis is also found in another cyclopean embryo, No. 201, in our collection, as shown in figure 3.

Wilder pictures and describes a cyclopean pig (Wilder's fig. 1, plate 2) in which the third nerve arises as a pair in the usual way, which unites after passing through the superior orbital fissure. The union is in the neighborhood of the orbital muscles. He also described the orbit of the cyclopean eye in a large double-headed fetus. By dissection of the head he found that the two third nerves, one coming from each brain, unite with each other and form a common trunk stretched transversely across the midline back of the eyeball. From this anastomosis small twigs arise to supply the muscles of the eyes (Wilder's fig. 6, plate 3). In his work on monsters (page 282), Ahlfeld states that Delle Chiaie described a specimen having a similar anomaly, which was published in Naples in 1840. Delle Chiaie gives an excellent illustration of this specimen, with a diagrammatic section of the head, showing the eye and its attachments. This picture is copied by Ahlfeld on plate 46, figure 18. It is somewhat difficult to identify all the structures given, but he apparently pictures the two third nerves anastomosing before they reach the single eye. He also pictures a branch of the fifth nerve passing into the snout, which, as in our specimen, contains a cavity. I have been able to find one more specimen in the literature in which there is an anastomosis of the two third nerves within the orbit in cyclopia. This is in the excellent description of Dr. Black on the nervous system of a cyclopean specimen at birth. Black alludes to the third nerve in a single sentence. He says (on page 204) that in the region of the central tendon the third nerve divides into a number of small branches, each of which communicates with its fellow on the opposite side. I have given these references, as they are the only ones which I can find in the literature, and they invariably accompany the description of the orbit in cyclopia. The anatomy of cyclopia in monsters is rarely given; and it may be remarked that until we have numerous good descriptions (like that of Black) of the eyes, central nerves, and face in cyclopia, we shall not understand fully the anatomy of this most interesting type of specimen.

In a *Janus* monster (No. 1178, *a* and *b*) at birth with cyclopia on one side, Dr. Theodora Finney has demonstrated a large anastomosis behind the orbital branches of the third nerve before they are distributed to the eye-muscles. An account of her specimen with a figure is given on page 30.

It remains to attempt to correlate what has been said above regarding cyclopia with the form of the brain and the optic vesicle in early human embryos. Before undertaking this attention is called to two papers by Tandler on the form of the early brain in *Tarsius* and in *Platydaetylus*. Tandler's papers are especially noteworthy for the reason that the topography of the forebrain has been determined with a greater precision than has been carried out in the human embryo, except possibly in the most recent work of His. There is sufficient material at hand to make similar studies upon the human brain, but until this is done I must content myself with what has already been published, alluding occasionally to several of the specimens in our own collection.

A specimen 3.2 mm. long, with 13 or 14 myotomes, was described with much care by His in his last publication upon the brain. He has illustrated this specimen by a sagittal median section through the body as well as by the external and

internal forms of the brain. These illustrations are given in the His monograph as figures 2 and 33, and they are also copied by Streeter in his article on the brain in the Manual. It may be stated that figure 33 shows the neuropore nearly closed, the optic stalk being still represented as a wide-open canal which reaches to the midventral line. Dorsal to the optic stem is a slightly marked pocket which reaches to the neuropore, and it is believed by His that this represents the beginning of the cerebral hemisphere. It is interesting to note that Keibel and Else state in their Normal Plates (page 100) that the cerebral vesicles are just beginning in embryos 4.5 to 5 mm. long. It may be that His exaggerates this pocket slightly in his model, but it is of great value to us to have his opinion regarding the location of the primordia of the cerebral hemispheres in the brain-tube before the neuropore is closed. According to Watt, the cerebral hemispheres arise much more dorsal than is indicated by His in his figures.

Keibel and Else give excellent illustrations of the form of the brain up to the time of the closure of the neural tube. No doubt the Kroemer-Pfannenstiel embryo, which contains five or six myotomes, represents a normal stage with medullary plates wide open, and in this embryo there is no indication whatever of an eye primordium. The same is true in the Kollmann embryo, containing 14 myotomes, which is illustrated by Keibel and Else as figure 4. This specimen also seems to me to represent a normal embryo, as we have in our collection a stage that is practically identical with it. Our embryo No. 391 contains seven pairs of somites, and has been carefully studied and published by Dandy. A model of it, as well as sections through the head, has also been pictured by Evans in figures 408 and 409, Volume II, of the Manual, and it shows no out-pocketings in the anterior part of the brain to represent the eye primordia. However, when we reach Keibel and Else's figure 5, which is taken from the Pfannenstiel III embryo, two marked diverticula are seen to arise from the front end of the neural tube, which Keibel, in his article on the eye in the Manual of Embryology, believes to represent the primordia of the eye.

As Keibel and Else have reproduced numerous figures of sections through the head of this embryo, it is easy to ascertain the exact form of its neural tube. However, I am of the opinion that we can hardly view the neural tube in this embryo as normal, as it is not sufficiently advanced in development for an embryo of this stage and as it corresponds very much in form with the brain of our embryo No. 12, which I believe to be pathological. At this time the neural tube should be nearly closed, while in Keibel and Else's specimen and in our No. 12 it is still wide open. The Pfannenstiel III embryo contains the same number of somites—that is, 14—as our embryo No. 12, and the form of the brain and of the eye-vesicles is very similar in both embryos. A picture of the external form of embryo No. 12 will be found in my article on the development of the intestines (plate 19, fig. 2). My reason for believing that the brain form in both of these embryos can not be viewed as normal is that in other young specimens published recently by Wallin and by Bremer quite a different form of the brain-tube is shown for this stage of development. The Wallin specimen, which contains 13 somites, has the brain-

tube pretty well closed up, leaving a large neuropore in the front. The Bremer specimen is slightly in advance of this. We have also in our collection a similar embryo (No. 470) in which the brain form corresponds exactly with that in the specimens of Wallin and of Bremer.

Furthermore, the twin specimens of Watt, which contain 17 or 18 somites, correspond very closely with the three above-named embryos. In the Watt specimen the neuropore is nearly closed and the eye-vesicles reach all the way across the front of the brain-tube. The Pfannenstiel III embryo and the Bremer embryo are found pictured by Bach and Seefelder, both in profile and in sections, but I do not think we should unreservedly accept their description of the beginning of the eye-vesicles as final for the human embryo.

It seems to me that our knowledge of the form of the forebrain before closure of the neuropore is much in need of revision, and towards this revision we have assembled several new models of young embryos. The first is a model by Dr. Bartelmez of our No. 1201 (University of Chicago, No. 87) from an embryo with 8 pairs of somites, which seems to me to bear very much upon this question. The neural plate flanges out into a large tongue with a slightly hourglass-shaped depression running across the midventral line (plate 2, fig. 6.) The larger lateral depressions no doubt indicate the foveola, and the groove connecting them across the midline is in the position in which the optic stalk develops later on. It would probably be better if we accepted Frierip's designation of torus opticus for this connection. The torus opticus seems very insignificant in this specimen, as shown in the illustration (*t. o.*), but when we consider to what extent the torus may be stretched, as illustrated by Bach and Seefelder (fig. 2, plate 13), we recognize the importance of this structure. I am inclined to believe that the form of the brain in the Bartelmez embryo must be viewed as normal, as it corresponds so well in a series with that of several older human embryos, namely, those recently described by Wallin and by Bremer and our No. 470.

There is another ridge already indicated in the brain-tube of His's specimen EB reaching across the midline just below the neuropore. This is the torus transversus of Kupffer; and those who are interested in this structure are referred for greater details to the articles by Tandler, Kupffer, and Johnston. The neuropore is found just closed in our embryos No. 148, published by Mrs. Gage, and No. 836, which has been modeled by Dr. Evans, as well as a specimen of the same size modeled by Johnston. Dr. Johnston has been good enough to send me photographs of his model, so that I am able to compare it with our own. It is clear to me that the large "optic vesicle," extending over the whole lateral wall of the front part of the neural tube, represents more than the optic vesicle, as it must also include the primordium of the cerebral hemispheres, since the torus transversus touches the lower border of the neuropore and the optic vesicles fall below this line. This large so-called optic vesicle must resolve itself into the optic vesicle and brain hemisphere in subsequent development. In this process the torus opticus gradually must become more pronounced.

It is somewhat easy to compare the medullary plate of the Bartelmez embryo No. 1201 with the medullary plate in amphibia. The lateral foveola corresponds with Eycleshymer's pigmented spots, and if these areas were cut out eyeless embryos should be produced, as Stockard found in his experiments on *Amblystoma*. If it is admitted that the eye primordia communicate across the midline through the torus opticus, then Stockard's experiments upon the medullary plate may be interpreted as follows:

No important organ develops from the midline of the medullary plate, and this is represented only by a thin layer of cells. It has been called by His the floor-plate. The motor nuclei arise from the thickenings on either side of the floor-plate, and these are known as the basal plates. The narrow, thin floor-plate really forms the ventral midseptum of the spinal cord of the brain, and subsequently commissural fibers grow through it to form the raphe. If we view the basal plate from above, as shown in embryo No. 1201 (plate 2, fig. 6), we find that this raphe should extend forward to the neuropore, at which point the raphe fibers are the anterior commissure within the torus transversus. Back of this we have the torus opticus, and its commissural fibers are the fibers of the optic nerve. At an early period in its development the torus opticus must widen rapidly and push through the rest of the brain, for the optic stalk appears quite suddenly, and an injury to the medullary plate at this time would probably make itself felt more upon the optic stalk than upon the eye, for the short period in which it grows very rapidly is its critical period. If we cut out the optic stalk or the torus opticus, as Stockard and Lewis did in their experiments, then the foveola would remain together and form cyclopia. Stockard and Lewis also note that in their experiments they frequently obtained embryos with but one eye which appeared to be quite normal. This is to be expected when experiments are made upon two primordia which lie very close together; and when either the left or right eye primordium is removed, left or right-eyed embryos are produced, but not cyclopia. Lewis had to destroy only the midline of the embryonic shield in order to produce true cyclopia. It may be added that the anatomical changes found in our small cyclopean human embryo, as well as in all cyclopean human monsters, can be explained by removal of the structures represented along the line of the raphe of the medullary plate reaching from the mammillary bodies to the neuropore. This includes the torus transversus, which naturally involves the olfactory region and the anterior commissure. Thus we can explain by a study of this specimen the anatomical changes of the brain found in human cyclopia.

STUNTED EMBRYO, GL 20, WITH CYCLOPIA, CARNEGIE COLLECTION, No. 201.

The second specimen of cyclopia in our collection is a pathological embryo, which is not very well preserved, measuring GL 20 mm. This specimen came from Dr. Schelly of Baltimore, who obtained it from an abortion on February 7, 1902. He brought it to Mr. Brödel, and subsequently, because it was pathological, it was given to me. The embryo was incased in an ovum covered with a ragged decidua, together measuring 80 by 60 by 50 mm. Upon opening this

ovum it was found filled with a jelly-like fluid forming a type of magma described in my paper upon this subject. Sections were cut of the fleshy chorion and it was found that the wall is composed of a true chorionic membrane, villi, maternal blood, fibrin, decidua, blood sinuses, and trophoblast, with an extensive infiltration of leucocytes often accumulating in large masses or small abscesses. These layers are not in any regular order, but are intermingled, and show various stages of disintegration. The mesenchyme of the villi is fibrous, and many of these are invaded by trophoblast cells as well as by leucocytes. The trophoblast also invades the blood-clot, and maternal-blood sinuses are frequently found filled either with trophoblast cells or with leucocytes. There are certain groups of tissue in which the intermingling of the trophoblast cells within the fibrinoid substance appears, in sections, very much like cartilage. Most of the decidua adjacent to active trophoblast on the tips of some of the villi has the usual fibrinoid layer characteristic of normal development. It may be added that the structures of the chorionic membrane, as well as those of the embryo, stain unusually well, which indicates that the tissues were active and alive at the time of the abortion.

After the embryo was received it was photographed from one side (plate 2, fig. 1), and this record proved to be very useful in making several reconstructions. The embryo was then cut into serial sections 50 μ m. thick; finally, in order to study more carefully the structures within the head, a reconstruction of its external form, as well as of the form of the brain and the eye, was made in plaster of paris at a scale of 40 diameters.

Most extensive changes have taken place within the embryo. The brain is greatly deformed and is severed from the spinal cord through a growth of tissue in the region of the medulla back of the deformed ear. In fact, a part of the brain is included within the cap-like body on top of the head. The spinal cord begins again quite abruptly in the upper cervical region and ends with the same abruptness in the upper lumbar region. At its lower end there is a curious fibrous tumor measuring half the diameter of the cord. The cord, so far as it is developed,



FIG. 2.—Reconstruction of the head of embryo No. 201. $\times 25$. The outline of the head was obtained from a photograph, the brain and eyes from a plaster reconstruction, and the nerves were added from the sections. *C. H.*, cerebral hemisphere; *M.*, midbrain; *H.*, hindbrain; *S.*, rudimentary snout; *V.*, fifth nerve; *VIII.*, eighth nerve; *E.*, external ear.

appears to be normal, but it is markedly dissociated. Below the upper lumbar region the spinal cord is wholly wanting and the spinal canal is filled with mesodermal tissue which is rich in blood-vessels. Where the cord is missing most of the spinal nerves still remain, and many dorsal ganglia can be made out, showing that the changes in the central nervous system in this region took place after the spinal nerves had been developed from it.

Most of the epidermis is intact, but it is broken through at the back of the head, where there is found an extensive ulcer or necrotic mass, which is very rich in blood-vessels and involves the walls of the brain but does not reach into its ventricle. At the highest point of the head the epidermis has developed into a papilliform body, and below this there is a large necrotic area in which there is a great quantity of yellow pigment granules.

The mouth is closed completely, although the alimentary canal, from the mouth to the stomach, is open and appears normal. The intestine is matted together; the cloaca and anus are obliterated. The epithelium of the upper portion of the intestine gives rise to marked growths into the matted mass.

The thoracic region, liver, and vascular system have undergone practically no change. The extensive dissociation of the tissues throughout the embryo has caused an extensive destruction and arrest in the development of the muscular system. This is marked by all kinds of secondary changes in the connective tissue, especially in that of the skin, where the change is pronounced, as may be seen in plate 2, figure 4. In this region the change is so great that it obliterates entirely the external auditory canal.

A reconstruction of the brain and upper part of the spinal canal enabled me to determine with greater precision the parts of the brain which are within the head of this embryo. Subsequently it was possible to find the remnants of the ganglion of the eighth nerve; then that of the fifth nerve was determined. Their position is shown in the profile diagram of the outlines of the brain and these nerves (fig. 2). It at once becomes apparent that the portion of the brain extending up in the necrotic cap on the top of the head consists of the midbrain and hindbrain. With this idea in mind it was possible to answer the question in the serial sections, as the walls of the midbrain are thick and the ventricle relatively small, and the walls of the hindbrain are thin and its ventricle relative large. The pointed process which reaches towards the ganglion of the fifth therefore represents the pontine flexure of the medulla. It is now easy to interpret the

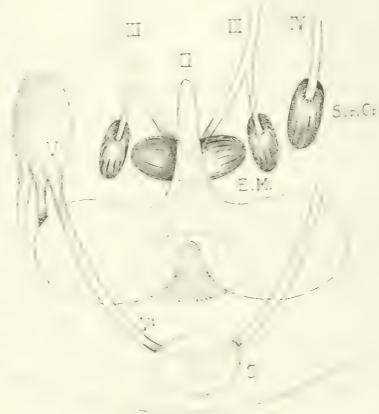


FIG. 3.—Diagrammatic reconstruction of the eye and the surrounding structures of embryo No. 201. $\times 25$. The cranial nerves are marked with Roman numerals. II, first branch of fifth nerve; S, rudimentary snout; E. M., eye-muscle; S. sup. Ob., superior oblique muscle.

isolated brain-mass between the eye and the large mass just described. By comparing these structures with a profile reconstruction of the forebrain of the cyclopean embryo No. 559 (plate 1, fig. 1), the larger mass just above the ganglion of the fifth undoubtedly represents the interbrain, as the free end of the optic nerve

reaches just to its base but does not enter it. The crescent-shaped mass of the brain in front of the interbrain is the unpaired single cerebral vesicle, which communicates freely with the ventricle of the interbrain. The structure of the wall of this degenerated cerebral vesicle corresponds very closely with that of the small vesicle in embryo No. 559. With these structures established, as shown in the profile reconstruction, it is possible to identify some of the re-



FIG. 4.—Section through the cyclopean eye of embryo No. 201. $\times 40$.
V, first branch of fifth nerve; S, snout.

maining structures of the orbit. However, the tissues are very greatly dissociated, and for the present it is impossible for me to follow them farther than is given in the following description. First of all, the three branches of the fifth nerve can be followed to their termination. The first branch reaches up to the rudimentary snout, where it anastomoses with its fellow from the opposite side—shown also in the diagram (fig. 3). This snout, by the way, is represented by a slight elevation in the middle of the face above the eye, and sections through it show that it is composed of a relatively large, irregular mass of cells which are separated more or less from the surrounding mesenchyme. Within the middle of this mass is a small pearl-like body about 0.1 mm. in diameter. Just behind this body the first branch of the fifth nerve anastomoses across the midline.

The second branch of the fifth runs below the eye on either side and reaches nearly to the skin, where it spreads out into several large branches. The third

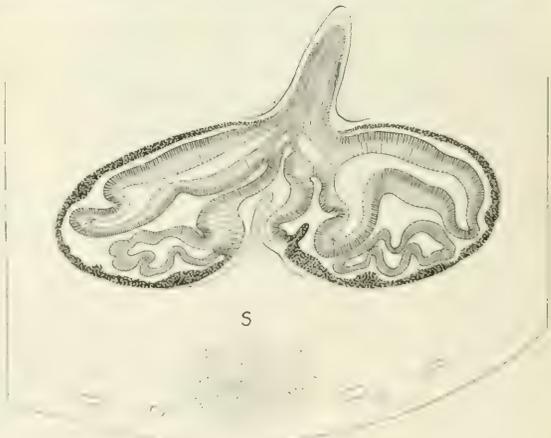


FIG. 5.—Section through the cyclopean eye and optic nerve of embryo No. 201. $\times 10$.

branch of the fifth nerve runs deep into the neck and is separated from the second by a pronounced ossification center representing, of course, the maxillary bone.

The two eyes are united, forming an hourglass-shaped body with a double retina, a single pigmented layer, and a single optic nerve which arises from the retinas as they approach each other (figs. 4 and 5). The tapetum is continuous over the superior surface of the eyes, but it is broken below, repeating the condition found in the eye of embryo No. 559. The optic nerve reaches to the base of the interbrain, where it ends abruptly. It is impossible to determine with precision the arrangement of the muscle masses of the orbit or of the nerves passing to them. That no trace of the sixth nerve could be made out is shown in figure 3, and this may be accounted for by the fact that the organ which gives rise to the sixth nerve has undergone extensive degeneration. However, the peripheral ends of the third and fourth nerves can be found, but they can not be traced back very far in the direction of their origin. The fourth nerve is thicker than normal and ends on the lateral side in an enlargement which may represent the superior oblique muscle. Below the optic nerve is a common muscle mass which crosses the midline, and to either side of this there are two independent muscle masses. Before the third nerve reaches these muscle masses lateral branches are given off, which pass to the second lateral muscle mass, as shown in the diagram. The two nerves then approach each other and communicate freely through the unpaired muscle mass, and then pass forward under the cyclopean eye and finally end just beneath the skin.

A comparison of plate 1, figure 1, and text-figure 1 with text-figure 3 shows that the first branch of the fifth nerve in both embryos anastomoses across the midventral line in the region of the snout and that the two third nerves anastomose with each other through the main muscle primordium of the orbit. Both of these anastomoses must be viewed as secondary, for the two nerves must have been single when they arose from the brain. This observation favors the theory that the eye promordia must also have been bilateral—that is, they must have been separated by a narrow strip of non-ocular tissue in the normal medullary plate.

FETUS COMPRESSUS WITH CYCLOPIA, CARNEGIE COLLECTION NO. 1165.

This embryo was sent to me by Dr. Ralph S. Perkins, of Exeter, New Hampshire. Only the embryo was received, which measures 43 mm. CR. It was found to be greatly distorted; the umbilical cord is of thread-like thinness, and the development of the different parts of the body seems to be unequal. Apparently some of the joints are dislocated, but at present it is impossible to say whether or not these distortions are due to mechanical manipulation after the embryo was aborted. This is possible, because the embryo had been wrapped in a towel some time before it was fixed in formalin, but a careful study of the sections demonstrates that the specimen is quite a typical fetus compressus.

The embryo came from a white woman, 35 years old. Her first child is 16 years of age; the second died at the age of 2, and the third is 12 years old. These are all by her first marriage. The first pregnancy of her second marriage

ended in an abortion at 5 months, and then she gave birth to a child which is now 21 months old. The next pregnancy resulted in an abortion at 5 months, and the last one gave the specimen under consideration.

Her last normal menstrual period began on February 25, 1915. The next period began on March 21 and continued for only one day; and this was followed by the abortion on May 2. There are no other data bearing upon this case except that 15 years ago the woman had an operation for suspension of the uterus.

Upon careful inspection of the head of the specimen a mechanical injury just below the lower jaw was found, as shown in figure 6. The ear seems to be distorted or abnormal, and in place of the nose and eyes there is a depression in front of the face, and running from it is a cleft reaching to the mouth. Apparently we have here a fetus compressus with cyclopia and hare-lip.

The head of the embryo was stained *in toto* in cochineal and embedded in paraffin. It was cut into serial sections 50 μ thick. The sections show that all the tissues are markedly dissociated, and in addition the brain is completely macerated. In fact, the brain-cavity appears like a bag filled with débris, which reaches down into the cervical region of the neck and terminates abruptly where the spinal canal is filled with a new formation of fibrous tissue. The primordial skull is composed of cartilages which have under-



FIG. 6.—Direct drawing of the head of embryo No. 1165. $\times 4.5$. The tissue of the lower jaw is injured. The depression from the cyclopean eye extends down into the mouth, forming hare-lip.

gone some fibrous changes, and their borders are not sharply defined, but grade over into the surrounding connective tissue. The cartilages at the base of the skull appear to be enlarged and extended; but this point can not be established without making an elaborate reconstruction. In the cervical region the bodies of the vertebrae are displaced backward into the spinal canal, which in turn is largely filled up with the newly formed fibrous tissue as well as with numerous round cells. The tissues of the various ossification centers have undergone a curious change, reminding one of necrosis. It appears as though the ossification centers had died while the surrounding cartilage had continued growing. It is difficult to define precisely the muscles and nerves in all of the various sections, while at points certain muscle groups seem to retain their normal form.

Most of the epidermis is wanting and in the region of the face are large skin protuberances composed principally of round cells. Such protuberances form the lids of the cyclopean eye, as shown in plate 2, figure 3. The orbital cavity lies upon the cribriform primordia of the maxillary bones and is filled with a single group of pigmented cells, which is surrounded by an infiltration of round cells. Back of this pigmented mass are the primordia of the eye-muscles, but their dissociation is so complete that it is impossible to locate the individual muscles, nor can any of the nerves be made out with precision. Aside from the pigmented mass there are no remnants of the layers of the retina, these having undergone complete dissociation. In the upper part of the orbital mass is a curious gland-like structure badly dissociated, which may represent the lacrymal gland. We have, therefore, in this specimen the remnant of a single median eye represented by an irregular but rounded mass of the tapetum situated below the depression of the skin. In turn this depression is partly covered with folds of dissociated tissue which may be recognized as the eyelids of the cyclopean eye.

CEPHALOTHORACOPAGUS MONOSYMMETROS WITH CYCLOPIA ON ONE SIDE,
CARNEGIE COLLECTION No. 1178 *a* AND *b*.

The double female monster, 205 CR and 350 GL long, weighing 1.624 grams was sent to us by Dr. J. I. Butler, Rodgers Hospital, Tucson, Arizona, on May 14, 1915. The mother is a Chinese woman, age 24, who has given birth to three children at term and has had two abortions. Apparently the uterus is normal and there is no history of venereal diseases. There is nothing else in the history that bears upon this case. The specimen has been completely dissected by Dr. Theodora Finney, who has given me the notes for the following description of the muscles of the orbit and the nerves of the cyclopean eye. A more detailed account of the anatomy of this interesting specimen will be published by Dr. Finney at some subsequent date.

The fetus is composed of two nearly complete bodies which lie with their anterior surfaces toward each other, and, as the name implies, are fused from the umbilicus up, forming one thoracic trunk and one head. There are two independent spinal columns, eight extremities, and two composite fronts, every symmetrical part of which is formed half of one and half of the other individual. There are also two faces, one of which is well formed, while the other is a synote with a cyclopean eye and snout situated above it. In dealing with the cyclops, then, it must be noted that its left half is formed from the left side of one individual, while its right half is from the right side of the other individual.

Internally the thoracic and abdominal viscera are double, with the exception of the esophagus, the stomach, and the upper part of the intestine, which are united with a single canal. There are two central nervous systems, separate and complete to above the level of the two hypophysi, where fusion occurs. As much tissue was lost from the region of the thalami in removing the brain, the mode of union of the base of the brain could not be determined. An optic chiasm, however, belonging to the well-formed face, remains *in situ* immediately behind the

orbits. This shows there is a true normal union for the two individuals at this point. In the cranial cavity behind the cyclopean eye one optic nerve-stalk, composed of two bundles pressed together, is observed.

The dome of the cranium is filled by three cerebral bodies; two of these are recognizable hemispheres, though much shortened antero-posteriorly. Their position is normal, behind the well-formed face. They possess well-defined but shallow cortical sulci. The third cerebral division consists of a kidney-shaped lobe. Its cortex is smooth, except for two or three atypical creases near the poles. It lies transversely across the cyclopean side of the cranial cavity with its two poles directed inferiorly, the convex portion between them straddling the single orbit. It represents fused cerebral tissue obtained from both individuals.

The cyclops has a well-formed eyeball to which four pairs of muscles are attached; their arrangement is shown in figure 7. These muscles can be identified by their nerve-supply as being the muscles of the upper and outer parts of the two fused eyes. These muscles are changed from their normal positions, so that they entirely surround the eye. The muscles are the superior obliques, the levator palpebræ, the superior and lateral recti. The two superior obliques lie near to the midline on the superior surface. Slightly lateral to these, though still on the superior surface, lie the two levator palpebræ. On the sides, in the place usually occupied by the lateral recti, lie the superior recti, which are shifted downward from their normal position through an arc of 90°. About the same amount of shifting causes the lateral recti to lie close together on the inferior surface of the eyeball. The inferior oblique muscles and the medial recti are completely eliminated. The inferior recti are entirely absent at the bulbar end. There is a short bundle of muscles underneath the proximal end of the lateral recti, which probably represents remains of the inferior recti. The lacrymal glands have participated in the change of position and fusion. Their tissues lie as a broad single gland-mass on the inferior surface of the bulb.

In order to be sure that the identification of the nerves passing to the muscles of the cyclopean eye was made correctly, they were carefully compared with the normal nerves of the eyes of the well-developed face. This comparison left no doubt as to which nerves were being traced to the single eye, as the points of origin

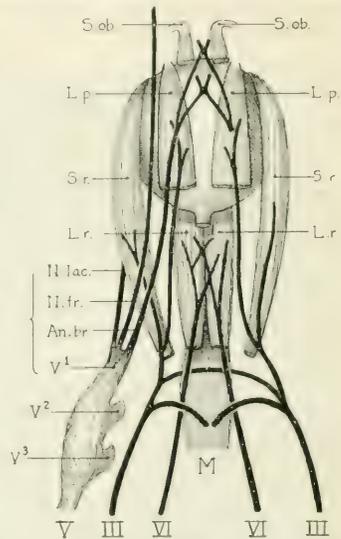


FIG. 7.—Diagram of cyclopean eye and its appendages of the *Janus* monster, No. 1178 *a* and *b*, from a sketch and dissection by Dr. Finney. For the sake of clearness the superior oblique muscles are moved forward. The cranial nerves are marked with Roman numerals. *S. ob.*, superior oblique muscles; *L. p.*, levator palpebræ; *S. r.*, superior rectus; *L. r.*, lateral rectus; *M.*, rudimentary muscle-mass, probably the remains of the inferior recti. It is noticed that the first branch of the fifth nerve gives rise to a trunk which anastomoses across the midline. The same is true of the third and sixth nerves.

from the brain-stem of the third, fourth, fifth, and sixth nerves were symmetrical for both faces. The arrangement of the nerves on the cyclopean side are as follows:

1. The olfactory nerves are absent.

2. The origin of the optic nerve was lost. Two small and flattened optic nerves, however, pass out together in the dura. These finally fuse into one stalk which ends in the bulb. This stalk, 2 mm. in diameter, is about the same size as the normal optic nerves of the well-formed face on the opposite side.

3. The two third nerves which belong to the cyclops are 0.5 mm. in diameter at their point of origin and throughout their course, while the third nerves on the opposite side which pass to the perfect face are twice that size. The cyclopean oculo-motor nerves pass into the dura, where they run toward each other to the place where the eye-muscles arise. Here these nerves lie within 3 mm. of each other. Branching occurs in this region. Two of these branches fuse immediately. There are two other pairs of main branches which innervate the levator palpebræ and the superior recti on each side of the single eye. There are some finer branches whose course could not be definitely ascertained.

4. The cyclopean fourth nerves are equal in size with those of the normal eye. They run as two fine threads to within a few millimeters of each other, when they turn anteriorly and run parallel on the surface of the superior oblique muscles, in which they terminate.

5. The two Gasserian ganglia of the cyclops are somewhat smaller than those of the normal face. Each has three divisions: ophthalmic, maxillary, and mandibular. The two ophthalmic divisions have each three main branches. One of these branches passes along the roof of the orbit and makes several χ -shaped anastomoses with its fellow near the front of the eyeball. Another runs forward, parallel with its fellow, out into the skin, where they are both cut; so if anastomosis occurred it could not be determined. The third and last branch, one on each side of the eye, ends in the lacrymal gland.

6. The sixth nerves of the cyclops, about 0.8 mm. in diameter, are equal in size with the sixth nerves, passing to the well-developed face. They converge to the base of the orbit when they run parallel to each other on the upper side of the lateral recti muscles, in whose substance they terminate after making several χ -shaped anastomoses.

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EXPLANATION OF PLATES.

PLATE 1.

1. Plaster reconstruction of the brain and cyclopean eye of embryo No. 559. $\times 25$. Cranial nerves are marked with Roman numerals. *o. v.*, optic vesicle; *s*, snout; *m*, mouth.
2. View of the right side of embryo No. 559. $\times 9$. Only the face region is worked out in detail. *U. v.*, umbilical vesicle.
3. Face of embryo No. 559. $\times 16$. The drawing is made from a plaster-of-paris reconstruction. *S*, snout.
4. View of the left side of cyclopean embryo No. 559. $\times 9$. The drawing was made directly from the specimen in formalin.

PLATE 2.

1. From the photograph of embryo No. 201. $\times 1\frac{1}{2}$.
2. Photograph of ovum, No. 559. $\times 1$.
3. Section through the cyclopean eye, No. 1165. $\times 40$. *E*, eye; *c. l.*, eyelid. Behind the eye are seen the ocular muscles.
4. Section through the external ear of embryo No. 201. $\times 75$. There is an invagination of the epidermis and tissues of the ear are dissociated.
5. Photograph of the ovum, No. 559, showing the embryo in position. $\times 3$. The exocoelom is filled with a dense magma.
6. Outline of brain of embryo No. 1201. $\times 100$. From a plaster reconstruction made by Dr. Bartelmez. The wide-open flange in front contains two depressions, the optic vesicle, which unite through a common groove, the torus opticus, as marked on the drawing. The depression behind this flange no doubt represents the cavity of the midbrain and hindbrain. This specimen is No. 87 of the collection of the University of Chicago and contains eight pairs of myotomes.
7. Photograph of section through the snout of specimen No. 559. $\times 60$. The frontal process contains the common cerebral vesicle, *c. v.*, and below the snout there is the upper jaw, *u. j.*

PLATE 3.

All the photographs are from the sections of the embryo No. 559. Figs. 1 and 2, $\times 50$; figs. 3, 4, 5, $\times 40$.

1. Section through the ocular muscle, showing the terminal fibrils of the third and sixth nerves. *Oc. mus.*, ocular muscle; *n*, notochord; *m*, mouth; *v*, fifth nerve.
2. Section through the eye at its attachment to the interbrain. *T. o.*, torus opticus; *r', v'', v'''*, branches of the fifth nerve; *ty*, terminal filaments of the fourth nerve ending in the primordia of the superior oblique muscle.
3. Section through the lower tip of the cerebral hemisphere. The peculiar tissue surrounding the nerve-body may represent a degenerated olfactory region. About this is seen a section of the interbrain, and below a process containing the cyclopean eye. *Fb*, first branch of fifth nerve; *m*, mouth.
4. Section through the middle of the cyclopean eye. *I. b.*, interbrain; *Fb*, first branch of fifth nerve; *m*, mouth.
5. Section through the cerebral hemispheres as they communicate with the interbrain. *I. b.*, interbrain; *c. h.*, cerebral hemisphere; *s*, snout; *u. j.*, upper jaw. Over the lamina terminalis is seen the peculiar thickening of the outside of the body which may represent the degenerated olfactory region also shown in the flat section of figure 3.

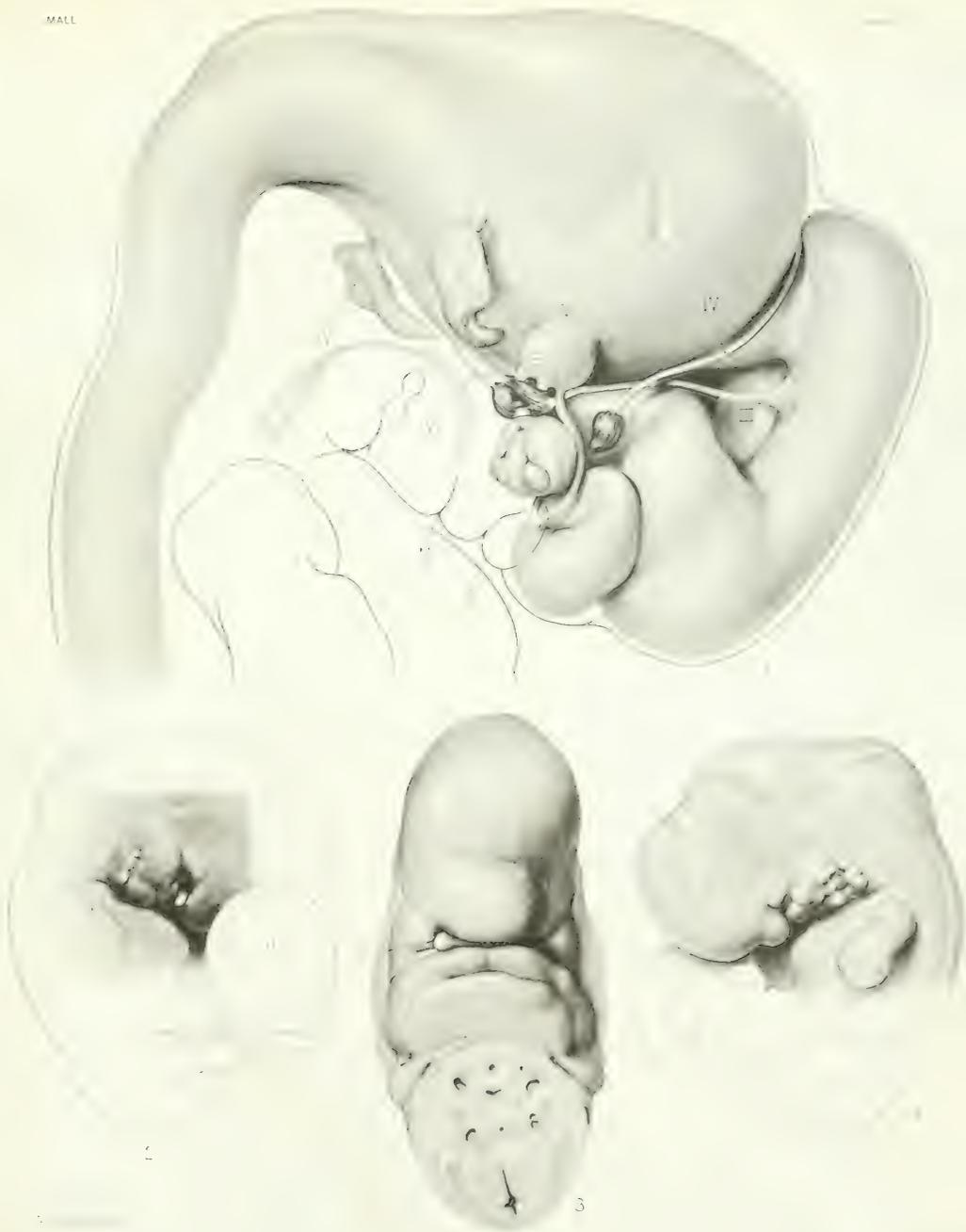


FIG. 1.



FIG. 2.



FIG. 3.



FIG. 4.

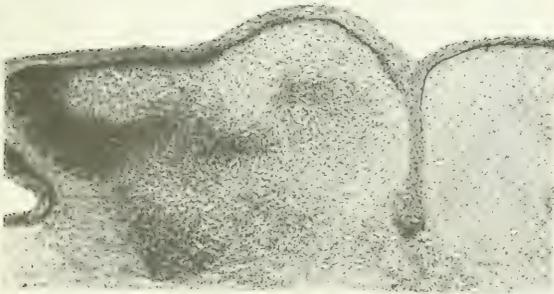


FIG. 5.



FIG. 6.

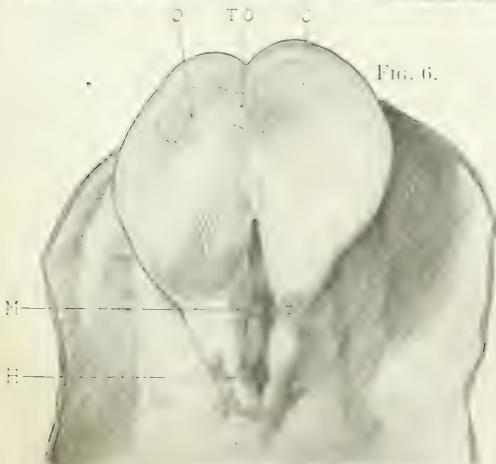


FIG. 7.

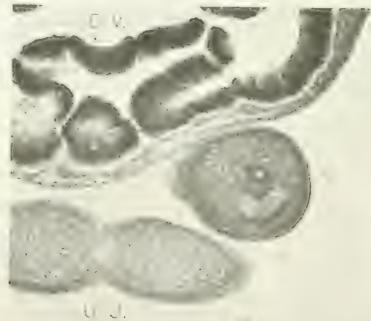




FIG. 1.

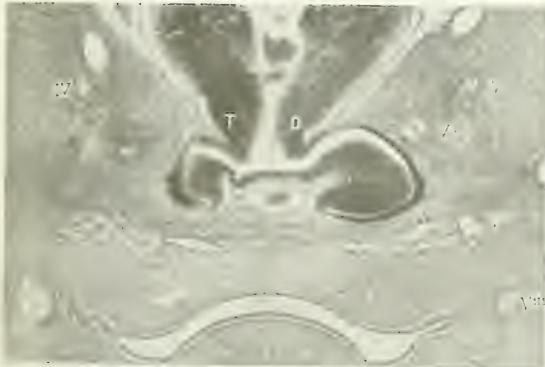


FIG. 2.

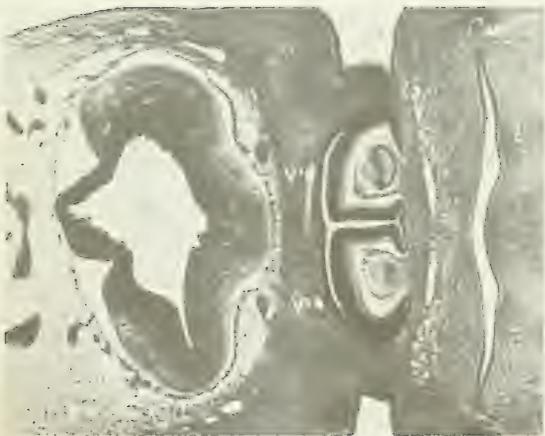


FIG. 4.

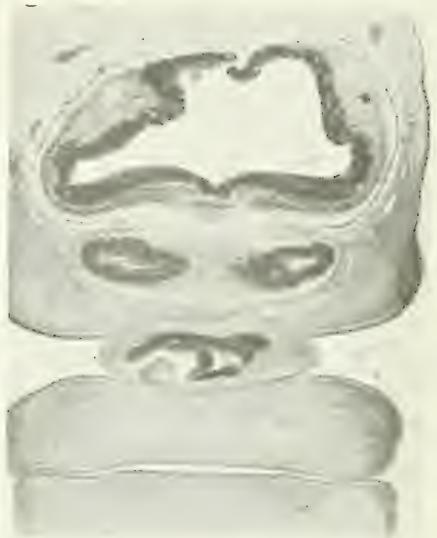


FIG. 3.



FIG. 5.

CONTRIBUTIONS TO EMBRYOLOGY, No. 16.

QUANTITATIVE STUDIES ON MITOCHONDRIA IN NERVE-CELLS.

By MADGE DEG. THURLOW.

One plate.

QUANTITATIVE STUDIES ON MITOCHONDRIA IN NERVE-CELLS.

BY MADGE DEG. THURLOW.

The hope of being able to establish a sound foundation for investigation into cellular physiology has inspired most of the work on cell constants. Perhaps the best known of these is the nucleus-cytoplasmic ratio of Hertwig (1902), which has already proved of great value in the investigation of changes in nerve-cell activities. On account of the reawakening of interest in mitochondria, the attention of investigators has been drawn in recent years to the cytoplasm. This is not surprising, as structural changes resulting from experimental variations are evident in the cytoplasm, for it is here that most of the products of differentiation are laid down and readjustments in response to changes in the environment take place.

Though the study of mitochondria has been carried far along many different lines, up to the present time no attempt has been made to place these cytoplasmic structures upon a quantitative basis. With this object in view the present work was undertaken, making use of a favorable method of technique. No attempt was made to establish a ratio between mitochondria and cytoplasm on the basis of relative volumes, the number of mitochondria per unit volume of cytoplasm being the basis of comparison. This relationship has served well as an adequate foundation for comparison of various nerve-cells and is thought to be of particular value in the case of the nerve-cell, inasmuch as it possesses no other cytoplasmic constituent lending itself to quantitative study; for the Nissl substance differs so widely in form and density that it is absolutely impossible with our present methods of technique to estimate its amount with any degree of accuracy, and the significance and form relations of the neurofibrils are not clear.

The original plan of the investigation was to determine whether by comparisons of the mitochondrial content of known motor and sensory cells there was a distinctive difference between cells of these categories. With this purpose in view, quantitative estimations of mitochondria in the nuclei of origin of the cranial nerves were made. The results were disappointing, in that they showed that the mitochondrial content could not be used as a basis of classification for motor and sensory cells, but they did show something that was not known before, viz, that the number of mitochondria per unit volume was constant for the nucleus of any cranial nerve.

MATERIAL AND METHOD.

The animal selected was the white mouse, and the observations were confined to the nuclei of the cranial nerves. The nuclei of the IX and XI nerves were not included in the investigation, owing to the difficulty of ascertaining with absolute certainty what cells constituted these nuclei in the nucleus ambiguus.

The method of fixation and staining has been described by Cowdry (1916a). The mitochondria, which take the fuchsin, appear as discrete, bright-red granules sharply outlined against a background of light-green Nissl substance, and, with proper optical and lighting facilities, may be readily counted.

Quantitative estimations were made by carefully counting the mitochondria occurring within a field of constant area. Such a field was obtained by placing within the ocular a glass disk upon which a single square had been ruled according to the method of Isaacs (1915). The actual area of the optical field covered by the square was determined by the stage micrometer and found to be 19.78 square micra. The thickness of the sections was 4 micra; hence the volume of the field counted could easily be determined, and from that the amount of mitochondria per cubic millimeter was calculated.

The lenses used were the Zeiss apochromatic 1.5 mm. objective and Zeiss No. 6 compensating eyepiece. Constant conditions of illumination were obtained by the use of the 40-watt Mazda lamp. A mechanical stage was employed. All counts and drawings were made under uniform conditions of magnification and illumination.

In order to avoid inaccuracies due to possible minor differences in thickness of the sections, the same number of fields was counted in every nucleus occurring in any one section. For example, in the same section may be found cells of the cochlear and vestibular nuclei of the VIII, of the VI, VII, and the mesencephalic nucleus of the V. If four fields were counted in one section in mesencephalic cells, four fields of all the other nuclei were counted in that section.

OBSERVATIONS.

Studies were carried out upon five brains which had been cut into partial serial sections. Only such nuclei as were well stained in each series were investigated and the results tabulated (table 1).

TABLE 1.

Nucleus.	Series 1259	Series 1348	Series 1237	Series 1260	Series 1257	Remarks.
Mesencephalic of V.	22.6	22.5	21.7	22.9	22.1	
VI.....	21.5	—	20.7	17.4	—	
VII.....	15.1	15.7	15.0	Series 1259, male, weight 9 grams, age 33 days.
VIII (vest.).....	17.6	Series 1348, female, weight 13.5 grams, age 61 days.
VIII (vent. coch.)..	18.8	18.9	Series 1237, male, weight 10 grams, age 25 days.
X (motor).....	14.1	14.7	Series 1260, male, weight 8.5 grams, age 31 days.
III.....	15.2	14.3	15.4	14.3	Series 1257, female, weight 9 grams, age 35 days.
IV.....	17.3	
					22.9	

The above figures represent the average number of mitochondria present in the field counted, the volume of which was 79.12 cubic micra. It will be noted that there are many gaps in the series. This is due to the fact that, owing to the great difficulty of cutting perfect serial sections 4 micra in thickness, some of the nuclei are missing. Again, perfect definition of outline of the mitochondria is required before they can be counted, and since it is impossible, even with the most expert technique in staining, to attain this ideal in every section, some of them could not be used.

At first 150 fields were counted in each nucleus and the average was taken, but as there was never any wide variation in the figures obtained for different fields the number was limited to 50, and later to 10. Most of the averages recorded here were based on 10 field counts—never on less. Lists were kept of the number of mitochondria in each field as they were counted, and such variations as the following were noted: In 35 fields observed in the VI nucleus the numbers ranged from 20 to 26; in 10 from the mesencephalic the range was 20 to 24; in 10 from the VII it was 14 to 17. This uniformity ran through all the nuclei of all the series, with a few exceptions that will be mentioned later.

In order to determine the percentage error in the total, 10 fields were re-counted in several cases and the error estimated, and it never amounted to more than 1.3 per cent. This maximum occurred in the cells most closely crowded with mitochondria; in cells having few mitochondria the recounts showed no error. Table 2 contains the detailed results of specimen counts and recounts.

TABLE 2.

Nucleus VII (motor).		Nucleus VI.		Nucleus XII.	Nucleus V (mesen- cephalic).	Remarks.
Original count.	Recount.	Original count.	Recount.	Original count.	Original count.	
15	16	25	24	17	22	Original counts of four nuclei are given. The figures illustrate the uniformity of the mitochondrial content of the individual nuclei and are typical of all the nuclei counted. In the case of nuclei VII and VI figures for the recounts are also given (right-hand column). These show little variation from the original count. In making the recounts, the slide was not moved, in order to have the limits of the field unchanged, the recount being made immediately after the original count.
15	16	25	26	15	24	
16	15	21	22	15	22	
16	17	27	26	15	25	
15	16	24	26	15	23	
14	15	28	27	14	23	
14	14	23	24	14	21	
16	17	22	20	14	21	
16	14	21	23	16	22	
17	16	25	23	17	22	

Care was taken to avoid cells whose limits could not be clearly defined. Inasmuch as the cell processes and the neuroglia possess mitochondria, it was necessary to choose sharply outlined cells, in order to eliminate errors due to counting mitochondria outside the cell-body.

It has been stated that the number of mitochondria in the cells of the same nucleus is quite constant, but that there are some exceptions. In one animal, in the VI nucleus some cells had fewer mitochondria than others, but as these appeared normal in other respects they were counted, the variation ranging in this instance from 13 to 21. The greater number, however, contained about 17 per field. In the case of the cochlear nucleus there was a group of cells dorsal and somewhat lateral to what appeared to be the main body of the nucleus. The dorsal group corresponds to current descriptions of the dorsal cochlear nucleus, and the larger group to the ventral cochlear nucleus. The cells of the dorsal nucleus contained practically no mitochondria, were slightly smaller, and possessed a cytoplasm clearer than those of the ventral nucleus. Only the cells of the ventral nucleus were included in the count, for it was felt that cells which

showed such constant and specific morphological differences could not be included in the same functional category.

Upon referring to plate 1 it will be seen that there are striking similarities between nuclei of different functional categories. For example, the mesencephalic nucleus (fig. 7) of the V (regarded as sensory by most authors—as Johnston, 1909, and Willems, reviewed by Donaldson, 1912) and the motor nucleus of the IV (fig. 4) have the same number of mitochondria per unit volume of cytoplasm; the visceral motor nucleus of the VII (fig. 1) and the somatic motor nucleus of the XII (fig. 5) have the same average; so, too, have the vestibular of the VIII (somatic sensory, fig. 2) and the motor of the III (fig. 6). Not only do nuclei of different categories agree in the number of mitochondria they contain, but those of the same category disagree, the numbers for the somatic motor nuclei being 14, 17, 20, and 22 mitochondria per unit volume of cytoplasm. Comparisons of the nuclei of the same general classification are made in table 3.

TABLE 3.

Somatic motor nuclei.		Visceral motor nuclei.		Somatic sensory nuclei.	
Cranial nerves.	Average number of mitochondria per field for all cells counted.	Cranial nerves.	Average number of mitochondria per field for all cells counted.	Cranial nerves.	Average number of mitochondria per field for all cells counted.
III....	17.3	X.....	14.4	V.....	22.3
IV....	22.9	VII....	15.3	VIII (vest.).....	17.6
VI....	20.5			VIII (vent. coch.)..	18.8
XII....	14.8				

No visceral sensory nuclei were studied. It may be seen that no distinction can be made between motor and sensory nuclei on the basis of their mitochondrial content.

In order to have some idea of the enormous number of mitochondria in the cells of the nuclei of the cranial nerves and of the really tremendous variations in number of mitochondria in cells of the different nuclei whose number of mitochondria per field vary only from 14.1 to 22.5, the results of some determinations as to the number of mitochondria per cubic millimeter of cytoplasm will be given. In nerve-cytoplasm containing 22.5 mitochondria per field, such as that of the cells of the mesencephalic nucleus (fig. 7), the number of mitochondria per cubic millimeter would be 284,378,159; the number for the cytoplasm of the cells of the nucleus of the X nerve (fig. 8), containing 14.1 per field, would be 178,210,313.

This study has been very carefully controlled. In the first place, several observers have briefly called attention to the fact that mitochondria differ in form not only in different nerve-cells, but also to some extent in the same cell. Nicholson (1916), working in this laboratory, has made a careful study of these morphological variations in mitochondria in the nerve-cells of the white mouse, the same form which I have studied. He, however, worked with cells other than those of

the cranial nerves, where the mitochondria are, for the most part, granular, as seen by the illustrations. Working particularly with the anterior-horn cells and the large cells of the reticular formation, he describes mitochondria which are filamentous, the filaments varying in length, some being so short as to be almost identical with the granular forms, as one proceeds from the periphery to the nucleus.

Naturally the question would arise as to whether, under these circumstances, quantitative variations such as those recorded in table I have any real significance. It is obvious that the method which I have adopted of counting the mitochondria would have to be accompanied by very accurate measurements in order to yield reliable information of the relative as well as actual amount of mitochondria in cells in which their size and shape differ to any appreciable extent. Filamentous mitochondria, though occurring in the cell processes, are rarely found in the cell-bodies of the cranial nerves and, since my observations are confined to the cell-bodies, these filamentous mitochondria do not constitute a source of error. A few of them are homogeneous throughout, but most can be resolved by careful focusing into rows of discrete granules, fairly uniform in size. For the work in hand this was a great advantage, for by counting the granules a more accurate index of the *amount* of mitochondria was obtained than would have been possible by counting the filament as a unit. Though the method of counting here used is not ideal in the case of cells characterized by great dimorphism on the part of their mitochondria, it does yield reliable results when care is taken to restrict its use to cells in which the size and shape of the contained mitochondria are practically uniform, as in the cells of the nuclei of the cranial nerves.

Despite the fact that the granules were of the same size, if there were lack of uniformity in their distribution, an accurate estimation of the mitochondria for any one cell would be impossible unless all the mitochondria in that cell were counted. It is because of their practically uniform distribution that the amount in any one field can be used as typical for the whole cell. Counts were made of different fields of a cell in one section, also of fields selected from the same cell in successive serial sections; and the numbers were practically identical. It is true that there is usually a slight crowding of mitochondria in the axon hillock, with a tendency for them to be arranged in filaments or rows of granules along the long axis of the cell-process (figs. 3, 4, and 5). In the canalicular system, which in these preparations shows white, there are no mitochondria; where they do seem to occur in the canals the appearance is due to their presence in the thin layer of cytoplasm surrounding the canal. Especially in figures 1 and 5 there appear to be large areas free from mitochondria. This is explained by the fact that all the drawings were made in one optical plane; on a different focus mitochondria would have appeared in the cytoplasm, which is now free from them. No variations were noted in the density of distribution of mitochondria other than those just mentioned. Any minor unevenness in the distribution of mitochondria would be obviated by the fact that the square used in counting is relatively large, taking in an expanse of cytoplasm extending in most instances from the nucleus to the periphery of the cell.

Again, it has been shown that mitochondria vary in their solubilities in acetic acid (Regaud, 1910; Nicholson, 1916). In view of this fact objection might be made that the variation in mitochondrial content in different types of nerve-cells was an artefact produced by the solvent action of the reagents used. Such criticism of the results obtained in this investigation is invalid, for no acetic acid was used in the preparation of the specimens, nor was there any other solvent for mitochondria involved in the fixation or staining. The formalin which was used as a fixative for the mitochondria was neutral, and the long mordanting in the bichromate prevented their subsequent solution in alcohol, although their solubility in alcohol does not seem to be marked. Hence there is no error in technique which could account for the striking variation in amount.

DISCUSSION.

Having established the certainty that there is, in the nerve-cells of the medulla of the species used, a definite amount of mitochondria per unit volume of cytoplasm, there remains to be determined the functional significance of such numerical variation. Other investigators have, in their work on the central nervous system, determined various cell ratios, among which might be mentioned the nucleus-plasma ratio. Dolley (1914) has found that the resting nerve-cells of corresponding type for the same species have a constant nucleus-plasma ratio which is altered temporarily through functional depression or permanently through functional senility. Having once established this constant, he could study pathological changes following experimental conditions. Donaldson (1911) found variations in the water content of the nerve-cells accompanying functional changes. He makes no further statement than this: the variation in water percentage of the nerve-cell is an index of functional activity.

The relationship between number of mitochondria and volume of cytoplasm is another such constant. In the animals studied the number of mitochondria per unit volume of cytoplasm was found to be constant for corresponding cells, not only of the same animal, but also of different individuals of the same species (table 1). In nerve-cells of the same type, therefore, we have a cell constant which is definite for animals of the species studied and which can be used for observations of pathological conditions resulting from experiment. That mitochondria do react to conditions which affect the cell has been demonstrated by several authors, as Policard (1910 and 1912), Homans (1915), Scott (1916), and others.

These observations may be given another application, viz, to the doctrine of neurone specificity. It would be reasonable to suppose that even if all the mitochondria were identical, such definite and constant variations as are here recorded would be closely associated with a definite and constant functional differentiation. Combined, however, with this quantitative difference are qualitative differences (Nicholson, 1916), and this combination serves to strengthen the theory that the activity of the nerve-cells themselves differs in some way. It would be rather extreme to assume that cells differing specifically with respect to so con-

stant an element in their cytoplasm were functionally identical, for although as yet the rôle played by mitochondria in nerve-cells is unknown there is evidence that they play an important part in other cells. Cowdry (1916b) has discussed briefly the literature bearing on the relations of mitochondria to cell metabolism. From such evidence it is safe to assume that they are not an unimportant constituent of nerve-cells and that their constancy in amount in the normal cell is definitely associated with its normal activity.

CONCLUSION.

There is a constant number of mitochondria per unit volume of cytoplasm in normal nerve-cells of a corresponding type in the mouse. This constant differs for nerve-cells of different types. Sensory and motor cells can not be distinguished on the basis of their mitochondrial content. The significance of constant variations can not be interpreted with our present meager knowledge of the rôle played by mitochondria, but there is support for the theory that nerve-cells are functionally differentiated in the evidence here advanced of their constant difference with respect to the number of mitochondria they contain.

Finally, I desire to express my cordial thanks to Dr. E. V. Cowdry, who kindly suggested the problem and provided the material for the investigation.

BALTIMORE, May 31, 1916.

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EXPLANATION OF PLATE.

The following drawings were made with the aid of a camera lucida, a 1.5 mm. apochromatic Zeiss objective, and No. 6 compensating Zeiss ocular. All drawings are at a magnification of 1,650 diameters.

The Nissl substance appears as a background of bluish-green masses, between which are the unstained canals of the canalicular system. Against this background the mitochondria appear as bright-red dots. All cells were drawn without changing the focus, so that sometimes the mitochondria are clumped within the Nissl substance and sometimes within the canals, although this latter appearance is due to their presence in the thin layer of cytoplasm surrounding the canals. If the focus were changed sufficiently mitochondria would occupy the spaces now free. The counting, however, was done by focussing through the whole depth of the section. Some of the mitochondria seem to be smaller and less definite than others; this, in all the drawings, is due to the fact that they were slightly out of focus, yet sufficiently clear to be included in the drawing. Where they occur as chains of granules they are probably broken filaments. The granules, when brought clearly into focus, are of approximately the same size.

PLATE 1.

1. A typical cell of the motor nucleus of the VII cranial nerve. Note its similarity to the cell of the XII nucleus (fig. 5), both with respect to the appearance of the Nissl substance and the number of mitochondria, the number per unit volume of cytoplasm for both being 15.
2. A cell from the ventral cochlear nucleus of the VIII nerve. The Nissl substance here is a diffuse violet, and the mitochondria stain more intensely than those in the other nuclei.
3. A motor cell from the nucleus of the VI nerve.
4. A motor cell from the nucleus of the IV nerve. It will be noted that there appear to be more mitochondria in the cytoplasm of the cell of the mesencephalic nucleus (fig. 7) than in this, although the counts show that the number per field is practically the same. This is due to the fact that the cytoplasm of the mesencephalic cell is much more transparent, lacking the great clumps of relatively opaque Nissl substance which characterizes the IV nucleus cell; and so in a single optical plane, mitochondria may be seen to a greater depth in the mesencephalic cell. Since focussing was done throughout the depth of the section, the Nissl substance did not interfere with the accuracy of the counts.
5. A motor cell from the nucleus of the XII nerve.
6. A motor cell from the nucleus of the III nerve.
7. A sensory cell from the mesencephalic nucleus of the V nerve. The mitochondria appear closely crowded, even on one plane.
8. A cell from the dorsal motor nucleus of the X nerve.



CONTRIBUTIONS TO EMBRYOLOGY, No. 17.

DEVELOPMENT OF CONNECTIVE-TISSUE FIBERS IN TISSUE
CULTURES OF CHICK EMBRYOS.

BY MARGARET REED LEWIS.

Two plates.

DEVELOPMENT OF CONNECTIVE-TISSUE FIBERS IN TISSUE CULTURES OF CHICK EMBRYOS.

BY MARGARET REED LEWIS.

Up to the present time the study of fixed and stained preparations of embryos has failed to decide the question of the origin of the connective-tissue fibers. This is partly because the methods used necessarily coagulate and distort the delicate cell processes and also the intercellular substances, and such results readily lend themselves to various interpretations by different investigators.

In the following observations on tissue culture an attempt has been made to study the formation of the connective-tissue fibers directly within the living tissue, and not only within living tissue, but within tissue which has developed in an environment entirely free from fibrin or any substances other than those within the cell which coagulates upon fixation.

The pieces of tissue to be explanted for the tissue cultures were washed through several changes of Locke's solution until such fibrogen as was present had become coagulated within the pieces themselves before they were explanted. The medium thus remained free from fibrin, for no fibrin network was observed in the medium of any preparation. In order to see whether the substance forming fibrin would be dissolved out from the explanted piece and deposited as a fibrin network in the medium of the culture, a few cultures were made in which the explanted pieces of tissue were not carefully washed. No fibrin network was found in these cultures, even after many days. However, in such preparations a delicate network formed over the growth (not within the growth) upon fixation, showing that some substance had been dissolved out from the explanted piece, which coagulated upon fixation. Although this network did not resemble fibrin network, or the delicate processes from the cells, or even the fibrous tissue itself, all such preparations were discarded in order to avoid any possible chance of error.

By this method it is taken for granted that such connective-tissue fibrils as form in the tissue-culture growths arise from the cells, either as a secretion formed by the cells and deposited in the form of fibrils and fibers, or from the transformation of the cytoplasm of the cell itself. As will be seen later, while there is evidence of a possible secretory activity of these mesenchyme cells, as Remaut (1904) and Remaut and Dubreuil (1906) have claimed, due to the presence of the "grains de segregation," or the so-called vacuoles of Lewis and Lewis (1915), nevertheless, in these tissue cultures, the connective-tissue fibrils formed by a transformation of the cytoplasm of the cells.

Tissue culture is not an entirely satisfactory method for the study of any highly differentiated tissue, owing to the fact that the cells which migrate out from the explanted piece and later increase by division attach themselves so closely to the cover-slip and become spread out in such a thin layer that the differ-

entiated structure loses its characteristic appearance. Also, the cells of the new growth have a tendency to migrate away as individual cells instead of developing into a differentiated tissue composed of numerous cells. In all probability the cells do not de-differentiate and become more embryonic, as has been claimed by Champy (1913) and others, but simply lose their characteristic differentiated appearance, due to their changed shape and position. This is interestingly shown by a study of smooth muscle-cells (plate 2, fig. 6). Where the cells are attached closely to the cover-slip they no longer contract and the myofibrils appear as irregular bundles composed of numbers of delicate fibrils (plate 2, fig. 6). However, where the cell is not so closely attached to the cover-slip it continues to contract, and in this case the myofibrils are arranged into the characteristic fibrils. Taking the possible loss of the characteristic appearance of the differentiated structure into account, the very thin and largely spread-out living cells of the tissue culture furnish an excellent means for the study from day to day of certain structures of the cell.

Just how much differentiation can take place in such cells in these tissue cultures is difficult to state. Certainly in a few cases, where the mesenchyme growth at 48 hours was composed of quite undifferentiated cells, this growth, when kept alive by frequent baths of fresh solution, did develop definite connective-tissue fibrils. Muscle fibers have been observed to become more differentiated; but in the case both of the muscle fibers and connective tissue there is some continuation of function, as the muscle fibers frequently contract, and the connective-tissue growth also occasionally contracts back around the explanted piece and later grows out again.

Fibrils did not develop in many of the cultures of connective tissue, owing to the fact that the cells remained spread out as individual cells until the death of the culture. In the few cultures that were kept alive for a sufficient length of time, and in which the connective-tissue fibers did develop, they could be clearly seen and studied in the living preparation from day to day, and their development could be traced from the earliest delicate fibril within the exoplasm of the cell to the more adult fibers, which appear to be free from the cells.

PREVIOUS WRITINGS ON THE LIVING CONNECTIVE TISSUE.

Whether the connective-tissue fibers arise within the cells or from an intercellular substance is still an open question. The weight of evidence seems to be in favor of a cellular origin, although certain text-books of histology present the question almost wholly from the intercellular point of view.

There are many reviews of the literature on both sides of the question (Fleming, 1891; Spuler, 1896; Mall, 1901; Rothig, 1907), and also various text-books, and since the technique used by other investigators is so different from that employed in the following observations no effort will be made to take up in detail the various papers upon the origin of the connective-tissue fibers.

While many observers have studied teased preparations of connective tissue, Boll (1872) was the first to study the development of the connective-tissue fibrils entirely from the living cell. Boll made his cultures by teasing out a few of the

cells of the tissue to be studied and placing them in a hanging drop of amniotic fluid. Although he did not obtain any growth of the teased-out cells, he was able to observe the connective-tissue fibrils in connection with the cells throughout the different stages of the development of the connective-tissue fibers, and he became convinced, by this study of the living cells, that the fibrils had their origin within the cells and continued through the exoplasm of one or more cells. Boll studied carefully the following tissues:

- Arachnoid of chick embryo of 4 to 19 days' incubation.
- Subcutaneous tissue of chick embryo of 7 to 17 days' incubation.
- Cornea of chick embryo of 4 to 21 days' incubation.
- Tendon of chick embryo of 7 to 21 days' incubation.

He concluded that in all of these tissues the connective-tissue fibers originated from the cells.

In the study of the connective tissue by the tissue-culture method, preparations such as those studied by Boll were used, and also many others, in which the tissue was either teased out, or flattened out, or suspended in a hanging drop of Locke's solution instead of amniotic fluid. Figures similar to those given by Boll were frequently observed (plate 1, figs. 2, 4, and 5), and his observations were corroborated. However, in all such preparations it is impossible to eliminate the possibility of fibrin or some other intercellular substance taking part in the formation of the fibrils, and for this reason these observations will not be given below, although they undoubtedly show the connection of the fibrils with the cells.

As no cultures containing fibrin have been studied, nothing can be said at this time in regard to Baitzell's observations (1915), by means of which he shows that certain fibers which resemble the connective-tissue fibers may form in a fibrin clot in the presence of a piece of tissue of a chick embryo after various periods of time. In the development of the embryo there can be no question of fibrin playing any part in the formation of the fibrous tissue, since, so far as is known, fibrin is not present in the uninjured tissue. Whether the cells of the embryo possess the power of secreting a substance which may act in the same manner as the injured cell to produce the formation of fibrin, or whether the connective-tissue cells in the developing embryo act directly upon the plasma, are questions which Baitzell does not discuss. He quotes the following experiments of Loeb, from Adami:

"When a drop of uncoagulated lymph is placed between two glass slides, the mere act of pulling one slide over the other leads to the appearance of fibrils, which grow in length and bulk; which like those of connective tissue are not only intracellular, but actually traverse cell bodies situated in their path; which show themselves first in immediate connection with the cells, the cells as we now hold liberating an enzyme that determines the modification of the more soluble protein into a precipitated or coagulated modification. But the lines of the precipitation are evidently along the lines of strain."

These experiments of Loeb are in a way comparable to those of Baitzell, except that in Baitzell's experiments the strain is brought about by the shrinkage of the plasma clot. It seems rather difficult to draw any conclusions in regard

to the manner of the formation of the connective-tissue fibrils in the embryo from results which are so obviously due to injured cells as are those of Loeb's experiments. However, there is a striking resemblance between the fibrous tissue obtained by Baitsell by means of a modification of the fibrin clot and the fibrous tissue of the embryo.

Baitsell's (1916) paper on wound-healing, in which he finds that very shortly after a wound has been made in the skin of a frog, fibrin fibers, which resemble connective-tissue fibrils, are deposited, and that these fibers persist and take part in the formation of the cutaneous tissue, opens the exceedingly interesting question as to whether what takes place in the process of wound-healing can be in any way comparable to the behavior of normal developing tissue.

So far as can be gathered from Isaacs's (1916) incomplete report of his observations upon the living connective-tissue fibers, his results correspond more or less with those of Loeb—that is, that various strains cause the intercellular substance to form fibrillæ. Just what part the cells play in this formation it is difficult to understand. Isaacs does not say that the cells form an enzyme, as Loeb claims, but states that the movements of the connective-tissue cells probably effect the distribution of the material through chemical or other action and cause the fibrillated structures of the adult fiber. From Isaacs's brief report it is evident that he had performed numerous experiments with the living connective tissue, and it is hoped that his complete paper will clear up many points.

Ferguson's (1912) observations upon the living connective-tissue cells in the fins of fish embryos are extremely interesting, since by his method the connective-tissue cells were studied under entirely normal conditions. Since there has been some question as to whether fibers actually exist or whether they are merely coagulations of a colloid within the tissue due to abnormal conditions, it is interesting to note that Ferguson describes fibers as well as cells as existing in the living embryo. He found, by the aid of preparation stained by Bielschowsky's method, that the fibers arise within the cell. Unfortunately he was not able to see the fibers in the embryonic cells of the living embryo and to determine whether they become separated from the cells, or how this takes place. His observations upon the movements of the connective-tissue cells show that the round and stellate cells may move up to and stretch along a fiber as a very thin, long spindle cell, and in a few cases he observed such a spindle cell to become stellate again.

Ebeling (1913) has for a period of two years or more kept alive certain of the cultures started by Carrel. The growth of these cultures consists mainly of connective tissue, and Ebeling claims that connective tissue may have a permanent life outside the organism when properly cared for. The method of keeping the culture alive is as follows: The entire culture in its plasma clot is freed from the cover-slip and washed in Locke's solution to remove any waste products and is then cut into four or more pieces, and each of these pieces is again explanted into a drop of fresh plasma. This procedure is carried out every other day; although many of the cultures die, a few survive and grow, and are again explanted as described above. In all probability there is no differentiation of the connective tissue into fibrils or fibers, as Ebeling describes the growth as though it consisted

of undifferentiated mesenchyme, which is what would be expected in any tissue that proliferates as rapidly as this tissue necessarily must. It would be interesting to see whether, if one of the cultures were kept alive without further explanation, it would again differentiate after a certain equilibrium of proliferation had been reached.

Some workers have claimed that certain substances present in the medium of tissue cultures prevent the growth of the connective tissues. For example, Walton (1914) states that liver extract inhibits the growth of adult mammalian connective tissue in plasma cultures, and Russel (1914) claims that gentian violet, in solution of 1/20,000 in the medium of tissue culture, prevents the growth of connective tissue but not of endothelium. The reason for this neither writer explains, nor does either state what structure of the cell is affected by the substance so that the cells do not grow out, or whether the medium may simply not attract the cells to migrate and that the cells themselves are uninjured.

Thus a review of the literature on the living connective-tissue cells shows that the study of the living tissue has not presented decided proof as to whether the connective-tissue fibers arise from the cells or are formed from an intercellular substance. Evidence is presented on both sides, and the question remains as completely at a dead-lock as when the observations were confined to fixed and stained preparations.

OBSERVATIONS IN GENERAL.

A few general observations as to what takes place in the tissue cultures of connective tissue are given here in order to show what factors influence the growth of the fibers.

Cultures from the subcutaneous tissue of chick embryos of various ages were made in the usual manner (W. H. and M. R. Lewis, 1915; M. R. Lewis, 1916). Lewis and Lewis have shown that while the cells in the new growth in tissue cultures are under somewhat abnormal conditions as regards environment and nourishment, nevertheless they are actively growing cells which undergo normal division and which grow out as definite types of cells— that is, nerve-cells, muscle-cells, heart-muscle cells, endoderm of the intestine, epithelial cells of the skin, and connective-tissue cells.

As has been stated, no fibrin is present in the medium, and no substance which coagulates.

The subcutaneous tissue can be removed as a thin, transparent sheath from the skin of chick embryos of 10 days or older. It proved difficult to isolate the subcutaneous tissue from embryos younger than 8 days; and in these embryos a piece of skin or one of the deeper skin fascias or the arachnoid tissue was used for explanation. The connective tissue of embryos less than 8 days old is composed of cells without definite fibrils; that of embryos of 11 days and over contains definite bundles of fibers. The new growth from an explanted piece of subcutaneous tissue of embryos of 8, 9, and 10 days proves very satisfactory for the study of the development of the fibrils. The growth can be kept alive and healthy by frequent baths of fresh Loëke's solution, plus 10 per cent bouillon, plus 0.25 per cent dextrose; and fibrils begin to develop in the new growth in from 48 to 72

hours and continue to develop until the growth is about 6 days old or over. Cultures of the subcutaneous tissue from an 11 or 12 day chick embryo also prove very satisfactory for study, for not only is the new growth available for study, but the explanted piece itself is so thin that the cells and fibers can be observed even with the oil-immersion lens.

The fibers in the explanted piece were not observed to grow either in length or bulk, and after a period of two weeks they remained much the same as when explanted. In no case was a fiber observed to pass out from an explanted piece over the new growth; such a fiber always remained curled up within the explanted piece.

New fibrils begin to develop in the new growth from an explanted piece of tissue from an 8 to 12 day chick embryo shortly after the new growth is 24 hours old, and definite bundles of fibrils may be developed when the growth is 5 or 6 days old. These fibrils develop more quickly in growths from the older embryos of 10 to 12 days than in those from the younger embryos of 8 to 10 days.

The new growth from the subcutaneous tissue is extremely sensitive and reacts to all sorts of changes in its environment, by contraction. Frequently while a membrane of connective tissue was under observation it would begin to contract from the outer edge of the growth and draw in towards the explanted piece. This contraction might stop at any period or it might continue until the entire new growth had contracted close to the explanted piece. The explanted piece was never observed to contract. The relaxation after such a contraction was exceedingly slow, and frequently a contraction that had taken no longer than 2 to 5 minutes required for relaxation from 1 to 6 hours. In fact, the process did not resemble relaxation, but rather a growing-out again of the new growth. Often, coincident with the contraction, there occurred a rolling-back of the edge of the growth, and in this case when the cells migrated out again many of them became changed in their relative positions. Thus it is evident that a decided strain is present during the development of the fibrils, though there is no fibrin and (so far as can be seen) no substance which coagulates surrounding the cells. Whether this strain or tension (often exhibited by the contraction above described) may in any way influence the separation of the fibrils from the cytoplasm of the cell, it is impossible to state. It was not certain that a preparation which contained well-developed fibrils had not contracted during the development of the fibrils. However, it can be definitely stated that no substance formed into fibrils during contraction, as might have been expected from the experiments of Loeb and of Isaacs. The new growth, when relaxed after such a contraction, never contained any suddenly formed fibers or fibrils, and such fibrils as were present were in very much the same state of development as that in which they were before the contraction took place. Also, many membranes in which no fibrils ever developed possessed the power to contract, and did contract more than once.

From these general observations it is evident that the fibers which form in the tissue cultures must arise from the cells; and since the cells are spread out in a thin layer the process of development of the fibers can be observed in the living cell undisturbed by any manipulation.

OBSERVATIONS IN DETAIL.

The growth from a piece of chick embryo of 6 to 8 days' incubation is usually in the form of a membrane closely attached to the cover-slip, and is composed of large, flat cells, either connected by numerous cytoplasmic processes (plate 1, fig. 1) or else crowded together so that the delicate processes from cell to cell are lost and a more definite cell-wall appears (plate 2, fig. 10). The growth from older chick embryos may also sometimes have the appearance of a membrane, especially where the cells are spread out in a thin layer along the cover-slip. When such a growth is treated with silver-nitrate stain the membrane becomes marked with more or less definite cell-walls, according to the amount of crowding of the cells (plate 2, fig. 10). Such a membrane has been described by Clark (1914), where the connective tissue is stimulated to grow out over a very smooth surface, which Clark interprets as showing that under certain conditions the connective-tissue cells may become transformed into endothelium. While the pattern which appears with the silver-nitrate stain is in many ways characteristic of endothelium, still growths from older chick embryos (8 to 10 days) in these tissue cultures exhibited the characteristic activities of connective-tissue cells, and in some cases fibrils were formed within the cytoplasm of the cells (plate 2, fig. 10).

The growth from an 8 to 10 day chick embryo usually has the appearance of a reticulum of cells (plate 1, figs. 7 and 8). Some of these cells are of the large, flat, stellate type, having processes on all sides, in which may develop bundles of fibrils, which pass in more than one direction through the cells (plate 1, figs. 7, 8, and plate 2, fig. 10); others are cone-shaped—*i. e.*, while the cell-body may have several short processes, most of the cytoplasm is drawn out into one long process (plate 1, fig. 2, and plate 2, fig. 4). Both the granular and the clear cytoplasm is continued out into the one long process, which practically always extends in the direction from which the cell has migrated, and although in many cases it continues back as a delicate thread, passing as many as twelve or more cells, it has always a protoplasmic end, either free or closely attached to another cell. These long processes usually contain mitochondria and other granules scattered along their length, and never in any case have they been observed to change into connective-tissue fibers. In many "film preparations" of the subcutaneous tissue studied while alive, such long, delicate processes have been observed to extend along the side or through the middle of a bundle of fibrils. This is probably due to the fact that, through some stress, the cell has been drawn out into this shape, either from migration or manipulation, and the fibrils are those which were originally in the exoplasm of the cell.

During the beginning migration (1 hour after explantation) of the cells in the explants from older chick embryos (10 to 15 days), when certain of the cells first begin to migrate it is seen that they are drawn out into exceedingly long and delicate processes which ramify in all directions, as though their cytoplasm had extended a great length along the fibers of the subcutaneous tissue (plate 1, fig. 3). As the cell continues to migrate towards the periphery of the explanted piece or out into the culture medium, these long processes are drawn into the cell, until finally

it becomes stellate in form and later divides by mitosis and may again develop long and delicate processes among the cells of the new growth.

From a study of this cell (plate 1, fig. 3) and of the cells shown in plate 1, figure 8, and plate 2, figure 4, it can be seen that in the embryo, where the growth is in all directions rather than in a flat plane (as in tissue cultures), a section must necessarily cut many of these delicate processes and cause the appearance of a network of isolated protoplasmic threads between the cells, because the connection of these threads with the cells to which they belong is not shown in the section.

Typical spindle-shaped cells never appeared in pure cultures of connective tissue, but always in those which contained muscle-cells; and in every instance a typical spindle-cell could be identified as a muscle-cell.

In certain of the explanted pieces spindle-cells were observed, but these appeared to be due to the pull which had been put upon the tissue during manipulation, for frequently parallel bands of fibers extended along these cells.

In some preparations from a 10-day chick embryo the cells were connected by so many delicate processes that a network of these processes was formed between them, which would have been difficult to identify as cellular in origin had it not been for the fact that during the mitosis of one of these cells all the delicate network connected with the cell was partly drawn into it, and the space around the cell became free from network (plate 2, fig. 5). It thus became clear that the protoplasmic network between these cells was not extracellular in origin.

The fibrils appeared first (after 24 hours' growth) as slightly more refractive lines within the cytoplasm of the individual cells (plate 2, figs. 1 and 9). The mitochondria were frequently stretched along these delicate lines; by careful study, however, it was seen that the mitochondria did not take part in the formation of the cellular fibrils, but that even though they stretched for a certain distance along a fibril they later separated from it.

As the growth became older (48 to 72 hours) the cells become more and more densely connected by delicate processes with cells at a distance; and the refractive line of the primitive fibril appeared more and more within the cell and became partly gathered into bundles at one point or another (plate 2, figs. 1 and 2). The cellular cytoplasm became separated into an endoplasm—that is, the granular cytoplasm which contains mitochondria, fat, neutral red granules, etc.—which immediately surrounds the nucleus, and an exoplasm, or the clear, non-granular cytoplasm of the more remote surfaces of the cell (figs. 10 and 13).

The delicate fibrils of the cytoplasm continued from one cell to another, usually through the exoplasm of the cell processes (plate 1, fig. 9 and plate 2, fig. 2) and appeared in the living cell as clear, slightly more refractive lines of exoplasm, extending from one cell to another, and frequently across or through the exoplasm of one or more cells.

As the fibrils developed from day to day the bundle became more definite and more independent of the cytoplasm of the cells, until finally it extended as a slender, clear fiber across several cells (plate 2, fig. 3), and except in cases where the

individual fibrils can be traced into a cell, the bundle, or fiber itself, appeared quite independent of the cytoplasm of the cells (plate 2, figs. 2 and 3).

No mitosis was observed in cells whose exoplasm was actively developing into fibrils during the time in which the exoplasm contained the fibrils. Mitosis, however, continued, and many cells were seen to undergo mitosis in regions where other cells were forming fibers. So far as can be determined from these observations, the cell may again undergo mitosis after the bundle of fibers has become independent of the cell cytoplasm. Whether in such cases the cell actually separates itself from that part of its exoplasm which has been differentiated into fibrils or whether it simply divides the undifferentiated cytoplasm and meanwhile remains attached to the differentiated exoplasm (or fibers) could not be determined. However, the cells which contributed fibrils to a fiber bundle gradually increased in number and extended over a wider territory, and the bundle became differentiated into a more and more definite fiber (plate 2, figs. 1 and 3).

The study of the living cell, as well as of the fixed preparation, led to the idea that the fibers became more and more separated from the cells, although it is quite possible that they may merely continue through the exoplasm and become more definite, on account of the separation of the cells. Certainly in no case, in these tissue cultures, did the fiber become so well developed that the ending of the various fibrils which made up the fiber could not be traced into the exoplasm of a cell (plate 2, figs. 2 and 3). No completely differentiated fiber was observed throughout its development, although in a few instances, where the cultures were kept in a healthy condition for several weeks, fibers which resembled those of an 18-day chick embryo were developed. It seems probable that the development of these fibers was by a continuation of the process described above.

Certain preparations which had been carefully studied during their growth and development were fixed and stained, and from these preparations most of the photographs and drawings have been made. A few of the living cells were drawn on successive days, and although it was frequently impossible to determine the exact cell drawn the day before, at least a cell in its near neighborhood was taken.

MITOCHONDRIA AND THEIR RELATION TO THE CONNECTIVE
TISSUE FIBRILS.

One of the most convincing arguments in favor of the view that the fibrils arise within the cytoplasm of the cells is the fact that frequently a few mitochondria are seen along a primitive bundle of fibrils (plate 2, fig. 2) and that occasionally a few are found isolated within a well-developed bundle of fibrils in the primitive fiber (plate 2, fig. 3). So far as is known, mitochondria can not exist extracellularly.

The mitochondria of the cells of the growth from a 6-day to 10-day chick embryo are usually of several types; that is, the granular, the short-rod, and the long-thread or filament type. The greatest number are filaments. Mitochondria are scattered throughout the cytoplasm and occasionally along the network of cell processes between the cells, and they may be arranged in a row along a cytoplasmic process (plate 1, fig. 6). In a few instances a single filamentous mitochondrion has been observed to lie along the length of such a process (plate 2, fig. 3). A mitochondrion may be stretched along a fibril in such a way that in a fixed preparation it would be difficult to determine whether or not it took part in the formation of the fiber. However, a study of the living cell shows that the mitochondria retain all their characteristic activities. They continue to bend, twist, and migrate, with the result that a mitochondrion, even though stretched for a time along a fibril so that it appears to be part of the fibril, very soon bends and later may move away. Mitochondria arranged in a row along a cell process do not necessarily remain there, but may migrate into the body of the cell again.

In the older cultures the cell processes are usually free from mitochondria. In these cultures the mitochondria are more or less centralized around the nucleus—*i. e.*, within the endoplasm of the cell.

There is present in certain of the cells another structure, which stains in the manner characteristic of mitochondria with the various mitochondrial stains—red with Bensley's anilin-fuchsin methylene green stain (plate 2, fig. 7), black with iron hematoxylin (plate 1, fig. 6), and purple with Benda's method. This structure is in the form of a deposit along certain lines of the surface of the cell (plate 1, fig. 6), and is not present in the cell in its early development, but appears later along the edge or on the surface of the cell, and in certain cells, although not usually in those of subcutaneous connective tissue, frequently seems to be associated with the formation of fibrils. It seems probable that it is this structure rather than mitochondria which Meves (1910) had under observation when he described the formation of the fibrils of the tendon as taking place from the mitochondria after they had become arranged along the surface of the cell. In the stained preparation this structure definitely resembles the mitochondria, and it would be difficult to determine whether mitochondria take part in its formation. However, the living cell shows clearly that the structure is along the surface of the cell and that the mitochondria do not take part in its formation. Also, while the structure is fixed and stained by the same methods which fix and stain the mitochondria, it is not necessarily destroyed by the agents which destroy mitochondria, but may be present in

preparations in which the mitochondria have been destroyed. It is very similar to the structure which forms the fibril of the muscle-cell (plate 2, fig. 6).

Mislavsky (1913) was able to differentiate a plasma fibril as well as mitochondria in the kidney tubule cells. He found that while the plasma fibrils stretched entirely across the cells as straight lines, the mitochondria did not pass to the walls of the cells.

In the cultures studied mitochondria did not fuse into strands or become arranged in rows to form the connective-tissue fibrils. In all of these observations, while the mitochondria at times remained caught within a bundle of fibrils, the fibrils themselves originated from the exoplasm of the cell.

OTHER GRANULES AND "GRAINS DE SEGREGATION" OF THE CONNECTIVE-TISSUE CELL.

The connective-tissue cell ordinarily contains very few fat globules, and frequently none at all. When present they are small, round, highly refractive globules, which usually lie near the nucleus and which stain in the manner characteristic for fat.

In addition to the mitochondria granules in the cells, there are a number of small, round granules, which can be distinguished from the granular type of mitochondria only by the rapidity of their movements and by certain vital dyes. These granules stain blue with pyrol-blue, purple with brilliant cresyl-blue, and red with neutral red. In the fixed preparation they frequently take certain of the mitochondrial stains, and especially do they take the same purple color as the mitochondria with Benda's stain.

The vacuoles of Lewis and Lewis (1915) correspond more or less with the *grains de segregation* of Renaut (1904, 1907) and Renaut and Dubrieul (1906), which these observers found to stain with neutral red and which they claimed formed fibrils through a secretory activity of the cell. These bodies are present in the connective-tissue cell, sometimes in large numbers (plate 2, figs. 2 and 3), but so far as could be determined they take no part in the formation of the fibrils.

TENDON.

Only a few growths of cells which could be definitely identified as tendon-cells took place, and in these growths the formation of the fibrils occurred in a manner somewhat different from that of the formation of the fibrils of the subcutaneous tissue. The tendon-cells were arranged as narrow, elongated cells, more or less parallel, and the fibrils developed as clear lines along the surface of the cells. These delicate lines joined into bundles from one cell to another in markedly parallel lines (plate 2, fig. 8). In preparations stained with Mallory's connective-tissue stain the fibrils stained blue.

AMNION.

A study of the fibrils of the amnion was undertaken in order to see whether the observations of Péterfi (1914) could be corroborated in tissue cultures. Péterfi observed vacuoles within the epithelial cells of the amnion, and concluded from his preparations that these vacuoles fused together and became more numerous in the cells of the amnion of chick embryos of from 3 to 5 days' incubation. According to Péterfi, the walls of these vacuoles contain a substance which is different from the remainder of the cytoplasm, and as the walls fuse together they form a network of elastic fibers over the epithelial cells of the amnion of a chick embryo of 7 days' incubation. My observations on the amnion in tissue cultures did not show this. The epithelial cells contained varying numbers of vacuoles, most of which stained with neutral red, though a few remained unstained. Fibrils are formed, but, so far as can be determined from these observations, they are formed from the exoplasm of the cell, regardless of the vacuole, in practically the same manner as are the fibrils of the connective-tissue cells (plate 2, fig. 9).

CONCLUSIONS.

1. The connective-tissue fibrils begin to develop in the subcutaneous tissue of chick embryos of from 9 to 10 days' incubation, and appear as well-developed fibers in the subcutaneous tissue of a 12-day chick embryo. The new growth from explanted pieces of subcutaneous tissue from chick embryos of 8 to 10 days' incubation proved the most satisfactory for the study of the connective-tissue fibers.

2. The cut fibers which are present in the explanted piece of 11-day to 15-day chick embryo subcutaneous tissue do not grow either in length or bulk in the tissue cultures.

3. The new growth of connective tissue is exceedingly sensitive and reacts by a contraction of the cell from the outer edge in towards the explanted piece. This contraction does not cause the formation of fibers in the new growth.

4. Fibers are not present in the 24-hour growth from even 12-day to 15-day chick embryo tissue, but develop in the cells of the new growth from delicate fibrils in the exoplasm of the cells after 24 hours.

5. The fibrils developed as delicate lines of the exoplasm of the cell; they became gathered into bundles which passed from cell to cell, and the bundles later passed over or through the exoplasm of several cells as a definite fiber. The fibers never became so adult that the individual fibrils which make up the fiber could not be traced into the cytoplasm of some cell, whether near or distant from the main body of the fiber.

6. Although the new growth, when closely attached to the smooth cover-slip, often takes the form of a membrane, and although this membrane exhibits the cell pattern which is characteristic for endothelium when treated with silver nitrate, nevertheless there is no evidence that these cells have become endothelial cells; they still retain the characteristics of connective-tissue cells, and many form fibrils.

7. The mitochondria do not take part in the formation either of the fibrils or of the fibers in these cultures of connective tissue.

8. There was no evidence that the fibrils are formed by a secretory activity of the *grains de segregation* (vacuoles) of the connective tissue.

9. The fibrils of the epithelial cells of the amnion appeared to form in the same manner as those of the subcutaneous tissue—*i. e.*, from the exoplasm of the cell, and not from the fusion of the walls of the vacuoles.

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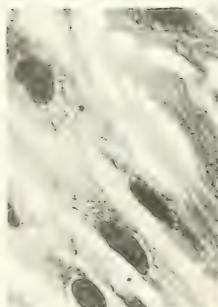
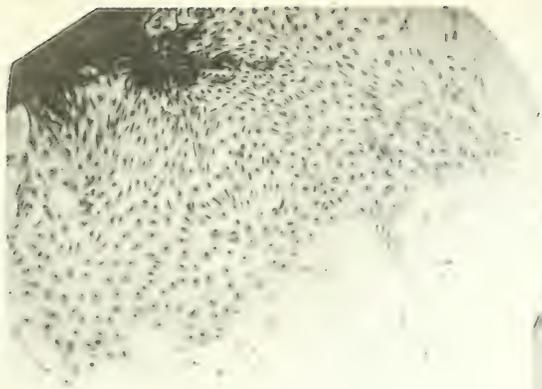
EXPLANATION OF PLATES.

PLATE 1.

1. Photograph of the membrane of connective tissue of 48-hour growth from a tissue culture of a piece of stomach of a 6-day chick embryo. 4 oc. and 4 mm. lens. Zeiss.
2. Photograph of cells and fibers in a film preparation of subcutaneous tissue of an 18-day chick embryo. Os. vap., iron hem. 4 oc. oil-imm. lens.
3. Camera-lucida drawing of a living cell from a culture of subcutaneous tissue of 14-day chick embryo, 3-hour growth. 6 oc. 4 mm. lens.
- 4, 5. Camera-lucida drawings of living cells in a hanging-drop preparation, after Bcll, of subcutaneous tissue from a 14-day chick embryo in Locke's solution. 4 oc. 4 mm. lens.
6. Retouched photograph of 48-hour growth from arachnoid tissue of 7-day chick embryo. Os. vapor, iron hem., 4 oc. oil-imm. lens.
7. Photograph of 48-hour growth from leg of 8-day chick embryo. Os. vap., iron hem., and eosin. 4 oc. 4 mm. lens.
8. Photograph of 48-hour growth from subcutaneous tissue of 10-day chick embryo. Os. vap., iron hem. 6 oc. 4 mm. lens.
9. Photograph of 48-hour growth from heart of 7-day chick embryo. Os. vap., iron hem. 4 oc. oil-imm. lens.

PLATE 2.

1. Camera-lucida drawing of cell, showing primitive fibrils in the cytoplasm. 48-hour growth from subcutaneous tissue of 9-day chick embryo. Os. vap., iron hem., and eosin. 4 oc. oil-imm. lens. Drawn by Miss J. E. Lovett.
2. Camera-lucida drawing of cells and fibrils united into bundles. 72-hour growth from subcutaneous tissue of 11-day chick embryo. Os. vap., iron hem., and eosin. 4 oc. oil-imm. lens. Drawn by Miss J. E. Lovett.
3. Camera-lucida drawing showing cells with fibrils within the cytoplasm and also fibers. 120-hour growth from subcutaneous tissue of 11-day chick embryo. Os. vap., iron hem., and eosin. 4 oc. oil-imm. lens. Drawn by Miss J. E. Lovett.
4. Camera-lucida drawing of cells from 24-hour growth of leg of 8-day chick embryo. Os. vap., iron hem., and eosin. 4 oc. oil-imm. lens.
5. Camera-lucida drawing of cell undergoing mitosis in 48-hour growth from subcutaneous tissue of 11-day chick embryo. Os. vap., iron hem., and eosin. 4 oc. oil-imm. lens.
6. Camera-lucida drawing of smooth muscle-cells, showing myofibrils from 48-hour growth of amnion of 5-day chick embryo. Os. vap., iron hem. 4 oc. oil-imm. lens.
7. Camera-lucida drawing of cell from 24-hour growth of 6-day chick embryo, showing deposit along surface of the cell, which is stained like mitochondria with Bensley's aniline fuchsin, methylene green stain. 4 oc. oil-imm. lens.
8. Camera-lucida drawing of tendon-cells from 72-hour growth of muscle of 9-day chick embryo. Zenker's fixation—Mallory's stain. 4 oc. oil-imm. lens.
9. Camera-lucida drawing of epithelial cells of 48-hour growth from amnion of 5-day chick embryo. Iodine-vapor fixation—Mallory's stain. 4 oc. oil-imm. lens.
10. Camera-lucida drawing of thin membrane of connective tissue from 72-hour growth of 10-day chick embryo. Silver nitrate and Ehrlich hematoxylin. 4 oc. and oil-imm. lens.





J. E. Lovett fec.

ARMANDO GILE MORE

CONTRIBUTIONS TO EMBRYOLOGY No. 18.

ORIGIN AND DEVELOPMENT OF THE PRIMITIVE VESSELS OF
THE CHICK AND OF THE PIG.

By FLORENCE R. SABIN.

With seven plates and eight figures in the text.

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ORIGIN AND DEVELOPMENT OF THE PRIMITIVE VESSELS OF THE CHICK AND OF THE PIG.

BY FLORENCE R. SABIN.

INTRODUCTION.

In this paper is given an account of the primitive vessels of the chick and of the pig, as made out by injecting living embryos, and, in the case of the chick, as seen growing in the embryo. Such studies must necessarily be accompanied by the study of sections. In the case of the mammalian embryo I have made injections in earlier stages than had been done heretofore and in the case of the chick I have carried the method of injection to the earliest stage in which it is possible. Below the stage at which they can be injected, the vessels of the chick can be studied in the living blastoderm by a technique which has developed out of the method of tissue-culture introduced by Harrison. The chick thus offers unusually valuable material for the study of vascular problems, as it is possible to use both the method of injection and that of direct observation of the living embryo in the same stage.

In the course of this study two fundamental ideas have been under consideration. The first concerns the most essential question in connection with the vascular system, namely, the relation of differentiation and growth of endothelium. According to one theory there is a limited period for the differentiation of angioblasts out of undifferentiated mesenchyme, and after this period all new blood-vessels arise from the growth or proliferation of older angioblasts. This theory seems to me to have the weight of evidence. The second theory is that angioblasts continue to differentiate out of mesenchyme indefinitely. If the former theory is correct and the period of differentiation of endothelium is a limited one, the fundamental problem concerning the early blood-vessels is to determine which differentiate and which are formed from preceding vessels. In practically every embryo chick observed, up to a certain stage new angioblasts can be seen differentiating and joining the older angioblasts, but the phenomenon becomes less and less frequent as older stages are studied. In the living embryo the aorta itself can be seen to differentiate out of mesenchyme, and at the stage when the heart begins to beat every chick shows a few isolated angioblasts along the mesial border of the aorta, which will be seen to join the aorta if the specimen be watched for a short time. I have some evidence also that some of the primitive vessels along the neural tube differentiate out of mesenchyme, the process being observed in the living embryo. On the other hand, one can watch the growth of the entire wall of a vessel by cell-division in the living embryo and the formation of new vessels from the walls of old vessels; so that the study of the early blood-vessels is gradually becoming a more exact problem, namely, the determination for each vessel, whether it differentiates *in situ* or develops from preceding vessels. My present material is not adequate for the solution of this question, but throws some light upon it.

The second question—which has proved of great interest—is the definition of the terms *artery*, *vein*, and *capillary* as they are used for the embryo. In the study of the vessels of the embryo particular stress should be laid on the time when circulation begins. That there is a very extensive development of blood-vessels before there is any circulation of the blood due to the beat of the heart was well known to the earlier embryologists; for example, to von Baer and later to His. Moreover, the heart beats for a considerable time before it starts any circulation. It is known that the blood-vessels spread over the body in definite and constant sheets of capillaries, and in these primitive vessels, after the circulation has begun, a vessel may serve as an artery for a time and then be reduced to a capillary plexus, in which the direction of the circulation is entirely different from that of the circulation of the original artery. Such a vessel, for example, is the subintestinal artery of the pig, which arises in a capillary plexus around the caudal end of the primitive gut and carries blood out to the arteries of the yolk-sac, where it must again pass through a capillary bed before it returns to the heart. This artery becomes broken into a capillary plexus in the wall of the gut, which makes new connections with branches of the omphalo-mesenteric veins within the wall of the mesentery, so that its blood, instead of flowing away from the embryo to the membranes, flows within the embryo toward the heart.

Again, a vessel may serve for a time as a vein in the return of blood to the heart and may subsequently receive new arterial connections and become an arterial plexus, with the direction of the flow of blood entirely changed. Such a vessel is the so-called *vena capitis medialis*. This is a primitive vessel along the hindbrain, which in the chick in the second and third days of incubation serves as a vein for the forebrain and midbrain, but as an arterial capillary trunk for the hindbrain; that is, it carries mixed blood and is the only vessel of the hindbrain, representing its entire capillary bed. Early in the fourth day it receives new arterial connections, a new vein develops to carry the venous blood for the forebrain and midbrain, and the primitive vascular channel of the hindbrain breaks into a capillary plexus in which the direction of the current of blood is at right angles to the direction of the original current. From these two examples it must be clear that in the study of the primitive vascular system it is very important to understand the function of the vessels at each stage of development, and any presentation of the vascular system which overlooks this point and is dominated wholly by the pattern of the vessels of the adult becomes difficult to follow and may be misleading. In the question of nomenclature a decision has to be made between two theories—that is, whether the vessels are to be named according to the function they perform at any given stage or whether they are to be named according to the vessels for which they form the primordia. If the latter method is chosen it must be remembered that a given vessel of an embryo often disappears entirely in giving rise to new vessels—for example, the primitive vessel of the hindbrain.

In this study I shall use terms as consistently as possible, in the following manner: By the term *artery*, in reference to an organ, I mean a vessel which brings blood to that organ but does not form any part of its capillary bed, and I have colored such vessels red. By the term *vein* I mean a vessel which carries blood from

an organ to the heart, provided it does not serve as the capillary bed for that organ or break up into another capillary plexus. Such vessels I have colored blue. All other vessels I have indicated in gray, and shall try to trace the complicated changes which such vessels undergo, serving at times as arteries, veins, or capillaries, or as vessels with a double function. This method of nomenclature is therefore based on the function of the vessel at the stage when it exists. It takes into account the very shifting course of the blood, as the vessels develop in the embryo better than does the method of making a too early identification of the adult vessels in those of the embryo. This usage of terms serves to restrict especially the term *vein* as applied to the embryo. The meaning of the terms *artery* and *vein*, as applied to the embryo, will become more clear in the discussion of individual vessels.

METHODS.

As has been stated, in this study I have followed two methods: first, that of the injection of embryos, and second, the method of studying the living blastoderm in the case of the chick in a hanging-drop preparation. A general account of the methods of injecting embryos will be found in my paper on the azygos veins published as "Contribution to Embryology, No. 7," by the Carnegie Institution of Washington in 1913. All of the injections of young embryos are made by blowing ink into the vessels through a very fine canula.

To inject the young chick the shell is opened and the embryo exposed to a strong light under a binocular microscope. A few drops of warm Locke's solution are placed on the blastoderm, and the vitelline membrane is removed. By the time the chick has 14 somites the sinus terminalis, or marginalis, is well developed in the edge of the area vasculosa and can easily be punctured with a fine canula. Nevertheless it is not easy to obtain complete injections of the blood-vessels of the chick through the veins until the embryo has about 16 somites. In stages between 9 and 16 somites more complete injections can be made by puncturing the aorta directly. This is a very interesting point in connection with the time of the beginning of the circulation. I shall show that, though the heart commences to beat about the time the tenth somite is forming, the circulation does not begin until about the stage of 16 somites. From the time the circulation begins it is easy to get complete injections by blowing a little ink into the vitelline veins and allowing the heart to pump the ink through the vessels of the embryo. If total specimens are desired it is well to dilute the ink one-half, so that the superficial vessels will not become so dense as to obscure the deep ones. I shall discuss in this paper the effects of injection in the embryo before the circulation has begun.

The earliest chick embryo which I have injected was one of 9 somites; and I believe this stage to be about the youngest to which the method is applicable. At the stage of 6 somites the dorsal aorta is in the stage of a plexus of angioblasts, many of which are still solid cells. This plexus of cells gradually acquires a lumen and becomes the aorta during the stages of from 6 to 9 somites. All stages up to 18 or 19 somites can be studied to great advantage in hanging-drop preparations.

Direct injections into the aorta can be made in the following manner: When the embryo is placed in a strong light the myotomes are of course very plainly visible, and along their lateral border can be seen a faint opaque streak, which is the intermediate cell-mass or nephrotome. Between the nephrotome and the lateral border of the myotomes is a thinner line which is more transparent. The cannula is then introduced between the lateral border of the myotomes and the intermediate cell-mass, with the point toward the head of the embryo. If the cannula enters the aorta, and only a slight pressure is used, there need be no extravasation at the point of puncture. After the embryo has been injected it is fixed by dropping Bouin's mixture on the specimen while it is still on the yolk. It is kept flooded with the fixing agent, and is not removed from the yolk until it is well hardened.

In regard to the injection of young mammalian embryos there are a few special points in technique which are of interest. In order to identify the young embryo pig the mucosa is examined carefully for long strings of chorion, which are so inconspicuous that they are more readily found by running the finger over the mucosa than by sight. These strings of chorion are then very carefully coiled on a glass slide or piece of filter paper until the embryo is found. In the case of the pig, I have never succeeded in puncturing the veins on the yolk-sac or the umbilical vein. The latter is so large that it might be punctured if it contained enough corpuscles to render it visible. The aorta, on the other hand, is readily punctured opposite the mid-body region. Here in early stages the two aortæ seem slightly dilated, or later are fused into a single vessel. The needle is introduced ventral to the myotomes. The injection mass in every case was india ink. Silver nitrate seems to damage the tissue much more markedly in very young embryos than in later stages. After injection the pigs were fixed in Carnoy's mixture of absolute alcohol 6 parts, chloroform 3 parts, and glacial acetic acid 1 part. They were then placed in 80 per cent alcohol, dehydrated in graded alcohols, and cleared by the Spalteholz method of benzine followed by oil of wintergreen.

The study of the blastoderm of the living chick embryo in a hanging-drop preparation depends on the methods originated by Harrison and developed by a large group of workers—Burrows, Carrel, M. R. Lewis and W. H. Lewis, and others. In 1912 McWhorter and Whipple applied the method to the study of the growing blastoderm of the chick, which was its first application to the entire embryo, so far as I am aware. These investigators mounted the blastoderm in clotted plasma and used the method to test the question as to whether blood-vessels arise from fusion of isolated vesicles. In 1913 Brachet published a study on the growth of a mammalian embryo in a hanging-drop preparation.

In studying the blastoderm of the chick by the method of the hanging-drop I have followed the technique of Margaret Reed Lewis, of growing the embryo in Locke's solution. In this way the embryo can be kept growing for several hours, and the cells which are nearest the cover-slip can be seen with great clearness and followed with an oil-immersion lens. The embryo is removed from the yolk and placed in a dish of warm Locke's solution and the vitelline membrane

and most of the yolk removed. It is more difficult to study the blastoderm with the dorsal surface against the cover-slip than from the ventral aspect, because the ectoderm does not adhere to the glass as well as does the endoderm, and it is necessary to have the embryo very flat in order to see the cells with higher powers. In regard to the blood-vessels, it is of course preferable to study the embryo from the ventral aspect, since the blood-vessels are nearer the endoderm than the ectoderm. In the study of the developing blood-islands it would be very advantageous to have the dorsal surface of the embryo against the cover-slip, as the blood-islands are farther dorsal and because the cells of the ectoderm over the area opaqua are much thinner than the cells of the endoderm over the same area. The cells of the endoderm of the area opaqua are so thick and so filled with globules of yolk that one can seldom focus through them in the living embryo. From the ventral aspect there is also an area of the axis of the embryo that it is very difficult to study with high-power lenses, namely, the portion of the axis just caudal to the head-fold, because the heart lifts the embryo from the cover-slip and the cells can then be studied only with dry lenses.

All of the chicks have been fixed in Bouin's mixture of saturated aqueous picric acid 75 parts, formalin (40 per cent) 20 parts, and glacial acetic acid 5 parts, for about 12 hours. They are then placed directly in 60 per cent alcohol. In the case of the chicks which have been growing on the cover-slip it is very necessary to have the embryo stick to the cover-slip throughout the fixation and dehydration. If the specimens are to be mounted *in toto*, they are mounted on the same cover-slip on which they were growing. If they are to be embedded and cut they can be removed from the cover-slip after they have been cleared in the oil of wintergreen. The specimens do not become as brittle in the oil as in xylol. In the blastoderms which are kept on the cover-slips it is possible to watch the effects of dehydration much more accurately than with free specimens. The edge of the specimen around the outer margin of the area opaqua clings very closely to the cover-slip; in fact, in mounting the embryo strands of tissue are pulled out which dry slightly and help the specimen to remain fixed. If the specimens are put into alcohol weaker than 60 per cent this outer margin will stick to the cover-slip, but the entire area pellucida will become free from the cover-slip and swell into a bleb. The space beneath fills in with fluid, and in the subsequent dehydration there is an uneven shrinkage which distorts the tissues. Thus, weak alcohol or water, or a dilute stain, macerates and swells the tissues and the subsequent shrinkage distorts the cells. On the other hand, if the specimens are placed directly in alcohol as strong as 60 per cent, a plexus of cells, which has been studied in the living specimen, can be readily identified in the fixed specimens. If the specimens are to be stained *in toto* they must be placed in a stain sufficiently strong so that the tissues will not macerate before they react to the stain. From such an experience one should avoid washing embryos in water and should also avoid the use of the lower grades of alcohol. I have used several changes of 60 and 65 per cent alcohol to eliminate the excess of picric acid. The dehydration can be done by changes of 5 per cent. If it is carried out too rapidly the specimens will crack, but the shrinkage with the higher grades

of alcohol is by no means as marked as in the case of older embryos. The early embryos are injured much more by maceration due to weak alcohol than by shrinkage due to too rapid dehydration in the stronger grades. The specimens are all cleared by the Spalteholz method of benzine followed by oil of wintergreen. Specimens can be embedded from oil of wintergreen if they are passed directly from the oil to a mixture of the oil and paraffin. The tissue does not become too brittle to cut even after remaining in the oil for a year or two.

VASCULAR SYSTEM OF THE CHICK.

GENERAL ACCOUNT OF THE VASCULAR SYSTEM OF THE CHICK UP TO STAGE OF 14 SOMITES.

In the study of the origin of the vessels of the chick I shall begin the detailed account with the stage of 6 somites. The study of the blastoderm in a hanging-drop preparation offers a valuable method for a study of the early stages. In the stages of the early somites there is a plexus in the area opaca which, by the older embryologists, Pander, von Baer, Remak, and later by His, was identified as the forerunner of the blood-vessels. Basing his studies on those of von Baer and Remak, His gave a description of the origin of blood-vessels which remains the foundation of our knowledge upon this subject (1868, pages 95 to 100). He described the first appearance of blood-vessels, or, as he later termed them, angioblasts, as occurring just before the appearance of the somites. He stated that the vessels began as a plexus of angular or spindle-shaped solid cells in the area opaca. These cells from the beginning were in the form of a plexus (Gleich von Anfang an ein geschlossenes Mosaik, page 98). The plexus was at first made up of solid cells without a lumen, and grew by processes of solid spindle-shaped cells, exactly similar to those which formed the original network. This plexus was in a definite layer—the vascular layer (das Gefässblatt) of Pander.

The vascular layer, His said, consisted not only of solid angular cells, but also of elements having a yellow color, or, in other words, it gave rise not only to blood-vessels but also to blood-cells. He regarded it as of the greatest importance that the first appearance of vessels was in the area vasculosa before the heart formed, and that these vessels arose entirely independently of any circulation. He then noted that the plexus of solid cells became transformed into vessels, the exact method of the transformation being impossible to determine; but that as the solid cells became the walls of vessels, their cytoplasm became less granular and their nuclei flatter. He then described the approach of the blood-vessels toward the axis of the embryo by means of the same type of solid processes which formed the original plexus and found that they approached the axis in two zones: first, opposite the myotomes, and secondly, along the splanchnopleure in the region opposite the future cephalo-mesenteric veins—that is, over the ventral surface of the two amnio-cardiac vesicles.

His noted that over the region of these amnio-cardiac vesicles there was a double sheet of vessels which approached the axis of the embryo, a more scanty sheet

in the somatopleure, and a more abundant sheet in the splanchnopleure. Because of the development of the head-fold and the heart, it was impossible that the approach of the vascular plexus should be uniform over the entire length of the embryo. For example, the most cephalic part of the head became cut off from direct lateral connection with the embryonic membranes, and the vessels which approach the heart gradually rotated from the direct transverse direction to an oblique angle. He then noted that the dorsal aorta developed in the mesial edge of the plexus of angioblasts of the area vasculosa along the line of the lateral edge of the myotomes. From these observations he concluded that the vessels of the embryo are derived from the vessels of the membranes, and that the portion of the axis which can not be seen to receive the plexus of primitive angioblasts from the membranes receives its vessels by a growth of the plexus which has already invaded the embryo at other places.

In following the differentiation of the vascular area by improved methods whereby one can watch the living cells growing under an oil-immersion lens, it is astonishing how accurate is this description of His, which must have been made by far cruder methods. To his description must be added that with finer methods it is seen not only that the plexus out of which the aorta develops is the border of the common plexus of the entire area vasculosa, but that new cells differentiate along the axis of the embryo as well, so that angioblasts differentiate over the entire zone from the outer edge of the area opaqua to the margin of the future aorta along the lateral border of myotomes. Thus His's description must be extended to include a differentiation of new angioblasts in the axis of the embryo itself.

In the living blastoderm over the area opaqua, the endoderm-cells are so thick and so filled with yolk that the development of the blood-vessels and the blood-cells beneath them can be followed only with great difficulty. In the area pellucida, on the other hand, the endoderm is thin, and during the periods when the endoderm cells are not dividing they are so clear that it is easy to focus through them.

At the stage of 6 somites the head-fold is well formed and the amnio-cardiac vesicles have met in the mid-ventral line. Along the axis of the embryo there is a zone of dense tissue radiating from the primitive streak and from the embryo cephalic to the streak. This denser mass of tissue divides the area pellucida into an inner thicker zone containing the axis of the embryo and an outer thinner zone. In sections it is clearly seen that this denser zone is due to the further development of the coelom nearer the embryo. Over the cephalic part of the denser zone the coelom has a wide lumen, and both its ventral mesoderm and the endoderm are thicker than the same membranes farther lateralward. This is very plainly shown in Duval's Atlas, plate xiv, figure 218, in the zone extending outward from his letter *b*. Farther caudalward in this dense band, opposite the undifferentiated myotomes and the primitive streak, there is no cavity of the coelom, and its dorsal and ventral mesoderm are fused and form a dense mass of cells. This entire thicker zone is difficult to study in the living chick, but the whole outer margin of the area pellucida is clear and the cells are so thin that

one can readily focus through them from the endoderm to the ectoderm, and see every cell of the entire zone. This is true, however, only when the endoderm is not dividing. The endoderm cells divide as a whole, and during the entire phase of cell division they are so opaque that it is impossible to focus through them. The phase requires about an hour, and to study the vessels beneath it is necessary to await until the cells become clear again.

In the entire outer margin of the area pellucida, at the stage of 6 somites, there are two plexuses. The dorsal plexus, which is the developing coelom of this area, appears to be composed of very large and flat vessels. Distinctly ventral to this plexus of the coelom is another plexus, much less abundant and made up of solid bands of cells, which are angioblasts. An exceedingly important point, which can be determined with great distinctness in the living specimen, is that the plexus of angioblasts connects by many tiny filaments with the plexus of the mesoderm of the coelom, but never connects by filaments with the endoderm. In sections the angioblasts of the vascular layer often touch the endoderm, but in the living embryo they are always separate. The living specimens also bring out very sharply the fact that the entire layer of angioblasts is distinctly ventral to the plexus of the mesoderm; in other words, the term *vascular layer* of Pander is an appropriate one, for the filaments of the angioblasts can be seen to dip down from the vascular layer to the mesoderm beneath. In the flat living specimen, and in sections which have been made from a specimen which was growing out flat on a cover-slip, there is no intermingling of the mesoderm and the vascular layer, such as is seen in Duval's plate xvi, figure 264. Such an apparent intermingling of the two layers is due to shrinkage. In other words, the angioblasts differentiate out of the mesoderm and form a new layer, which is throughout ventral to the mesoderm. These two plexuses were well known to His, who recognized them in their relations to the coelom on the one hand and to the angioblasts on the other in his work published in 1868, but described them more fully in the *Lecithoblast und Angioblast* published in 1900. His stated that the two plexuses were at times very hard to analyze.

The plexus of angioblasts is, then, distinguished first by its more ventral position, and secondly by the fact that the cytoplasm of the angioblasts is slightly more granular and reacts slightly more intensely to basic dyes than does the mesoderm. The following criterion, however, is the one which I have found most useful. In the living specimens there seems to be a sort of rhythm in cell division. I have already referred to the fact that the entire endoderm may divide and become so opaque that none of the cells beneath can be seen. At other times the entire plexus of angioblasts over a very extensive zone will pass into the phase of cell division. In this condition the cytoplasm of the plexus of angioblasts becomes very highly refractile and opaque, so that it can be distinguished from the plexus of the coelom with great ease, even with low powers of the microscope. The protoplasm shows this change for about an hour before the chromosomes pass on to the spindle: so, in order to obtain the nuclear figures characteristic of cell division, one must watch until a few areas in the plexus begin to clear and

then fix the specimen. The time required for the nuclear changes is much less than the time taken for the cytoplasmic changes. According to M. R. Lewis, the nuclear phase lasts about 5 minutes, while the cytoplasmic change takes about an hour. The facts that not every nucleus divides at the same moment and that the cytoplasmic changes have not been recognized explain the failure to note the rhythm of cell division.

Using the criteria for distinguishing angioblasts which have just been indicated, I will now describe what has been made out concerning the vascular system at the stage of 6 somites, both in the living specimen and in sections which have been made from a blastoderm in which the cells had been charted in the total specimen before the sections were cut. For this description the axis of the embryo may be divided into four zones: (1) that part of the head which is covered by the head-fold, as seen from the ventral aspect; (2) the head between the head-fold and the first myotome; (3) the zone of the myotomes; (4) the zone caudal to the myotomes. As has been described, there is a dense band of tissue on either side of the axis of the embryo which divides the area pellucida into an inner dense zone and an outer thinner zone. The area opaqua, on the other hand, is denser along its outer margin. Beginning with the area opaqua, in its outer margin there is a large marginal plexus of vessels partly filled with blood-cells which cling in large masses to the dorsal wall of the vessels. The blood-cells can be distinguished from the angioblasts by the fact that in the edges of the masses they tend to separate from the mass and have a definitely round contour. Angioblasts never have a round contour. In this marginal zone the cœlom is clearly seen, with its dorsal and ventral mesoderm, and the ventral wall of the blood-vessels is very plainly distinguished from the endoderm; but the dorsal wall of the blood-vessels is closely attached to the ventral mesoderm, and in places can not be distinguished from it.

The inner margin of the area opaqua and the outer margin of the area pellucida have two definite plexuses: the dorsal plexus of the cœlom and the scantier ventral plexus of solid angioblasts. Over the dense area on either side of the myotomes the cœlom is no longer in the form of a plexus, but has a complete lumen; for there the body-cavity is well formed. The plexus of angioblasts covering this area is continuous with a plexus of angioblasts along the lateral margin of the myotomes. Caudal to the sixth myotome, the plexus extends for a short distance along the undifferentiated mesoderm, curving a little to the side. Very interesting appearances are to be made out near the first myotome. Extending forward from the lateral border of the first myotome, the chain of angioblasts representing the aorta can be seen up to the margin of the head-fold, when it disappears under the fold. Opposite the first myotome, and extending forward from its mesial border, there is also a chain of angioblasts along the hindbrain, and this chain of angioblasts connects with the aorta above the first and between the first and second myotomes. The chain of cells along the margin of the hindbrain I should not recognize as angioblasts in sections; but in the living blastoderm they have exactly the appearance of the angioblasts of the aorta and connect with them by slender filaments.

In the region of the head, which can not be analyzed in the living blastoderm, the angioblasts representing the heart are well known and easily identified. The two cardiac primordia have met in the mid-ventral line and can be followed a short distance into a ventral cephalic aorta, which gradually becomes too indefinite for recognition. The dorsal cephalic aorta is very clear opposite the region of the heart, gradually disappearing farther forward. Thus, within the embryo there are chains of angioblasts representing the heart, most of the ventral cephalic aortæ, and a part of the dorsal cephalic aortæ. Opposite the region of the heart the two dorsal aortæ are definite, tiny vessels which emerge from under the head-fold and are continued partly as a plexus of solid angioblasts and partly as a vessel along the ventro-lateral border of the myotomes. The entire plexus which is exposed on the ventral aspect connects with the plexus of angioblasts of the area pellucida. In this account I wish to emphasize the very early appearance of angioblasts along the hindbrain—the forerunner of the so-called vena capitis medialis, which I prefer to call the primitive vessel of the hindbrain. I have not yet a sufficient number of observations to prove, first, whether the transitory vessel of the hindbrain does differentiate *in situ* while the aorta is differentiating, and secondly, whether it is established earlier than the vessels of the forebrain; but both of these propositions seem to me to be very probable.

In this study I have found Williams's (1910) very careful description of the vascular system of the early chick embryo of great value. His specimen of 6 somites is clearly a little farther advanced than mine. He found that at 6 somites the aortæ were established, but were still small and irregular. He then observed a vessel along the neural tube (hindbrain) connected with the aorta in the first and second interspaces, the vessel in the first interspace being nearly as large as the aorta itself.

It is now important to consider how the plexus of angioblasts increases. This occurs first by cell division and secondly by the differentiation of new angioblasts. Cell division in the plexus of angioblasts is very extensive, for in watching the living specimens it is seen that large areas of the plexus divide at the same time, and in these cycles of cell division every cell of the plexus divides. Besides this very extensive cell division new angioblasts differentiate and join the plexus. This process can best be observed along the mesial border of the dorsal aorta itself, near the lowest myotome. Here practically every blastoderm between 6 to 10 somites will show one or two isolated angioblasts which are very readily marked from the dense mesoderm beneath. Out in the zone of the developing ectoderm the distinction is by no means so easy. These angioblasts are either single, spindle-shaped cells or clumps of two or three cells. When observed they are seen to put out tiny filaments toward the wall of the aorta, which at once responds by putting out a filament toward the young cells. These tiny filaments meet halfway, and the new angioblasts thus join the wall of the aorta. They gradually approach the wall and become incorporated into the vessel. As the new cells become a part of the wall their protoplasm becomes less granular and they acquire a lumen. The exact process by which angioblasts acquire a lumen is extremely difficult to determine, and concerning this point nothing has yet been added to the original description of His.

These observations on the origin of the aorta, as well as the observations indicating that the transitory vessel of the hindbrain differentiates from angioblasts *in situ*, at once lead to the general question of the origin of the vascular system. All are agreed—on the foundation of the work of von Baer, Remak, and His—that certain cells of the embryo differentiate to form angioblasts or vasoformative cells in the early stages of embryonic life, and that these angioblasts increase by cell division. There has, however, been a wide divergence of opinion as to whether the differentiation of new angioblasts continues throughout life or whether there is a limit to the period of differentiation, after which all the new angioblasts must come from the growth of preceding endothelium.

It is in relation to these two theories that I am making these studies on the living blastoderm. It is, I think, clear that the study of blood-vessels in the stages of their differentiation does not prove that they continue to differentiate out of mesoderm throughout life, any more than the finding of several primordia for the thymus proves that new thymus glands continue to arise throughout life. The question of the origin of the blood-vessels is now an exact one—namely, which vessels arise in the embryo (as does the aorta, at least, in part) by differentiation of angioblasts, and which grow from previous vessels. In other words, how long does the period of differentiation of angioblasts continue?

His formulated the theory that the embryo itself is invaded by angioblasts from the yolk-sac. This theory was based on the following observations: First, that along the myotomes in the early stages angioblasts can be seen streaming toward the axis of the embryo from the outer margin of the area pellucida; second, that he observed no such streaming of angioblasts toward the axis of the embryo in the zone between the head-fold and the first myotome (here, as a matter of fact, a few angioblasts can be found in early stages, but are much scantier in number than lower down); and third, that the most cephalic part of the head does not receive angioblasts from the membranes. From these observations he concluded that the vessels of the axis of the embryo must arise from a growth of the angioblasts which could be seen to enter the embryo at certain places.

Although these observations of His are for the most part correct, that a differentiation of new angioblasts does take place along the axis of the embryo was shown by two series of experiments. First, those of Hahn, who cut out the membranes of one side of a chick in the stage of the primitive streak and obtained a few specimens in which the membranes were entirely lacking, but the aorta was formed on the injured side. Second, the experiments of Reagan, in which he cut off a part of the head of the chick in the stages just before and just after the head-fold is visible, and allowed the isolated parts to remain in the egg and develop. In these isolated fragments he obtained vessels.

The fact that angioblasts do differentiate in the axis of the embryo is conclusively proved by my observations, having watched certain cells differentiate and join the aorta in the living blastoderm. In what I have called the second zone of the axis of the embryo—that is, the zone between the head-fold and the first myotome—the process can not be followed with such minute detail as is

possible opposite the myotomes, because in the former case the heart lifts this zone of the embryo from the cover-slip; but every specimen shows chains of angioblasts which are apparently differentiating *in situ* in this area. None of the experiments or observations here recorded take into account the ultimate point of origin of the cells which differentiate into angioblasts.

By the time the chick has 9 somites the dorsal aorta is readily seen behind the head-fold as a complete vessel if the living embryo be viewed from the ventral aspect. Opposite the upper myotomes the aorta is directly ventral to the myotomes, but it gradually curves outward, so that opposite the ninth myotome and the undifferentiated mesenchyme it lies along the lateral border of the myotomes. Along its entire lateral border it is connected with the plexus of the area pellucida. As has been shown by Evans, the entire caudal portion of the aorta is a part of the capillary plexus of the area pellucida. In summing up the question of the origin of the aorta it may be said that it differentiates as a part of the plexus of angioblasts, extending over the entire area vasculosa, and is increased by the addition of new angioblasts along the axial line of the embryo.

By the time the chick has 9 somites the aorta can be injected; it forms from the plexus of angioblasts while the seventh, eighth, and ninth somites are forming. While the dorsal aorta of the region of the myotomes is best seen in the living chick, the cephalic aorta is best observed in an injection. As can be seen in plate 1, figure 3, the heart is a simple tube. In some specimens, even with 10 somites, it is in the exact mid-ventral line; in others, as in plate 1, figure 3, it is slightly to the right. In some of my injections the ventral aorta has numerous mesial and lateral sprouts; in this particular specimen these sprouts are more numerous along the dorsal cephalic aorta.

In one of my injections the heart itself shows a little of the primitive plexus. The dorsal cephalic aorta shown in plate 1, figure 3, is still in the form of a plexus; from the arch of the aorta two very constant sprouts extend to the ventral surface of the forebrain. The development of these sprouts is well shown in a figure by Evans from a duck embryo with 13 somites (fig. 398) in the "Manual of Human Embryology" (Keibel and Mall).

The mesial sprouts do not form permanent vessels; but in one very interesting abnormal embryo which I injected these mesial sprouts had formed anastomoses across the mid-line. They are thus, in this specimen, analogous to the vessels

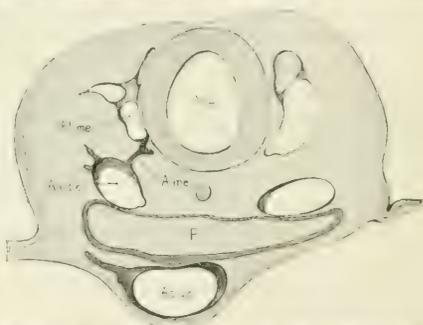


FIG. 1.—Transverse section of an injected chick of 12 somites, passing through the middle of the mesencephalon, to show the vascular plexus on the mesencephalon. On the left, the dotted area shows how far the ink passed through a dorsal artery from the aorta into the plexus on the midbrain. The section is from the same series as those in figures 2 and 3, and it is to be compared with the total preparations shown on plate 1, figure 2, and on plate 2, figure 1. The section is 50μ thick and is unstained. $\times 133$. A. me., artery to the plexus on the mesencephalon; A. d. c., aorta dorsalis cephalica; A. v. c., aorta ventralis cephalica; Me., mesencephalon; P., pharynx; Pl. me., plexus on the mesencephalon.

which cause the fusing of the two aorta lower down. Opposite the region of the heart some of the lateral sprouts extend out in the somatopleure, as shown in plate 2, figure 1, and in sections in Duval's plate xvii, figure 276. Opposite the midbrain some of these lateral sprouts may connect with the superficial plexus.

The dorsal cephalic aorta itself, as seen in plate 1, figure 3, is very large. From the dorsal aspect it is broad and flat and is placed in a nearly exact transverse axis instead of in the oblique position which it subsequently assumes.

The next stages in development, including the relations of the primitive cerebral vessels and the cardinal system of veins up to the stage of 14 somites, I shall describe with the aid of two total preparations from chicks of 12 and 14 somites and three sections from the stage of 12 somites (figure 2 of plate 1, figure 1 of plate 2, and text-figs. 1, 2, and 3).

At the stage of 12 somites the aorta is very readily injected. The vessels to the brain, however, though they connect with the aorta, are difficult to inject. In plate 1, figure 2, is shown the usual result of injecting a small quantity of ink into the omphalo-mesenteric veins at the stage of 12 somites; the ink passes through the heart and the aorta into the capillaries, which are the fore-runners of the omphalo-mesenteric arteries. This is true, even though vessels to the entire brain—that is, to the forebrain, midbrain (text-fig. 1), and hindbrain (text-fig. 2)—are present and connect with the aorta, although the common cardinal vein is present down to the twelfth interspace (text-fig. 3) and the entire lateral border of the aorta opposite the myotomes is connected with the plexus of the area vasculosa. In plate 1, figure 2, the only branch of the aorta injected is an unusual dorsal branch opposite the tenth somite, passing out into the somatopleure. In order to fill these different branches of the aorta in the stages shown in figure 2 of plate 1 and figure 1 of plate 2 before the circulation has begun, it is necessary to introduce the needle into the aorta and inject, as it were, backwards. In this way the pressure in the aorta is probably raised, the heart being sufficiently stimulated by the ink to force the injection mass into the tiny channels that would otherwise remain empty. Indeed, after the circulation has begun, if only a very small quantity of ink enters the heart it will return to the area vasculosa without injecting the branches of the aorta within the embryo. These fill up only as the injection is continued and the heart becomes well filled with ink.



FIG. 2.—Transverse section of an injected chick of 12 somites, passing through the first interspace to show the relations of the primitive vessel of the lamellum, the transverse vein of the first interspace, and the anterior cardinal vein. The section is from the same series as those of figures 1 and 3, and is to be compared with the total preparations shown on plate 1, figure 2, and on plate 2, figure 1. The sections 50 μ thick and is stained 1. = 133. A. d. = aorta dorsalis cephalica; C. = oesophagus; P. = pharynx; V. a. v. = v. cardinalis anterior; V. om. = v. omphalo-mesenterica; V. so. = v. of the somatopleura; V. t. = v. transverse of the first interspace; V. p. = v. primitiva rhombencephali; V. v. = ventriculus cordis.

That there are vessels within the embryo at the stage of 12 somites which can be injected from the aorta is proved by three sections from an injected chick of this stage (text-figs. 1 to 3). These sections are best followed by comparing them with the specimen shown in plate 2, figure 1, from a chick with 14 somites. The section shown in text-figure 1 passes through the midbrain and shows a plexus of vessels on the midbrain fully as large as the aorta itself. On the left side of the section (right side of the embryo) is a slender artery containing ink, connecting this plexus with the aorta. This plexus of large vessels on the midbrain, shown in text-figure 1, also connects with a single longitudinal vessel along the hindbrain at this stage.

These neural vessels, which at this stage are connected with the aorta, have no vent, which probably explains the great difficulty in injecting them. They are full of fluid, and though the ink enters them from the aorta, it does not penetrate far (text-fig. 1). This point is, I think, interesting in connection with the time of the beginning of circulation. As is well known, the heart begins to beat early in the second day. I have made a number of observations which show that it beats at the stage of 10 somites. In one instance I injected an embryo of 10 somites in which the heart was not beating, and when a small amount of the ink entered the heart it was stimulated to beat. In another instance I had been watching an isolated blastoderm of 9 somites for over an hour when the heart began to beat. This occurred just as the tenth somite was beginning to appear. It is therefore quite certain that the stage of 10 somites marks the beginning of the heart-beat.



FIG. 3.—Transverse section of an injected chick of 12 somites passing through the twelfth interspace to show the relation of the posterior cardinal veins to the aorta. The section is from the same series as figures 1 and 2, and is to be compared with the total preparations shown on plate 1, figure 2, and on plate 2, figure 1. The section is 50μ thick and is unstained. $\times 133$. A., aorta; N, nephrotomic; V. c. p., v. cardinalis posterior.

At the time the heart begins to beat its venous end connects with the extensive capillary plexus of the area pellucida in which the omphalo-mesenteric veins arise, and the entire aorta opposite the myotomes is connected with the capillary plexus in which the omphalo-mesenteric arteries arise. In other words, there is a plexus of vessels covering the entire area opaca and area pellucida which connects with the venous end of the heart and with the entire dorsal aorta of the embryo opposite the zone of the myotomes. In the area pellucida this plexus of vessels is filled with fluid, but there are very few free cells in the vessels. After the heart begins to beat most of the isolated blastoderms show occasional wandering cells of various types that float into the vessels of the area pellucida, showing that these vessels are full of fluid; and when one of these cells approaches the heart in the omphalo-mesenteric veins it oscillates back and forth with each beat. It is thus very strikingly apparent that the circulation does not begin for a considerable time after the heart begins to beat. It is difficult to note the exact time of the beginning of the circulation while the chick is on the yolk, for the few red blood-corpuses that are forced into the aorta are inconspicuous with the powers of the microscope that can be used. In the isolated blastoderms the earliest chick in which I have seen the circulation begin was one of 17 somites. At the beginning of circulation a few corpuses are shot into the aorta with each

beat of the heart. The mounting of the blastoderm on a cover-slip, however, interferes with the circulation much more than with the heart-beat, because the flattening of the blastoderm tends to flatten the vessels and thereby impede the circulation. This is often strikingly shown when through mechanical difficulties the circulation is entirely cut off on one side of an isolated blastoderm and not on the other. It is therefore probable that the circulation begins in the chick about at the stage of 15 or 16 somites. It is interesting to note that it is at this stage that the duct of Cuvier breaks through into the omphalo-mesenteric veins, whereby the dorsal aorta and the veins of the embryo become connected with the venous end of the heart.

It is thus clear that at the stage of 12 somites, when the head of the embryo contains a complete aorta and a neural system of vessels which consists of a plexus of large vessels on the forebrain and midbrain, and a single channel on the hindbrain, there is no circulation through these vessels due to the beat of the heart.

The connections of the vessels of the brain with the aorta are of importance. The arteries connecting the vessels of the forebrain with the aorta consist of a group of vessels just at the primitive arch of the aorta. These are shown in plate 2, figure 1, and have been thoroughly demonstrated by Evans. These arteries connect with the neural vessels at the base of the optic cup, in the groove representing the line between the telencephalon and the diencephalon. Subsequently this group of vessels divides into two arteries, one of which encircles the optic stalk and the other extends caudalward along the ventral border of the thalamus and the midbrain (plate 6). Opposite the midbrain there is a group of tiny arteries connecting the plexus with the aorta, one of which is shown injected in a chick of 12 somites in text-figure 1.

It is very clear (in the section of text-figure 1) that the vessels to the neural plexus are direct, dorsal branches of the aorta. The vessel along the hindbrain connects with the aorta by two groups of tiny branches, one cephalic and the other caudal to the otic vesicle. These branches are also for the most part direct dorsal branches. In one of my sections, however, two arteries to the vessel of the hindbrain are placed with reference to the aorta, as are the vessels on the left side in text-figure 2—that is, one is dorsal and the other dorso lateral. These connections between the aorta and the primitive vessel of the hindbrain are shown injected, by Evans, in his figure 393 in the "Manual of Human Embryology" (Keibel and Mall).

As far as the vessels which connect the vascular channel of the hindbrain with the aorta are concerned, it has been shown that they differentiate as angioblasts at the stage of 6 somites, while the aorta and the neural vessels are differentiating. The origin of the primary plexus of deep vessels on the surface of the forebrain and midbrain requires more careful study during the stages of from 6 to 12 somites. It is probable that these vessels differentiate, and that their connections with the aorta differentiate, as does the preliminary vascular channel of the hindbrain. The development of the deep neural vessels and the origin of the superficial plexus of vessels opposite the brain, as well as the origin of the primary head-vein, will be taken up subsequently.

ORIGIN OF THE CARDINAL VEINS.

It is now important to consider the cardinal veins—how they arise, how they become related to the primitive neural vessels, and how they become connected with the heart through the duct of Cuvier. The general relations of the cardinal veins are best shown in plate 2, figure 1, from a chick of 14 somites, but their origin can be traced back to the stage of 9 somites. They form as a longitudinal anastomosis which connects diverticula of the aorta that project dorsalward between the somites. In 1906 these dorsal diverticula were described by Grafe, who stated that the cardinal veins arose from sinus-like projections from the aorta. That the cardinal veins arise from dorsal intersegmental branches of the aorta was shown by Rabl in 1892 and by Hoffmann in selachians in 1893.

The condition of the aorta just before the diverticula arise is of importance. Up to the stage of 9 somites it is clear that the entire aorta which can be seen from the ventral aspect in the living blastoderm is connected with the plexus of the area vasculosa through so-called ventral branches which extend lateralward. Even cephalic to the first myotome a few chains of angioblasts connect the aorta with the plexus of the area vasculosa. However, these tiny branches all along the lateral border of the aorta are seldom injected, except opposite the caudal end of the aorta (plate 1, fig. 2).

When the chick has 9 somites a new set of aortic branches begins to form, which are very distinct from the lateral vessels. In the living blastoderm of from 9 to 12 somites it can be seen that diverticula of the aorta project dorsalward into the interspaces. The more cephalic of these diverticula are dorso-lateral, as shown on the right side in text-figure 2, from a section through the first interspace; the more caudal ones are distinctly dorsal, as seen for the twelfth interspace in text-figure 3. This is due to the fact that at the stage of 12 somites the aorta is obliquely placed with reference to the lateral margin of the myotomes. As shown in text-figure 2, in the first interspace the lateral margin of the aorta is in the lateral line, while in the twelfth interspace, as shown in text-figure 3, the aorta is directly under the lateral line. The first two of these diverticula have been seen at the stage of 9 somites; and they are present in all of the interspaces at the stage of 12 somites. In a total preparation of a chick of 12 somites the ink lodges in these dorsal diverticula and forms dark streaks across the aorta from the dorsal aspect; these streaks are very characteristic, but are difficult to indicate in a drawing. The specimen of plate 1, figure 2, shows such streaks across the aorta in the interspaces.

The diverticula begin at the time when the first two somites lie within the arch formed by the two omphalo-mesenteric veins where they join the heart. In this connection I have tried to determine whether there is a constant relation in regard to the time when the cardiac or head fold reaches the level of the first somite; and in this regard the figures in His's "*Untersuchungen ueber die erste Anlage des Wirbelthierleibes*" (1868), plate XII, and those in Lilly's "*Development of the Chick*" (1908) are the most helpful. In general, at the stage of 9 somites the position of the first somite is about as shown in Lilly's figure 61, page

106; but I have chicks of 10 somites in which the first somite is farther from the head-fold than in the usual specimen of 9 somites. As a rule the head-fold is along the cephalic border of the first somite when the embryo has 12 somites; but in some specimens, such as in His's plate XIX, figure 20, there is at this stage an interval between the first somite and the head-fold. After the cephalic curve of the midbrain has formed, as shown in plate 2, figure 2, the embryos are not as flat from the direct dorsal aspect and the point can not be tested with the same definiteness.

The longitudinal vessel which connects these diverticula in the lateral line of the embryo is the common cardinal vein (plate 2, fig. 1). The cardinal vein has two fundamental relations—on the one hand to the primitive vascular channel of the hindbrain and on the other hand to the venous end of the heart. As is shown in plate 2, figure 1, and in the section in text-figure 2, the cardinal vein becomes connected with the neural vessel by two cross-anastomoses in the first and second interspaces. Of these vessels the one in the first interspace is the larger and more important. The cardinal vein itself is not shown on the left side in text-figure 2 (right side of the embryo), since the transverse vein of the first interspace is slightly oblique, as is plainly seen in plate 2, figure 1.

The transverse vein of the first interspace has been described and illustrated by Evans; and has been traced back to the stage of 6 somites by Williams. In the chick it is an important channel in the second and third days of incubation, for it is the channel by which all of the blood for the brain drains into the cardinal vein and thence to the heart. The transverse vein of the first interspace is characteristic of the chick. It does not form in the pig where the transitory vessel of the hindbrain connects with the cardinal vein in front of the first somite instead of in the first interspace.

At the stage of 12 somites the dorsal diverticula of the aorta are present in all the interspaces, but there is not yet a continuous vein connecting them opposite the lower interspaces. The cardinal veins begin to form at a very early stage, when the zone along which they form is close to the aorta (text-fig. 3), so that the primitive common cardinal vein is an accompanying vein to the aorta. It is this accompanying vein of the aorta which connects with the venous end of the heart, forming the duct of Cuvier. So close is its relation to the aorta that the duct of Cuvier may be regarded as a direct connection between the dorsal aorta and the omphalo-mesenteric veins.

The position of the duct of Cuvier is well known, and is shown in plate 2, figure 1. At the stage of 14 somites, as shown in this figure, the common cardinal vein opposite the second, third, and fourth somites is in the form of a plexus; and it will be noted that there is a vessel extending lateralwards from this plexus opposite the cephalic border of the omphalo-mesenteric veins, and a similar vessel opposite the caudal border of the vein. These two vessels are in the somatopleure dorsal to the omphalo-mesenteric veins. This is very clearly shown in the section in text-figure 2. The more cephalic of these two vessels (*V. so.*) develops, as I shall show for the pig, into veins which drain the body-wall over

the region of the heart cephalic to the duct of Cuvier. They receive their blood from lateral branches of the aorta (of which the lateral artery opposite the heart, shown in plate 2, fig. 1, may be one) and are analogous to the branches of the umbilical veins below the duct of Cuvier.

Of the veins of the somatopleure, those which are opposite the caudal border of the omphalo-mesenteric veins join the omphalo-mesenteric vein in the septum transversum of His, as shown on the right side of text-figure 2. The connection, which has not taken place in the specimen in plate 2, figure 1, at the stage of 14 somites, occurs at the stage of 15 somites, as was shown by Evans. In making injections at the stage of 15 or 16 somites it sometimes happens that the ink first injected does not fill the neural vessels, but runs from the aorta into the duct of Cuvier through direct aortic branches, such as that shown in the third interspace in plate 2, figure 2.

One of the most interesting points in connection with the duct of Cuvier is that it forms just about the time or just before the time when the circulation begins, which is probably of great importance from the standpoint of the physiology of the embryo. Thus plate 1, figures 2 and 3, and plate 2, figure 1, represent the blood-vessels of the chick before the circulation has begun, while figure 2 of plate 2 and figure 1 of plate 3 represent a series of chicks in which the circulation has commenced. Inasmuch as the duct of Cuvier has not connected with the omphalo-mesenteric veins (sinus venosus) at the stage shown in plate 2, figure 1, the longitudinal plexus and vessel of the lateral line at this stage is a common cardinal vein which will be divided into an anterior and a posterior division by the position of the duct of Cuvier. From a comparison of figures 1 and 2 of plate 2, figure 1 of plate 3, and plate 6 it is clear that the anterior cardinal vein must increase in length at the expense of the posterior cardinal vein as the heart shifts caudalward. In these figures it is plainly shown that the cardinal system opposite the duct of Cuvier continues in the form of an extensive plexus (see also plate 1, fig. 1, of the pig) and that the plexus ultimately gives rise to the umbilical veins.

This completes the general account of the blood-vessels of the chick before the circulation has begun; that is, up to the stage of plate 2, figure 1. I shall take up, under two headings, the study of the further development of the primitive blood-vessels in the stages in which the blood is circulating; first, the vessels of the brain and their relation to the primary head-vein; second, the vessels of the spinal cord. The primitive vessels of the nephrotomes will be taken up in connection with the pig embryos. It is of course evident that the two divisions overlap, for the vessels of the brain begin in the period before the circulation commences.

VASCULAR SYSTEM OF THE BRAIN AND THE PRIMARY HEAD-VEIN.

As has been shown, the neural vessels begin to form very early, before there is any circulation, and indeed before the heart has begun to beat. The primitive vessel of the hindbrain differentiates at the stage of 6 somites as a chain of angioblasts along the border of the hindbrain, and at the time it is differentiating

connects with the aorta by chains of angioblasts which are forerunners of direct dorsal branches of the aorta.

Exactly when the angioblasts along the forebrain and the midbrain can be identified has not been determined, but at the stage of 12 somites there is a plexus of large vessels along the lateral surface of the forebrain and midbrain extending to the ventral surface of the forebrain at the base of the optic vesicle and anastomosing with the primary vascular channel of the hindbrain. This plexus connects with the aorta just at the base of the optic vesicle, as was shown by Evans in his figure 398 for a duck embryo of 13 somites in the "Manual of Human Embryology" (Keibel and Mall). At the stage of 12 somites this plexus also connects with the aorta opposite the midbrain, as shown in text-figure 1.

This deep primary plexus, which I have uniformly represented in a gray tone, soon gives rise to a superficial plexus opposite the region of the forebrain and the midbrain, as shown in text-figure 1. In this superficial plexus there develops a venous channel for the forebrain and the midbrain, as will be seen in plate 2, figure 2, from a chick of 16 somites, which is the stage when the blood begins to circulate. The superficial plexus opposite the forebrain and the midbrain arises, for the most part, from the deep plexus (text-fig. 1), but I have also injected a few tiny connections between the superficial plexus and the aorta itself in early stages. These, however, disappear and the superficial plexus drains only the deep plexus.

The vein which develops within the superficial plexus is characteristically placed, and is very adequately shown by Evans for the stages of 17 to 25 somites (Anatomical Record, 1909, III, figs. 3 to 6). At the stage of 29 somites this primitive cerebral vein is clearly shown in plate 6. Owing to the flexure of the midbrain, the primitive cerebral vein (*v. cap. p. 1*) runs directly across the thalamus; and it receives a very interesting series of branches. It is obvious that a very large number of the primitive veins opposite the cerebrum drain the eye. Beginning with the position of the Gasserian ganglion, as seen in plate 6, there is a plexus of veins which I have called the primitive maxillary veins (*v. m. p.*), which drain the inferior part of the eye and the most anterior border of the cerebrum. These veins have usually been called the primitive inferior ophthalmic veins, and, according to the function which they actually perform at the stage of plate 6, this would be perhaps a more logical name. However, the stage when this plexus drains mainly the eye is very transitory. Soon the capillaries of the maxillary arch develop and the plexus of veins which, at the stage of plate 6, clearly lies in the maxillary arch, drains all of the structures of that arch, the roof of the mouth, and the nose. The position of the maxillary vein and its corresponding artery in the maxilla is shown for the pig in plate 7. In the chick of the fourth and fifth days of incubation this group of veins clearly drains the entire maxilla and receives branches from the most anterior part of the cerebrum and a group of inferior ophthalmic veins, of which one of the most important runs in the optic stalk. Therefore I have preferred to limit the name primitive inferior ophthalmic veins to the branches of the primitive maxillary vein instead of calling

the entire trunk the ophthalmic veins. The emphasis on the fact that this group of veins belongs in the maxilla, bringing it into line with the veins of the mandibular arch and with the veins from the rest of the aortic arches, is interesting in connection with the origin of the middle segment of the primary head-vein.

Cephalic to this plexus of maxillary veins is an extensive series of veins from the marginal vein of the optic cup. The vein in the margin of the optic cup is very characteristic. Above these is a smaller, but very important, group of veins which drain the cerebrum proper. As can be seen in plate 6, they tap the deep plexus of the cerebrum at their tips; they gradually creep dorsalward on the deep plexus until they meet with those of the opposite side in the mid-dorsal line. The anastomosis of these veins in the mid-dorsal line will ultimately give rise to the superior sagittal sinus, as has been shown by Mall and Streeter. On account of the relation of the primitive veins of the neural tube to the ultimate formation of the dural sinuses, this process of the creeping of the primitive veins toward the mid-dorsal line on the deep plexus is very important.

Over the thalamus at this stage is the main root of the primitive cerebral vein and one large accessory root. For the midbrain the superficial branches of the primitive cerebral brain have not yet appeared, and all the blood of the midbrain drains through the deep plexus toward a characteristic deep vessel along the cerebellar ridge, which joins the primitive vessel of the hindbrain. In plate 2, figure 2, it is clearly shown that at the time when the circulation begins all the venous blood of the forebrain and midbrain must pass through the deep channel of the hindbrain and the transverse vein of the first interspace in order to reach the heart. This figure (plate 2, fig. 2) shows that the vessel of the hindbrain is mesial in position both to the primitive cerebral vein and to the anterior cardinal vein. Plate 6 shows that it is also farther dorsal than either of these veins; and also, what is well known, that the primitive vessel of the hindbrain is mesial to the Gasserian ganglion, the acoustic complex, the otic vesicle, and the ganglion of the glosso-pharyngeus. The cephalic end of the ganglion of the vagus, on the other hand, is mesial to the transverse vein of the first interspace; that is, the primitive vessel of the hindbrain runs down to the region of the cephalic end of the ganglion of the vagus.

The primitive vessel of the hind-brain serves as a transitory vein for the brain of the chick during the second and third days of incubation, as is very evident in any living chick. On the other hand, it serves as the only channel for the blood to the hindbrain, and it can receive arterial blood directly from the aorta through tiny branches. These branches are so small and are so seldom fully injected that it is probable that only a small amount of blood actually passes through them in the living chick into the primary channel of the hindbrain. The permanent arteries for the hindbrain develop later, as will be shown.

Plate 6 shows how the primary vascular channel of the hindbrain ceases to serve as a vein for the forebrain and midbrain, and how the true head-vein, the *vena capitis prima*, develops. The specimen here shown also indicates the fate of the primary vessel of the hindbrain. The deep plexus of the cerebrum, the

thalamus, and the midbrain has now made an almost complete covering for that part of the neural tube. Over the midbrain the plexus is farthest developed and has anastomosed across the mid-dorsal line with the plexus of the opposite side; over the thalamus and the cerebrum the deep plexus has almost reached the mid-line. The primary artery of the brain which supplies this extensive plexus divides into two branches—first, into a large arterial plexus which curves around the dorsal margin of the optic stalk and leads to the plexus around the eye and to the plexus of the cerebrum, and partly supplies the plexus of the thalamus; second, into an artery which curves along the ventro-lateral border of the thalamus and the midbrain, and is approaching the hindbrain. This artery will soon meet the ascending artery seen along the ventro-lateral border of the rhombencephalon.

Opposite the hindbrain the development of the vessels, both the arteries and the veins, is most interesting. As is shown in plate 6, there is now a most important new vein. This is as yet a tiny, irregular vessel, hardly larger than a capillary, which connects the veins of the maxillary, the mandibular, and the second aortic arch with the anterior cardinal vein. The primitive vessel of the hindbrain is a vein for the brain only; this new capillary develops out of the capillaries of the visceral arches and by means of the relation of the maxillary veins to the primitive cerebral vein it receives the blood of the primitive cerebral vein and hence it becomes a true head-vein. We shall call this new vein, which is usually called the *vena capitis lateralis*, the middle segment of the *vena capitis prima* (*v. cap. p. 2*), and will say that as soon as this anastomosis between the primitive maxillary veins and the anterior cardinal veins takes place we can speak of a primary head-vein which extends from the region of the thalamus to the duct of Cuvier and drains the structures of the head, namely, the brain and the tissues of the visceral arches.

The specimen in the drawing of plate 6 is not shown from an exactly lateral aspect, but is tilted slightly to show the ventro-lateral surface of the hindbrain; but even with this tilting it is clear that the general position of the superficial vessel is such that it can become a direct line between the primitive cerebral vein and the anterior cardinal vein. This direct line is very plain in plate 2, figure 2. In other words, it is a more favorable vessel for the drainage of the large primitive cerebral vein than is the primitive vessel along the hindbrain.

The exact course of this tiny chain of new capillaries is most interesting, because it conforms so closely to the structures that are present before it develops. In this connection the relation of this new capillary to the Gasserian ganglion is important to note, because it has been so little understood. As is well known, the ganglion arises from the wall of the pons at the point shown in plate 6, grows ventralward, and becomes adherent to the skin, making the placode of the *trigeminalis*. If sections from specimens at the stage of plate 6 are studied, it will be seen that it is this attachment of the Gasserian ganglion to the skin, occurring at the stage when the tiny capillaries that give rise to this superficial vein begin, that renders it impossible for the new capillaries to pass lateral to the ganglion; hence they grow mesial to it. The primitive vessel of the hindbrain is mesial

to the Gasserian ganglion, but lies against the hindbrain; the primary head-vein is also mesial to the ganglion, but lies ventral to the hindbrain.

On the other hand, the new capillaries pass dorsal to the placode of the acoustic complex, and the slight dorsal curve of the primary head-vein (which is very evident in plate 6) indicates this adjustment of the vein. The placode of the acoustic complex is indicated in plate 6 by a film over the primitive vessel of the hindbrain opposite the root of the eighth nerves. The vena capitis prima passes ventro-lateral to the otic vesicle and again curves slightly dorsalward opposite the ganglion of the glosso-pharyngeus.

In another injected specimen of this stage the superficial vein is a slender capillary plexus spanning the gap between the second aortic arch and the anterior cardinal vein, and not yet connecting with the veins of the maxillary arch. Thus this middle segment of the vena capitis prima (the so-called vena capitis lateralis) begins as an irregular capillary plexus between the aortic arches and the anterior cardinal vein. It becomes a true head-vein, in the sense that it drains the entire head, whereas the primitive vascular channel of the hindbrain (vena capitis medialis) is a true neural vessel draining the brain only and not the entire head.

Up to the stage when the capillaries of the visceral arches develop, the primitive channel of the hindbrain serves as the only drainage channel in the head, and this means practically for the brain alone; but as more structures in the head differentiate, a new vascular channel develops to drain these structures. This new chain of capillaries which receives the blood of the primitive cerebral vein by means of the relations of the maxillary veins is so direct and so favorable a channel for the blood of the primitive cerebral vein that the vena capitis prima develops very rapidly at the expense of the primitive vessel along the hindbrain.

By far the most interesting way to follow this transformation is by watching the living chick. As is very clearly shown in plate 6, there is a stage when there are two venous channels for the head of the embryo—a large, deep channel along the hindbrain and a superficial tiny capillary chain farther ventral and farther lateral. While this more lateral channel is very tiny, it is hard to see it in the living chick, because there are few if any blood-corpuscles in it, and it is by the injection of blood, as it were, that one sees the vessels. In one chick opened toward the close of the third day and kept in a warm box, the two veins were of equal size when first observed, but in the course of about 2 hours the ventral channel had become by far the larger. This important change can be followed in the living chick either by opening a number of eggs at the close of the third day of incubation and observing the veins by the blood within them or by keeping a single chick of the right stage under observation for 4 or 5 hours.

In such living specimens it can be seen that the deep vessel of the hindbrain, which remains as a single vessel for 2 days, becomes a capillary plexus as soon as the mass of venous blood from the forebrain and midbrain becomes shunted through the superficial vein. In an injection many interesting details of this process can be made out which are not so clearly seen in the living chick. In a stage still earlier than that shown in plate 6, the deep vessel of the hindbrain

begins to show very characteristic dorsal branches which conform to the surface of the hindbrain and to the roots of its nerves. In fact, the first of these branches, as can be seen in plate 6, tend to surround the root of the trigeminus, the root of the eighth nerves, and the otic vesicle. While these branches of the primitive vessel of the hindbrain are forming, the vessel itself also becomes a plexus. I have injections which show how this takes place. At first the single channel gives rise to a plexus of very large vessels which tend to run longitudinally, following the pattern of the original vessel. Gradually the vessels of this plexus become smaller and the longitudinal pattern is lost. I have not illustrated the development of the plexus on the hindbrain for the chick, but this point is well shown for the pig in plate 7, and the principles are the same in both forms. The plexus on the hindbrain ultimately covers the hindbrain as completely as the plexus on the midbrain shown in plate 6, but the pattern of the plexus is modified by the structures of the hindbrain: (1) by the roots of the nerves and their sensory ganglia; (2) by the otic vesicle, which for a time lies close to the hindbrain; (3) by the special vascular structure of the roof of the fourth ventricle. As has been said, the plexus into which the primary vessel of the hindbrain first breaks up tends to have a longitudinal pattern; the ultimate plexus over the hindbrain, on the other hand, tends, like the rest of the neural plexus, to show indistinct transverse lines. This is, I think, plain in plate 7, and it leads to the subject of the new arterial supply for the vessels of the hindbrain.

A most important point in the history of the transformation of the primitive vessel of the hindbrain concerns its relation to the neural arteries, and this point is well brought out in plate 6. Taking into consideration the entire neural tube, it is originally supplied by a series of arteries from the aorta: (1) a group of vessels to the forebrain, that is, to the cerebrum and the thalamus, at the base of the optic vesicle from the primitive arch of the aorta; (2) a few small arteries opposite the midbrain; (3) a series of small arteries to the primitive vessel of the hindbrain; (4) a series of intersegmental arteries, of which the most cephalic is in the first interspace. In plate 6 an artery is shown on the right side from the primary arch of the aorta, which is growing along the ventro-lateral surface of the thalamus and the midbrain, and this artery is approaching a new artery, which is at the same time growing forward along the hindbrain. This new artery is very important; it starts as a longitudinal anastomosis along the neural tube between the segmental arteries. In plate 6 it connects the first, second, and third segmental arteries, which are occipital vessels, and is growing forward, making more and more new connections with the deep vessel of the hindbrain. Plate 6 shows none of the primitive arteries which connect the primitive vessel of the hindbrain directly with the aorta; but in plate 7, from a pig of a still older stage, it is very interesting to note that two of these original arteries still persist and take part in the formation of this new longitudinal artery. This longitudinal artery grows rapidly forward until it joins the corresponding descending artery opposite the midbrain. It is very clear in plate 6 that the longitudinal neural artery along the hindbrain is originally along the ventro-lateral border of the hindbrain, and thus that there

is one on each side. In plate 6 this vessel is labeled the basilar artery (*a.b.*), which is an illustration of the fact that the relations of the arteries of the adult may have too great an influence on the naming of the embryonic vessels. This vessel is not even a capillary which will become the basilar artery, because it is not in the mid-ventral line; it is rather a vessel which will become a part of a capillary plexus that will gradually reach the mid-ventral line, where the basilar artery will form. At the stage of plate 6 there are bilateral longitudinal arteries along the thalamus, the mid-brain, and the hindbrain, as can be proved by a direct ventral view of the specimen. The relations and the importance of this vessel would be emphasized by calling it a part of the primary longitudinal neural artery. On the other hand, the vessel shown in plate 7 from a pig embryo of an older stage is in the mid-ventral line and is thus the true basilar artery.

During the fourth day of incubation the longitudinal artery seen opposite the first, second, and third somites in plate 6 grows caudalward along the ventro-lateral surface of the spinal cord on either side, to the caudal end of the neural tube. These ventro-lateral arteries develop as a longitudinal anastomosis between all the segmental arteries of the spinal cord. At the stage of the fourth day of incubation it is clear that the vascular plexus along the entire surface of the neural tube is supplied with blood by bilateral ventro-lateral arteries which extend from the groove between the cerebrum and the thalamus to the caudal tip of the tube. These two longitudinal arteries are originally in the form of a plexus on either side of the subthalamus, as is still better shown in plate 7 for the pig, and are more definitely a single channel along the rest of the course.

This longitudinal neural artery receives its blood from the forerunner of the carotid arteries on either side and from the segmental arteries. It is easy to see that it is these important longitudinal arteries which will ultimately give rise to the circle of Willis, the basilar artery, and the anterior spinal artery.

The development of the anterior spinal artery has been worked out in the pig by Evans (1909 and 1912). In the chick the anterior spinal artery does not form until the fifth day of incubation. During the fourth day there are two ventro-lateral arteries along the spinal cord which are placed on either side of the notochord and are not connected except by an occasional capillary across the mid-ventral line; they make a sharp ventral boundary for the lateral plexus on the spinal cord. These two longitudinal arteries are just mesial to the point where the spinal arteries meet the spinal cord, as can be seen in Evans's figure 437c in the "Manual of Human Embryology" (Keibel and Mall). They give rise to the characteristic anterior arteries which penetrate the spinal cord. During the fifth day of incubation these two longitudinal arteries become connected with each other across the mid-ventral line, which is the beginning of the formation of the anterior spinal artery. The stage of the fourth day of incubation for the chick in which there are bilateral longitudinal arteries along the ventro-lateral border of the entire neural tube from the point of origin of the carotid artery to the tip of the spinal cord is an important stage for understanding the blood-supply of the nervous system.

It must be made very clear indeed that the longitudinal artery seen along the hindbrain in plate 6 is a neural artery and is not the vertebral artery. This is a specimen of the third day of incubation and the artery shown in this specimen forms along the neural tube at a stage when the occipital arteries supply only the neural tube. On the fifth day of incubation, on the other hand, these same arteries also supply the corresponding myotomes with vessels, and there then forms a second longitudinal anastomosis on either side along the upper segmental arteries which is nearer the aorta than the neural vessel. These second longitudinal vessels become the vertebral arteries. These arteries form at the stage of the fifth day of incubation in the chick and are present in a pig measuring 15 mm., a very much older stage than the one shown in plate 7, which measured 6.5 mm. The vertebral arteries form as the heart is shifting farther caudadward; and indeed it is clear that the basilar and anterior spinal arteries together, as well as the vertebral arteries, provide for the arterial supply of the hindbrain when the shifting relations in the neck interfere with the direct arteries from the aorta. The fundamental relations of the neural arteries to the plexus on the surface of the neural tube has now become clear. This plexus is fed with arterial blood from bilateral longitudinal arteries which are along the ventro-lateral border of the plexus and eventually come to lie for the most part in the mid-ventral line. Over the surface of the subthalamus the vessels remain bilateral.

It is now necessary to consider how the neural plexus becomes related to the veins. In the study of the development of the veins of the brain as distinct from those of the spinal cord, it is of primary importance to study how the deep plexus of vessels becomes related to branches of the primary head-vein. This point I have worked out more in detail in the pig and shall therefore take up its consideration later. The fundamental points are, however, (1) that the branches of the primary head-vein opposite the forebrain and midbrain are transverse veins superficial to the deep plexus which constantly tap the deep plexus at their tips and grow toward the mid-dorsal line; (2) that the transverse veins of the hindbrain are profoundly influenced by the presence of the ganglia of the hindbrain and by the otic vesicle. The sensory ganglia become as completely surrounded by a capillary plexus as the neural tube itself, and each of these plexuses gives rise to a vein or group of veins. Moreover, the same is true for the spinal ganglia.

In this account of the origin of the neural vessels great stress has been laid on the development of the vessels of the hindbrain, on account of the peculiar relations of the primitive vessel of the hindbrain to the drainage of the forebrain. In the course of the development of the vessels of the hindbrain the direction of the circulation of the blood is ultimately exactly at right angles to its original course. This change takes place, (1) by the completion of the true head-vein, by which the pial vessel is relieved of a great volume of venous blood from the brain; (2) by the development of a new longitudinal arterial channel, by which it can receive a much greater arterial supply. By these changes the blood over the hindbrain soon runs from the ventral toward the dorsal border, at right angles to its original course from the cephalic to the caudal border.

In the transition from the stage in which the primitive channel of the hind-brain serves as the vein of the brain to the stage when the new lateral superficial vessel—the true primary head-vein—is complete, it is clear that the primitive transverse vein of the first interspace is cut out of the main line of drainage for the head. It does not form a part of the primary head and neck vein of the embryo. Thus the primary head-vein, from the standpoint of development, consists of three parts: an anterior division, which is the primary cerebral vein; a second portion, which is a true head-vein draining the entire brain, forebrain, midbrain, and hindbrain, as well as the visceral arches; and thirdly, the anterior cardinal vein. The transverse vein of the chick persists as a root of a characteristic vein of the hindbrain—namely, a vein which arches caudalward along the lateral surface of the medulla. This vein of the medulla will be followed farther in the pig. It was called the posterior cerebral vein by Mall. The position of the transverse vein of the chick embryo in the first interspace is also just opposite the cephalic end of the ganglia of the vagus nerve. As soon as the superficial vein—the primary head-vein—is formed, the vascular channel of the neck straightens out, and there is then no longer any way of distinguishing the exact place where the second segment of the primary head-vein joins the third segment or the anterior cardinal vein, for the two become a single, continuous channel. From now on, the place of transition can be indicated only in a general way by the root of that vein of the medulla which follows the roots of the vagus nerve along the medulla; and it is well known that veins are shifting landmarks. Stated in other words, the anterior cardinal vein extends along the entire zone of the occipital myotomes, and as the occipital muscles develop these myotomes become indistinct landmarks.

The first interspace is thus a transitory landmark, and in later stages and as soon as the superficial head-vein connects directly with the anterior cardinal vein and eliminates the transverse vein of the first interspace from the direct line of drainage for the blood of the brain, the distinction between the head-vein and the anterior cardinal vein becomes less obvious. The cephalic portion of the head-vein develops to drain the forebrain and midbrain; the middle portion develops to drain the brain and the gill-arches. The vein of the anterior part of a chick of the fourth day of incubation is therefore a composite structure, so far as development is concerned. However, at the fourth and fifth day of incubation there is a single long vein extending from the groove between the cerebrum and the thalamus down to the duct of Cuvier. This vein receives branches from all the various structures of the head. The neural branches come from the cerebrum and the eye from the thalamus, the midbrain, and the hindbrain. The branches from the hindbrain are especially modified by the ganglia of the hind-brain and the otic vesicle. On the ventral aspect this vein receives branches from the developing visceral arches and from the somatopleure opposite the heart. The entire vein may thus be called the embryonic head-vein, or the *vena capitis prima*.

As far as the relations of the vena capitis prima to the vessels of the adult are concerned, it has been shown by Mall and Streeter that only a very small portion of the primary head-vein persists within the skull cavity—namely, the segment just mesial to the Gasserian ganglion which becomes the cavernous sinus. The neural branches of the primary head-vein ultimately give rise to the other dural sinuses.

In regard to the relations of the anterior cardinal vein of the embryo to the internal jugular vein, it is interesting to note, in plate 6, that the entire anterior cardinal vein is opposite occipital myotomes; that is, it is entirely within the head. The caudal part of the anterior cardinal vein will become a vein of the neck when the duct of Cuvier shifts into the zone of the cervical myotomes. The cephalic end of the anterior cardinal vein of the embryo is opposite the upper zone of the medulla. The cardinal system of veins in general covers the entire zone of the myotomes, which includes a part of the head as well as the entire body of the embryo.

In closing this account of the origin of the primary head-vein, it is important to emphasize again the relation of the new vessel, the middle portion of the vena capitis prima, to the various structures related to the hindbrain—that is, to the otic capsule and to the ganglia of the hindbrain. The middle portion of the head-vein develops after these structures are formed and must conform to their position. It grows in as straight a line as possible, and passes mesial to the placode of the trigeminus, lateral to the acoustic complex, to the otic capsule, and to the ganglion of the glosso-pharyngeus. It is entirely a new vessel, and has no remnants whatever of the preliminary vascular channel of the hindbrain which arises and runs along the neural tube. As is seen in plate 6, there are two entirely distinct vessels in the head of a chick of the early part of the fourth day—the so-called vena capitis mesialis, a neural vessel, and the so-called vena capitis lateralis, a true head-vein.

After following this account of the origin of the primary head-vein of the chick, it will be of value to consider the long series of previous studies upon which it has been based. The observations which seem to me to lead to a clear understanding of this subject are those of Salzer, Mall, Grosser, Evans, Williams, and Streeter. The view first held in regard to the development of the veins of the head was that the external jugular vein was the primary vein of this region. This view, which was incorrect, was based on the work of Rathke. In 1887 Kastsechenko described a remarkable relationship between the jugular vein and the cranial nerves in the chick. He stated that up to the end of the third day the cranial nerves were lateral to the jugular vein (primitive vessel of the hindbrain), and noted that this vein was not in the form of a plexus. At the end of the third day the facial and glosso-pharyngeal nerves became mesial to the vein, and on the sixth day the vagus became mesial. He thought that the nerves cut through the veins, as it were, without the latter losing their continuity.

In 1895 Salzer published an article on the development of the veins of the head in the guinea-pig, which forms the basis of the correct interpretation of this

difficult subject. He described the head-vein, in an embryo guinea-pig 2.5 mm. long, as a vessel running from the region of the optic cup, close to the neural tube (primitive vessel of the hind-brain), to the level of the first vertebra, where it turned lateralward and lay lateral to the aorta (anterior cardinal vein), ending in the duct of Cuvier. This vein (the primitive vessel of the hindbrain), was mesial to the cranial nerves, and Salzer called it the anterior cardinal vein. It was a transitory vein, for by the time the embryo was 2.8 mm. long he found a second vein, lateral to the nerves, from the region of the acoustico-facial complex forward. This second vein he called the *vena capitis lateralis*, and concluded that, not only in the guinea-pig but in vertebrates in general, the anterior cardinal vein (deep channel of the hindbrain) is the first vein to develop in the head, and that it is replaced by a *vena capitis lateralis*, which as the neck develops is continued into the neck as the internal jugular vein. This description of the veins of the early embryo by Salzer is nearly correct, and was a great step in advance, though more complete studies give a different interpretation and naming of the veins.

The next step was made in 1907, by Dr. Mall, who studied the cerebral sinuses in the human embryo and, on the basis of this work of Salzer, demonstrated that the first drainage canal for the head (primary head-vein including the anterior cardinal) gives rise to the cerebral sinuses and the internal jugular vein. This drainage canal (the *vena capitis prima*) he called the anterior cardinal vein, using the term in its generally accepted sense as applying to the entire head-vein and neck-vein of the embryo.

In the same year Grosser made it clear that the first vascular channel for the head (deep vessel of the hindbrain and the anterior cardinal) can be analyzed into two parts: a cephalic part which lies close to the neural tube, and a caudal part which has an entirely different position—namely, ventral to the myotomes and lateral to the aorta, in the same position as the posterior cardinal vein. He limited the term “anterior cardinal vein” to this caudal portion, and analyzed the cerebral portion into a primary vessel (the *vena capitis medialis*) and a secondary vein (the *vena capitis lateralis*).

At this point Evans gave his beautiful injections of early blood-vessels, published in 1909. He showed the form of the primitive vascular plexus of the brain and also how this plexus covered the surface of the forebrain, encircling the large optic vesicle with a chain of capillaries and spreading over the surface of the thalamus and midbrain. He described how this extensive plexus became a single slender channel along the wall of the hindbrain, leading down to the transverse vein and the duct of Cuvier; and also demonstrated the connections of the plexus of the forebrain and the single vessel of the hindbrain with the aorta.

Streeter has recently published a study dealing with the later stages of the *vena capitis prima*. It was from the branches of this vein that Dr. Mall had shown that the dural sinuses were derived. Streeter has worked out the development of the dural sinuses more in detail and has shown that the only part of the *vena capitis prima* to persist is the part mesial to the Gasserian ganglion which

becomes the cavernous sinus. The rest of the dural sinuses come from branches of the primary head-vein. The sinuses of the mid-dorsal line arise from the anastomoses of the veins of the two sides; the basal sinuses arise for the most part from veins which border the Gasserian ganglion and the otic capsule.

In 1916 Stracher published an article on the veins of the head of the chick, in which he deals with the fate of the vena capitis medialis and the origin of the vena capitis lateralis. In this work he uses the method of reconstruction in preference to the method of injection in a form in which it is easy to obtain abundant injected material, on the ground that with reconstructions the relations to the surrounding tissues can be better analyzed. Stracher's own work, however, suffers from the limitations of his method. His reconstructions show the larger trunks, which are not always the most important ones, and do not show certain tiny channels which are essential to an understanding of the relations of the vessels. He shows the stage at which the primitive vessel of the hindbrain (vena capitis medialis) and the primary head-vein are both present in the same specimen and equal in size. This had not been done previously, and is an important point. He also shows in part how the middle segment of the primary head-vein arises, but misses several points that are essential to an understanding of this vein. In his text-figure 2, from a chick of 30 somites, he shows a short branch from the anterior cardinal vein and a branch from the maxillary (ophthalmic) veins, and recognizes that these two branches become connected and form the vena capitis lateralis. He speaks of the branch from the inferior orbital vein (my maxillary vein) as arising from a swelling on the vena capitis medialis, not realizing that it is a new outlet, not for the blood of the vena capitis medialis but for the blood of the primitive cerebral vein, as is plainly shown in plate 6. In discussing the origin of the vena capitis lateralis from the lower border of the Gasserian ganglion to the anterior cardinal vein he says (page 55):

Kastschenko gibt keine Abbildung, die ihre Entstehung zeigen würde, seine Tafel stellt sie da, nachdem ihre Ausbildung vollendet ist. Nach seiner Schilderung "durchschneiden" die Nerven die Vene. Demgegenüber ist zu betonen, dass der eben geschilderte Teil der Vena capitis lateralis - es folgt später noch die Ausbildung weiterer caudal und cranial davon gelegener Strecken - frei im Gewebe, ziemlich entfernt von der Vena capitis medialis entsteht.

Thus he realized a part of the method of origin of the vena capitis lateralis, but missed entirely its relation to the capillaries of the visceral arches. In regard to the relations of the portion of the primary head-vein in the region of the Gasserian ganglion, Stracher's models are better than his interpretations. The essential facts are that the vena capitis medialis is a vessel on the hindbrain, the vena capitis lateralis is a more superficial vein which lies ventral to the hindbrain; both are present in the same specimen at a given stage; both are mesial to the Gasserian ganglion, one as a part of the system of vessels of the pia mater and the other as a part of the primary head-vein.

Stracher shows both the vena capitis medialis and the vena capitis lateralis in their correct position mesial to the Gasserian ganglion, and then concludes

that the medial vein of this area becomes transformed to make the lateral vein. His own figures do not warrant this conclusion, which was formed through not following the fate of the primitive vessel of the hindbrain. Thus his diagram (page 68), which should show the primary head-vein coming, embryologically, from three segments—namely, from (in his nomenclature) the vena cerebralis anterior, the vena capitis lateralis, and the vena cardinalis anterior, shows it coming from five: (1) the vena cerebralis anterior; (2) a short stretch of the vena capitis lateralis; (3) the vena capitis medialis; (4) the vena capitis lateralis again; (5) the vena cardinalis anterior. He observed the beginning of the breaking of the vena capitis medialis into a plexus, and then missed the plexus as it became finer, so that he lost the very important point of the fate of the primitive channel of the hindbrain. These points are covered in his summary (page 67):

“Sodann entwickelt sich eine neue Venenbahn (Vena capitis lateralis), die parallel zur medialen Kopfvene, aber lateral vom Nervus acustico-facialis, glosso-pharyngeus und dem Hörbläschen verläuft. Sie verbindet das Stück der Vena capitis medialis, das sich medial von der Trigemiusanlage findet, mit der vorderen Kardinalvene. Zur selben Zeit weiten sich Gefäße des Venennetzes am Hinterhirn zu einer Bahn aus, die bedeckt von der Trigemiusanlage beginnt, an der Seite des Hinterhirns dorsal von Hörbläschen im Bogen verläuft und in der Gegend des Nervus glosso-pharyngeus wieder zur medialen Kopfvene zurückkehrt (Vena capitis dorsalis). Sie tritt mit Beginn der Obliteration der Vena capitis medialis auf und verschwindet wieder, sobald die Vena capitis lateralis vollständig ausgebildet ist. Die Vena capitis medialis verodet zuerst im Bereich des Hörbläschen, dann caudal davon in der Gegend des Nervus glosso-pharyngeus. Weiterhin entwickelt sich dadurch um den ersten Ast des Nervus trigeminus ein Venenring, dass lateral vom Nerven eine Vene entsteht, die rostral vom Nerven aus dem Stamm austritt und sich caudal wieder mit ihm vereinigt. Der mediale Schenkel dieses Ringes verschwindet alsbald. In ähnlicher Weise entwickelt sich auch um den Nervus vagus ein Ring mit Beihilfe der dorsal einmündenden Zweige. Auch hier verodet die alte medial vom Nerven gelegene Bahn. Damit ist die Vena capitis lateralis vollständig ausgebildet, und die Kopfvene ändert ihren Verlauf, was ihre Lage zu den Nerven anlangt, nicht mehr, da die Nervi accessorius und hypoglossus beim Huhn auch im ausgebildeten Zustande lateral von Vena jugularis interna ziehen.”

It is, I think, clear that the primary blood-vessels which arise in the head are neural vessels. These neural vessels form a continuous plexus of capillaries which closely invests the brain. Along the hindbrain angioblasts probably appear first, but here they form a single, characteristic long channel which serves temporarily as a vein and does not take the form of a plexus characteristic of the neural vessels until relatively late. This single, large, primitive vessel does not extend the full length of the rhombencephalon, however, but at the zone of the cephalic roots of the vagus nerve, or, in other words, opposite the first occipital myotome, becomes a plexus on the side of the medulla which gradually extends the full length of the cord and connects with every intersegmental artery and vein. The neural system of vessels becomes connected with the venous end of the heart by means of the two cardinal veins. These connections are very characteristic: the most cephalic, which is either in front of the first occipital myotome (as in the pig) or between the first two occipital myotomes (as in the chick), is always the largest and drains the entire brain. All the other connections are

small intersegmental veins. Thus it may be said that two organs determine the early blood-vessels, the neural tube, and the nephrotome. Soon a third set of organs (the visceral clefts) develop and give rise to capillaries, which connect on the one hand with the anterior veins of the brain and on the other with the cardinal veins; and in this manner the head-vein of the embryo is completed.

The method of nomenclature of the primitive vessels of the head is certainly open to discussion. The primitive vessel of the hind-brain, of which I have shown the origin, the relations, and the fate, is the vessel seen by Kastschenko in 1887 and more clearly by Salzer in 1895, and recognized by all who have since worked on this subject as being the first long vein in the head region and as lying along the wall of the hindbrain. Grosser gave it the name of "*vena capitis medialis*," a name which has been universally accepted.

It may be argued that it is a mistake to attempt to change a name of this type which has been generally adopted; but on the other hand a name which would emphasize the essential point in regard to this vessel—namely, that it is a neural vessel and that it develops into neural vessels—rather than the accessory fact that it serves temporarily as a vein for the head, would, I am convinced, clear up much of the confusion in regard to the primitive veins of the head. It does serve for two days in the chick as a vein for the forebrain and the midbrain, but at the same time it is the entire capillary bed of the hindbrain and ceases to be a single long channel as soon as the cerebral blood is shunted through another channel. It then develops, as have the rest of the neural vessels, into an extensive capillary plexus in the position of the pia mater. I therefore wish to avoid the use of the term *vein* in connection with it and to reserve the term *vein* for the *vena capitis lateralis*, for which I shall use the term *vena capitis prima*, because this is the first vascular channel of the head which is purely a vein and because it is the first vessel which drains the head and not the brain alone. I have therefore called the *vena capitis medialis* the primitive vessel of the hindbrain. The term *vessel* is more indefinite than the term *vein*, but for that very reason it applies better to a channel which serves both as a vein and as a capillary at the same time, and ultimately becomes a capillary plexus, out of which both arteries and veins will arise. I propose to call the long vein of plate 6, extending from the region of the thalamus to the duct of Cuvier, the primary head-vein. This primary head-vein develops in three segments—a cephalic segment which is the primitive cerebral vein, a middle segment opposite the hindbrain, and a caudal segment which is the *vena cardinalis anterior*. At the stage of plate 6 this vein is entirely within the head, because the duct of Cuvier is still opposite occipital myotomes. As soon as the duct of Cuvier shifts into the neck region this vein will become the primary head and neck vein. The terms *vena capitis medialis* and *lateralis* have the sanction of usage; but it seems to me that the terms *primitive vessel of the hindbrain* and *primary head-vein* better express the function of these vessels.

DEVELOPMENT OF THE SPINAL ARTERIES.

It will now be necessary to go back and follow the development of the spinal arteries. This can be done in a series of injections of the stages from 15 or 16 somites upward, or the entire process can be followed in any one chick up to the stage of about 30 somites. The process is easier to illustrate after the embryo has rotated so that the lateral instead of the dorsal surface is presented. The entire process can thus be readily followed in plate 3, figure 1, from a chick of 25 somites, with six sections from two different series of chicks of 25 and 30 somites.

The general stage of development of the vessels of the head of the embryo at the stage of 25 somites can be seen in Evans's figure 6 (*Anat. Record*, 1909, III, p. 505); or can be estimated from my plate 6, the stage of 25 somites being just before the superficial capillaries which make the middle segment of the primary head-vein begin. The deep vessel of the hindbrain is still the vein for the brain, and is shown in its relation to the capillary plexus on the lateral surface of the spinal cord in plate 3, figure 1.

The general development of the area vasculosa at this stage is also of interest in following the vessels in sections. The roots of the omphalo-mesenteric arteries at the stage of 25 somites are opposite the twentieth and twenty-first somites. As was indicated above, in the earlier stages the entire lateral border of the aorta opposite the somites was originally connected by direct lateral (that is, ventrolateral) branches with an arterial plexus of the area vasculosa. In this plexus, on either side of the embryo, the omphalo-mesenteric veins gradually extend caudalward from the region of the sinus venosus and thus are formed two veins, or a plexus of veins, with direct short connections with the aorta. This process explains the large veins of the splanchnopleure shown in figure 3, plate 2, and figures 2 and 3, plate 3.

In the chick the spinal arteries do not arise as direct dorsal arteries from the aorta to the cord, but the direct dorsal arteries make a primary arch to the dorsal border of the nephrotome, where they give rise to the cardinal veins. The spinal arteries then arise from these arches instead of from the wall of the aorta itself.

In following the development of the spinal arteries I shall begin with the more caudal segments in plate 3, figure 1, because they show the earlier stages. As has been described in connection with the origin of the cardinal veins, the first dorsal branches of the aorta are direct dorsal diverticula of the wall of the aorta into the interspaces, as is shown best in text-figure 3 for the stage of 12 somites. Figure 2 on plate 4 and figure 4 on plate 2 are both from the lower segments of a chick of 30 somites. They are both taken below the origin of the omphalo-mesenteric arteries in the zone where the arteries of the posterior limb-buds are forming. The cardinal veins are also developing in this area. Figure 2 on plate 4 passes through the twenty-fifth interspace, and plate 2, figure 4, is still lower down and passes through the twenty-seventh interspace. In plate 4, figure 2, it can be seen that even in later stages the dorsal branches start as direct diverticula of the aorta. These diverticula soon arch lateralward and, as can be seen in plate 2, figure 4, dilate slightly just dorsal to the nephrotome. These dilated portions of the arches

become connected with similar dilatations in the other interspaces and make the cardinal veins. These observations indicate that the cardinal veins begin with dilatations of the dorsal branches of the aorta—that is, that they start as an outgrowth from the wall of the aorta in the different interspaces and that these intersegmental vessels become connected along the lateral line.

Very soon the vascular arches which give rise to the cardinal veins give off sprouts which extend toward the spinal cord, as is shown from the tenth to the seventeenth interspaces in plate 3, figure 1. The position of these sprouts is shown in section on plate 2, figure 3. This section passes through the twenty-first interspace of a chick of 30 somites. These neural sprouts soon reach the spinal cord, as is shown in section on plate 3, figure 4, which is to be compared with the seventh interspace of plate 3, figure 1. The section shown in plate 3, figure 4, is from a chick of 25 somites—from another series than that of all the other sections on the plates. It is from a series of nearly the same stage as that of plate 3, figure 1; it passes through the seventeenth interspace. It was selected because it shows so well the double dorsal arch to the posterior cardinal vein, the primary direct one and the secondary neural one. One has only to imagine the primary direct arch disappearing to obtain the well-known pattern of the spinal arteries shown in section on plate 3, figure 2, and in the upper interspaces of plate 3, figure 1.

From this study, it is, I think, clear that the spinal arteries of the chick arise from dorsal intersegmental vessels which give rise to the cardinal veins, and not directly from the aorta. In plate 3, figure 1, it is very evident that the capillary plexus which forms along the lateral surface of the spinal cord is a direct continuation of the primitive vessel of the hindbrain. The original simple chain of capillaries on the lateral surface of the cord, such as is shown in plate 3, figure 1, from a chick of 25 somites, very soon becomes a plexus on the neural tube, as indicated opposite the second somite in plate 6. By the fourth day of incubation this plexus covers the entire lateral surface of the spinal cord. The relation of this plexus to the spinal arteries on the one hand and to the spinal veins on the other is very regular and characteristic. Every spinal artery, on approaching the cord, bifurcates into a short ventral branch and a longer lateral branch (plate 3, fig. 2). The ventral branch leads to a longitudinal neural artery which at the stage of the fourth day lies on the ventral surface of the cord just lateral to the notochord. In other words, there are symmetrical ventral longitudinal arteries. These arteries form a ventral border for the plexus along the lateral surface of the cord. The lateral arteries run in the plexus on the surface of the cord; they lie just cephalic to each spinal ganglion and extend nearly to the dorsal border of the cord. The veins which accompany these transverse arteries, in contrast to the arteries, are lifted off from the surface of the cord, as it were. They also correspond to the cephalic border of each ganglion, and they are more superficial in every case than the corresponding artery. I am emphasizing the fact that the arteries lie in the plexus on the surface of the cord and that the veins are more superficial, because the same is true for the primary arteries and veins of the brain, as can be seen very clearly in plate 7.

The series of sections of injected chicks shown on plates 2, 3, and 4 allow an interesting comparison of the primitive branches of the aorta. It is clear that the branches of the aorta can be described best according to what organs they supply rather than by regarding their exact point of origin in the wall of the aorta. The primary branches extend to the splanchnopleure and are primitively directly lateral branches, as can be seen on the left side of text-figure 3. That they come to be ventro-lateral and then ventral branches is well known and is shown in plate 2, figure 4, in which it is clear that there are three sets of arteries on the right side of the section. The first is a dorsal branch to the cardinal vein; the second is a lateral branch to the somatopleure, and the third is a ventral branch to the splanchnopleure. The branches that extend to the cardinal veins need careful attention. There are in the first place the original dorsal arches that give rise to the cardinal veins, such as are shown in figure 4 of plate 2 and figure 4 of plate 3. These branches are strictly intersegmental; moreover, the intersegmental branches are the first arteries related to the cardinal veins because they are the only ones present at the stage of 12 somites. In later stages (for example, at the stage of 25 or 30 somites) there develop a few direct dorso-lateral arteries to the cardinal veins, and these arteries may lie opposite the somites instead of between them. Such an artery, for example, is shown on the left side of figure 3 of plate 3. This section is the next one below that of figure 2 of plate 3 in the series and indeed the edge of this artery is shown in the latter section. Similar direct dorso-lateral arteries to the cardinal veins are shown on both sides of figure 4 of plate 3. This latter figure demonstrates that these dorso-lateral arteries are new vessels and not remnants of the original dorsal arches. On the left side of figure 4 of plate 3 blood reaches the cardinal vein in three ways: from the aorta along the surface of the cord, from the aorta along the primary dorsal arch, and from the aorta through a dorso-lateral artery. It must also be brought out that these dorso-lateral arteries to the cardinal vein are not the same as the direct lateral arteries to the tubules of the pronephros and the metanephros, which develop later and are quite differently placed, as can be seen in text-figure 8 from a chick of 35 somites. These dorso-lateral arteries to the cardinal veins are of importance in connection with the extension of the cardinal veins caudalward and are very important in comparing the chick with a form like the pig, where the dorso-lateral branches are more numerous.

It may be well to enumerate here the different types of branches of the aorta which may be found in the embryo from the standpoint of the structures they supply: first, there are the arteries to the splanchnopleure; second, mesial branches which connect the two aortae; third, lateral arteries to the somatopleure leading to the umbilical veins; fourth, dorso-lateral arteries to the cardinal veins; fifth, lateral arteries to the limb-buds; and sixth, lateral arteries to the nephritic tubules.

THE VASCULAR SYSTEM IN YOUNG PIG EMBRYOS.

In the study of the vascular system in a mammal it is not as easy to obtain young stages for injections, as in the case of the chick. The material, however, offers valuable opportunities for comparison with human embryos, and to obtain injections in much earlier stages than have ever been injected in human specimens. I shall follow the development of the vessels in the pig by the aid of six figures of injected embryos, and shall describe the specimens and follow the development of the vessels under six headings: First, the form of the heart; second, the ventral branches of the aorta, including the allantoic arteries and the subintestinal artery; third, the umbilical veins and the vessels of the thoracic body-wall; fourth, the vascular system of the nervous system and the formation of the primary head-vein; fifth, the cardinal veins; sixth, the vessels of the pronephros and the mesonephros.

THE FORM OF THE HEART.

The youngest pig which I have injected is shown on plate 4, figure 3. This is from a specimen which measures 4 mm. in oil and which has 14 somites. It corresponds in development with a human embryo, No. 470 of the Carnegie collection, which measures 3.3 mm. and is in the fourth week of development. In this embryo pig an injection was made into the aorta opposite the origin of the omphalo-mesenteric arteries. The point of injection was obscured by extravasation, so that it is not shown in the drawing. The stage of development of the specimen can be judged by the form of the brain, the otic vesicle, and the form of the heart.

The extensive venous plexus covering the anterior or cephalic wall of the yolk-sac converges on either side into large right and left omphalo-mesenteric veins, which meet in a conjoined tube, the sinus venosus. The sinusoids of the liver have not yet begun to form, so that the sinus venosus stands out clearly. The sinus has a marked diverticulum, which Tandler called the horn. The dorsal wall of the sinus shows a series of sprouts, representing the duct of Cuvier, which is probably developed at this stage, as indicated by the posterior cardinal vein, but is incompletely injected. The most caudal of the sprouts form a small plexus representing the umbilical vein in the somatopleure.

Above the sinus venosus is a well-marked groove between the sinus and the atrium. The atrio-ventricular canal, on the other hand, is only just indicated. The form of the heart corresponds closely with the description by Tandler (*Manual of Human Embryology*. Keibel and Mall, page 536) which is based on the studies of Born, in which he says that the heart becomes a horizontal loop, the two limbs of which are separated by an almost horizontal bulbo-ventricular cleft into two parts, a ventricular limb and a bulbar limb. In my specimen the bulbar limb consists of three parts: first, the bulbus cordis; second, a short constricted portion of the tube, the *fretum Halleri*; third, the large truncus arteriosus, which gives off the two aortæ. In the use of the term *fretum Halleri* I am following the usage of His (*Anatomic menschlichen Embryonen*, pages 131 and 140). He describes this portion of the tube as the portion which ultimately gives rise to the semilunar valves.

In connection with the development of the heart, figure 1 of plate 5 and figure 1 of plate 1 are very interesting. The specimen from which the former was taken was one of a litter of five, all of which were injected. It measures 6 mm. in oil, that is, after fixation and dehydration, and has 20 somites. The specimen on plate 1, figure 1, was one of a litter of six embryos, all of which were injected. It measured 7 mm. when fresh and is 6.2 mm. long in oil. It has 23 somites. It should be noted that these embryos do not have a caudal flexure, so that these measurements must not be confused with the same measurements of older specimens after the flexure has formed. The number of somites gives more valuable data in regard to the stage of development in these stages than do measurements.

If comparisons are made with human embryos at the stage of 23 somites it will be noted that at this stage the human embryo has two very marked flexures, shown, for example, in the R. Meyer embryo No. 300, represented in Felix's figure 531, in the "Manual of Human Embryology" (Keibel and Mall), and hence it is very much shorter.

In figure 1 of plate 5 the changes in the heart from the stage shown in plate 4, figure 3, are readily followed. The direction of the ventricular arch has changed from the horizontal to an oblique position. The atrio-ventricular canal has become the characteristic long, slender channel, and there is a marked constriction between the ventricle and the large bulbus cordis. The fretum Halleri is now a long, slender tube, and both the bulbus cordis and the truncus arteriosus are shown in maximum distension.

In plate 1, figure 1, the form of the sinus venosus is not clear, as it is concealed by the injection of the sinusoids of the liver. In all of the six specimens of this litter the sinusoids of the liver are farther developed on the left side than on the right. In all of the other specimens, however, and on the right side of this specimen, there is a marked constriction between the liver and the sinus venosus just below the upper large opening of the umbilical vein. At this stage the umbilical vein connects with the liver and with the sinus venosus by large openings, and with the duct of Cuvier by an extensive capillary plexus in the somatopleure. There is a constriction between the sinus venosus and the atrium, and a well-marked atrio-ventricular canal. The bulbo-ventricular cleft gives the effect of an hour-glass constriction of the heart. This is true of all the specimens of the litter, but in one the contraction of the bulbar portion is particularly marked. The differences in the form of the heart in figure 1, plate 5, and figure 1, plate 1, are partly due to the fact that the hearts in these specimens were fixed while beating and were caught at different phases of the beat. For example, in plate 5, figure 1, the bulbus cordis and the truncus arteriosus show a maximum distension, while in plate 1, figure 1, the bulbus cordis and the truncus arteriosus are contracted and there is a general distension of the cephalic aorta. On the other hand, in plate 1, figure 1, is shown the beginning of a torsion of the ventricular loop, by means of which the beginning of the fretum Halleri will come to be opposite the ventricular end of the atrio-ventricular canal.

This torsion is more clearly seen on figure 2 of plate 5 and figure 1 of plate 4. These two specimens are from the same litter. They measure 7.1 mm. in oil, and have 27 somites. Figure 1 of plate 4 is given because of an extravasation in the vessels of the head in the specimen of figure 2 of plate 5. In this latter figure the sinusoids of the liver have markedly developed. The sinusoids of the left side anastomose across the ventral line with those of the right side. The opening of the left umbilical vein into the liver is directly mesial to the umbilical vein itself and is hidden by it, while the opening into the duct of Cuvier is plainly visible. There is also a plexus from the umbilical vein in the somatopleure connecting it with the posterior cardinal vein and with the duct of Cuvier; but this is omitted in the drawing.

There is a well-marked constriction between the sinus venosus and the atrium. The change in the heart is due to the twisting of the obliquely placed ventricular arch, whereby the point which marks the beginning of the fretum Halleri comes to lie exactly opposite the opening of the atrio-ventricular canal into the ventricle. The bulbus cordis lies far to the right and its connection with the fretum Halleri is hidden by the ventricle, while the opening of the auricular canal is far to the left. These relations as seen from the other side are shown in plate 4, figure 1. From these two figures it is obvious that a still further twisting of the heart must take place before the arterial orifice comes to lie directly anterior.

VENTRAL BRANCHES OF THE AORTA, INCLUDING THE ALLANTOIC ARTERIES AND THE SUBINTESTINAL ARTERY.

One of the most interesting subjects in connection with these injections has been the study of the ventral branches of the aorta, or the branches to the yolk-sac, the gut, and its derivatives.

The study of the early vessels of the embryo emphasizes the fact that the vessels should be considered in relation to the organs which they supply. The fundamental relations of the ventral branches of the aorta to the yolk-sac and to the allantois are shown in two total preparations of injected pig embryos (plate 5, fig. 1, and plate 1, fig. 1) and in two sections (text-figs. 5 and 6). Plate 5, figure 1, is from a specimen of approximately the same stage as in Evans's figure 394 in the "Manual of Human Embryology," which shows the state of development of vessels of the brain at this stage.

The position of the embryo should be carefully noted. The caudal half of the specimen is seen from the direct ventral aspect, while the cephalic half is from a direct lateral view. The place of rotation is around the ninth somite.

Extending from the level of the eleventh somite to the caudal end of the embryo there is a series of tiny ventral arteries from the two aortæ. These are of uniform size and are placed at regular intervals, approximately one opposite an interspace and one opposite a somite. In this particular embryo only a few of these ventral branches are injected; but other specimens show that the entire length of both aortæ gives rise to branches like those shown opposite the twelfth, thirteenth, and fourteenth somites. From the region of the eleventh to the four-

teenth or fifteenth somite these tiny branches from the two aortæ unite in a plexus of large arteries on either side of the stalk of the yolk-sac, which join and give rise to the omphalo-mesenteric arteries on the yolk-sac. The large arteries are seen only on one side in plate 5, figure 1, and plate 1, figure 1, but are shown on both sides in figure 2 of plate 5. From the fourteenth somite caudalward the ventral branches of the aorta are uninjected in this specimen (plate 5, fig. 1), but show in other specimens leading to a single artery which arises in the caudal end of the embryo. Opposite the caudal end of the embryo the ventral branches of the aortæ form a sheet of capillaries on either side of the alimentary canal, which deserves careful consideration. These two sheets of capillaries form a plexus which completely surrounds the entire caudal end of the gut cephalic to the allantois, the stalk of the allantois, and the blind end of the gut, caudal to the allantois. This capillary plexus gives rise to two arteries, the paired allantoic arteries and the single subintestinal artery. Thus, we have here examples of arteries in the embryo which arise in a capillary plexus and end in a capillary plexus. The primitive allantoic arteries arise in a plexus around the stalk of the allantois and pass to the capillaries of the body of the allantois; the subintestinal artery arises in a capillary plexus around the gut and runs to the capillaries of the yolk-sac.

The allantoic arteries, as seen in plate 1, figure 1, extend into a plexus on the ventral or cephalic surface of the allantois; this plexus arches around the dome of the allantois, though not completely shown in the drawing, and reaches the veins on the caudal surface. The two allantoic veins join the umbilical veins at the point where the stalk of the allantois is fused with the body-wall. A section through the allantoic arteries from an injected embryo of the same litter as the specimen of plate 1, figure 1, is shown in text-figure 6, and shows the allantoic arteries following the wall of the gut into the allantois. In the series from which text-figure 6 is taken there are a few tiny capillaries extending dorsalward from the allantoic arteries just at the point where these arteries pass ventral to the coelom. These capillaries grow lateral to the coelom, and when the posterior limb-buds begin they will anastomose with the iliac arteries. These capillaries will become the umbilical arteries in the somatopleure.

These observations on the pig agree with the findings of Hochstetter in the rabbit (1890) and show that in these forms the primary allantoic arteries are vitelline vessels, while the central ends of the umbilical arteries are vessels of the somatopleure, which appear later and anastomose with the primitive allantoic arteries.

In the study of the R. Meyer human embryo 300, Felix (1910) gives an exceedingly interesting reconstruction of the vascular system of a human embryo which is of the same stage as my figure 1 of plate 1. This reconstruction (fig. 7, *Morph. Jahrb.* 1910, XLI, p. 590) shows that the primitive artery of the fetal membranes at the caudal end of the embryo arises in a capillary plexus around the gut, just as is shown in my figure 1 of plate 5 and figure 1 of plate 1. The position of this plexus in the wall of the gut is shown in section in Felix's figure 9, which is to be compared with my text-figure 6. The same relations are shown for the chick in Duvál's *Atlas*, plate XXXIII, figure 372. In the human embryo this artery

has been traced back as a vitelline vessel to the stage of 5 somites by Felix (1910), and to the stage of 8 somites by Dandy (1910). This artery in the wall of the gut, which is the primitive allantoic artery in the pig, has been called the umbilical artery in the human embryo on account of the insignificance of the allantois and the earlier vascularization of the chorion. The relations of these two vessels in connection with the human embryo were summed up by Evans (1912, page 595) in the phrase that the umbilical artery is merely a modified vitelline vessel. The entire question of the relation of the arteries for the fetal membranes at the caudal end of the embryo has centered around the position of the central end of the arteries with reference to the coelom, as can be seen in text-figure 6; that is to say, whether the artery is mesial or lateral to the coelom. In general, both in birds and in mammals there is a primitive artery mesial to the coelom; that is to say, a splanchnic vessel, and a secondary vessel, the umbilical artery, lateral to the coelom running in the somatopleure. Thus the vessels develop in the same manner in the different forms, for there is a primitive splanchnic artery followed later by an artery in the somatopleure, but there are variations in the relative importance of the allantois itself.

Besides the two arteries of the allantois, the two sheets of capillaries of the wall of the caudal end of the gut give rise to another artery. Extending forward from the stalk of the allantois, as seen in plate 5, figure 1, the two plexuses meet in a capillary plexus ventral to the caudal root of the yolk-sac. This plexus is continued as a single, ventral, subintestinal artery which joins the omphalo-mesenteric plexus opposite the fourteenth or fifteenth somite. The point where the subintestinal artery joins the omphalo-mesenteric plexus is the well-known intestinal landmark where the stalk of the yolk-sac joins the gut. A figure which gives a very clear understanding of these relations is Tandler's figure 1 in the *Anatomische Hefte*, 1904, 1²³, page 192.

This subintestinal artery in the pig is the more interesting in view of the corresponding subintestinal vein in the chick, discovered by Hochstetter in 1888 and accurately described by him. He described its relations not only to the omphalo-mesenteric veins, but also to the intestinal and the allantoic vessels, and noted that it disappeared and that the left vein was larger than the right. A complete understanding of the development of this vein in the chick can be gained from the figures of Popoff (1894). As was mentioned in connection with the chick, during the early hours of the third day of incubation the entire capillary plexus of the area vasculosa caudal to the omphalo-mesenteric arteries must be regarded as an arterial capillary plexus down to the marginal vein, as shown in Popoff's plate v. During the last hours of the third day, as seen in Popoff's plate vi, branches of the omphalo-mesenteric vein gradually extend caudalward on either side of the embryo in the wall of the yolk-sac, and arch around the posterior end of the embryo; the left vessel starts ahead of the right and is always larger than the right. As these veins gradually extend backward into the territory of the pre-existing arterial plexus, forming more and more new connections with the plexus, they change the direction of the current of the blood in the

plexus (which has been away from the heart) to a direction towards the heart. The vein on the left side quickly extends to the marginal vein, making the single posterior vein of the yolk-sac of Popoff, which lies a little to the left of the mid-line, as shown in Popoff's plate VIII. The two lateral veins form an arch around the posterior end of the embryo; this arch is just cephalic to the point where the stalk of the allantois will develop.

On the third day of incubation there is a very extensive capillary plexus on either side of the posterior end of the gut, and beginning at the very caudal tip of the gut on either side are symmetrical ventral veins, which unite in a loop just cephalic to the base of the allantois and then run forward, at first as two veins and then as a single ventral vein in the ventral wall of the yolk-sac. The sub-intestinal vein is thus the primitive vein for the entire posterior end of the gut, for the caudal tip of the gut, the allantois, and the entire rectum and intestine up to the margin of the yolk-sac. Caudal to the allantois these vitelline veins receive the most caudal branches of the posterior limb-bud. This relation has been described by Evans (*Anat. Record*, 1909, iii). On the third day the umbilical artery develops around the somatopleure in connection with the posterior limb-bud and anastomoses with the primitive allantoic capillary plexus in the wall of the allantoic stalk. By the beginning of the fourth day the vessels in the stalk of the allantois show an exceedingly interesting relation. On either side there is one large artery coming from the aorta and now running in the somatopleure instead of in the splanchnopleure; but this artery is fed also from a capillary plexus in the wall of the splanchnopleure, which completely surrounds the stalk of the allantois and the caudal tip of the gut, and by a few capillaries of the somatopleure from the tail of the embryo, which capillaries, however, tend to drain more and more into the posterior cardinal veins.

These relations are clear in the light of the development of these vessels. There is at first a plexus of capillaries arising from the aorta and running in the stalk of the allantois, in which arise the primitive allantoic arteries; and secondarily, a capillary plexus in the somatopleure of the caudal end of the embryo, in which an umbilical artery develops. The umbilical artery joins the original allantoic artery in the fused area of allantois, somatopleure, and amnion (see text-fig. 6), and then the primitive allantoic arteries from the aorta become reduced again to a capillary plexus. Thus the allantois has a double arterial supply and a double venous drainage, the former in the wall of the gut and the latter in the somatopleure. The primitive allantoic arteries arise in a plexus of the splanchnopleure, and the corresponding venous return is through the sub-intestinal vein; the subintestinal vein anastomoses with the allantoic veins, but the direct continuation of the allantoic veins is into the umbilical veins, which develop in the somatopleure. Finally the umbilical arteries develop in the somatopleure, connect with the allantoic arteries, and soon bring most of the blood to the allantois.

The fate of the subintestinal vein in the chick is very interesting. If an injected chick of the fourth and fifth days be dissected so as to expose the caudal end of the gut and the straight posterior segment of the gut which leads up to the open bell of the yolk-sac, it will be seen that the entire wall of the gut is surrounded by a capillary plexus. At the caudal end of the gut and just cephalic to the stalk of the allantois the ventral vein has entirely disappeared in this capillary plexus, while farther forward it is still clear in the ventral wall of the gut, though freely connected with the plexus. It is clear also that this posterior segment of the gut is receiving new arterial and venous connections which grow in along the dorsal border at the cephalic end of the segment. The new artery is a branch of the omphalo-mesenteric artery given off just at the root of the yolk-sac; it extends caudalward along the dorsal wall of the gut and anastomoses with the aortic branches which are the forerunners of the inferior mesenteric arteries. The new veins are branches of the omphalo-mesenteric veins within the mesentery, the forerunners of the portal system. The entire subintestinal vein gradually disappears as a single channel by developing into the plexus of the wall of the gut. In this plexus it is clear that the direction of the flow of the blood in the wall of the gut is from the ventral toward the dorsal border, at right angles to the direction of the stream in the subintestinal vein.

It may seem curious that the pig should have a subintestinal artery in place of the well-established subintestinal vein of the chick. As has been shown, the subintestinal vein in the chick develops as a part of the process by which the primitive circulation of the yolk-sac, with arteries and veins as far apart as possible, becomes changed so that every zone of the area vasculosa is invaded by veins. The pig of plate 5, figure 1, represents the more primitive condition for comparison with Popoff's plate IV, in which the caudal part of the yolk-sac is still arterial.

The subintestinal artery of the pig can be seen in section in text-figure 5 from a pig of the same litter as the one shown in figure 1, plate 1; it receives numerous ventral arteries from the aorta, as does the corresponding vein in the chick; but it joins the omphalo-mesenteric arteries at the point of loop of the mesenteric arteries instead of the veins. This same artery is still present in the pig measuring 9 or 10 mm. after the caudal flexure has formed, at which stage it is breaking up into the capillary plexus within the wall of the gut. By the time the pig is 15 to 17 mm. long there is a new longitudinal artery in the dorsal wall of the gut, extending from the superior mesenteric artery caudalward and anastomosing with all the ventral aortic branches which represent the inferior mesenteric artery. At the same time the accompanying venous plexus from the omphalo-mesenteric vein extends along the dorsal border of the gut. As this new blood-supply for the lower half of the intestine develops, the ventral vein of the earlier stages of the chick, or the ventral artery of the pig, becomes reduced to a part of the capillary plexus in the wall of the gut. It is interesting to note that in a pig of 9.5 mm. the ventral artery of the gut is also accompanied by a plexus of ventral veins, which correspond to the single ventral vein in the chick. Thus the difference in the two forms becomes readily understandable, for the invasion of that

part of the gut by the veins is merely relatively later in the pig, and the veins are thus much more transitory.

Branches of the omphalo-mesenteric veins growing down the mesentery begin early in the pig. These are shown in plate 4, figure 3. They are not seen in plate 1, figure 1, because uninjected in the specimen. In other specimens from the same litter there is a vein in the mesentery underneath the umbilical vein, as seen from the side, and joining the main omphalo-mesenteric vein at the lower margin of the liver. These branches are shown in plate 5, figure 2. The veins in the root of the mesentery anastomose with the mesial cardinal (subcardinal) vein as soon as it develops. This anastomosis was described by Hochstetter.

The ventral subintestinal artery here described was discovered by Ravn in 1894 in the rat and mouse. The vessel was also described by Evans in the pig (Manual of Human Embryology, Keibel and Mall, foot-note 56 on page 656). Ravn's description can be readily followed in my plate 5, figure 1, as he described the main omphalo-mesenteric artery arising in the caudal end of the embryo. Both Ravn and Evans describe this subintestinal artery as arising from the umbilical artery. My specimens, however, are from still earlier stages, and prove that this vessel arises, as does all the rest of the omphalo-mesenteric system, in the wall of the yolk-sac or gut; that it is a true vitelline vessel. Its anastomosis with the umbilical arteries in the somatopleure occurs later. Thus the subintestinal artery in the pig and the subintestinal vein in the chick are vitelline vessels. They disappear as single channels and help in the formation of the primitive plexus in the wall of the gut in connection with the changes by which the gut receives its permanent blood-supply and in connection with the gradual reduction of the yolk-sac.

The study of the ventral branches of the aorta in the human embryo is based on the work of Mall, who in 1891 published an account of a human embryo 7 mm. long, in which he described two main ventral branches, a cœliac axis and an omphalo-mesenteric artery, and a series of small ventral branches in the lumbar region, making a capillary network in the mesentery. He noted that the position of both the cœliac axis and the omphalo-mesenteric artery was farther forward than in the adult, and analyzed all the available material in a study of the shifting of the arteries caudalward along the aorta. In this study he recorded human embryos with the cœliac axis opposite the first, second, fourth, and sixth dorsal nerves, as compared with the position in the adult opposite the twelfth nerve. In 1897 he made a further study of the ventral arteries, especially in a human embryo 2.1 mm. long. In this specimen he showed a series of ventral branches extending from the seventh somite to the caudal end of the aorta. These vessels he grouped together as the omphalo-mesenteric arteries. In the reconstruction he showed that the upper arteries tended to be opposite the middle of the somites rather than between the somites, as are the dorsal intersegmental vessels. In a second analysis of the ventral aortic branches he showed that there is a constant shifting of the cœliac axis and omphalo-mesenteric arteries caudalward. A double origin of the omphalo-mesenteric arteries in one embryo suggested the method of the wandering of the vessels.

The same idea of the shifting of the arteries caudalward was further developed by Tandler in two papers in 1904 and by Broman in 1908. These workers extended their observations over a long series of embryos, Broman giving a study of 41 specimens. In one of the youngest specimens in his series, an embryo measuring 3.4 mm., the upper ventral branch was between the sixth and seventh interspaces. He described the branches as tending to occur between the interspaces, there being two or three to a somite. He found that the coeliac axis and the superior mesenteric artery are not segmental vessels (that is, opposite the interspaces), while the inferior mesenteric artery is sometimes opposite and sometimes between the somites. Broman gives an analysis of the literature and an extensive discussion of the methods by which the shifting of the ventral arteries may take place.

In the human embryo ventral branches of the aorta have been described from about the seventh segment caudalward. In the pig these ventral branches are very numerous—approximately one to a segment and one to an interspace. They are originally of uniform size and about equidistant apart. They unite into an extensive plexus of larger vessels in the more cephalic region and into a long artery in the caudal region. It is easy to follow the method of the shifting of arteries from such a primitive pattern; that is, any of the vessels of the original system could easily enlarge and the blood-stream be increased or decreased according to the development of the region of the organ supplied. The entire wandering of the arteries can be understood without presupposing the development of any new vessels, but rather through the shunting of the blood through different channels already present in response to the varying development of the parts supplied by these arteries. Moreover, it is plain that the point brought out by Evans is of importance—namely, that the so-called wandering of arteries takes place while the vessels have the structure of capillaries; that is, while their wall consists of endothelium alone. From the position of the primitive ventral arteries it is also easily seen that there might be variations as to whether the ultimate ventral arteries of the older embryo came opposite an interspace on the same level as the dorsal arteries or opposite a somite.

THE UMBILICAL VESSELS.

My series is not very complete in regard to the umbilical veins, but it shows a few interesting points. In plate 4, figure 3, the relation of the somatopleure to the fold of the amnion is very plain. In the somatopleure is the beginning of a capillary plexus representing the umbilical veins. In plate 5, figure 1, the umbilical veins are not injected, but they are well shown in plate 1, figure 1, in which it is clear that the return flow of the blood from the caudal end of the embryo is in part through the subintestinal artery in the splanchnopleure and in part through the umbilical veins in the somatopleure. At the stage of plate 1, figure 1, the umbilical veins have established their connections with the liver, though they still connect with the sinus venosus. In figure 1 of plate 1 and figure 2 of plate 5 it is clear that cephalic to the duct of Cuvier there is also a capillary plexus in

exactly the same position as the umbilical veins; that is, in the somatopleure. In the specimen of plate 1, figure 1, this venous capillary plexus connects with a tiny lateral aortic branch shown just opposite the zone of the second aortic arch. This lateral artery is not the second aortic arch, which arises from the ventral rather than from the lateral surface of the aorta. From this tiny lateral artery a straggling chain of capillaries is injected within the somatopleure, out over the heart, and down to the duct of Cuvier; they are omitted in the drawing. It is clear that they are vessels for the body-wall analogous to the vessels which drain into the umbilical veins; but they are cephalic to the duct of Cuvier. The venous end of the plexus is injected in plate 5, figure 2. It was shown in the chick that the corresponding vessel of the somatopleure over the heart develops very early. In later stages these vessels in the somatopleure over the heart anastomose freely with a plexus of capillaries lateral to the occipital myotomes, as shown in text-figure 5 in my article on the Origin and Development of the Lymphatic System, 1913.

NEURAL BRANCHES OF THE AORTA AND THE PRIMARY HEAD-VEIN.

In connection with the neural vessels, I have no specimens of embryo pigs corresponding to the chicks of 6 somites in which to trace their beginning. I have one litter of very young pigs, measuring 3 mm., in which the heart and aorta are present; the neural folds are open at the cephalic end, and I can find no angioblasts along the closed hindbrain.

At the stage of figure 3, plate 4, the vessels to the forebrain can be injected; and the vessel of the hindbrain must be present, for it is seen in a human embryo of the same stage of development. Opposite the third and fourth somites the lateral plexus of the neural tube has been injected from the aorta. I found only one specimen of the litter of figure 3, plate 4; but the fact that the posterior cardinal vein is almost completely injected indicates that the anterior cardinal vein is present and that it connects with the deep vein of the hindbrain. At the stage of plate 5, figure 1, the vessels of the head are in about the stage of development of those of plate 1, figure 1, as is proved by the injections of the same litter. In one specimen of the same litter as plate 5, figure 1, the anastomosis of the capillaries around the optic stalk is complete, just as was shown by Evans for the later stage of three aortic arches in his figure 395 (Keibel and Mall, *Manual of Human Embryology*, II, p. 579).

The best view of the early neural vessels in my series is given in plate 1, figure 1. In order to analyze the relations of the vessels of the head, I have used gray to indicate all of the capillaries which are true neural vessels, in the sense of lying close to the wall of the neural tube and giving rise to the vessels of the subsequent pia mater.

As can be seen in plate 1, figure 1, the deep capillary plexus of the forebrain and midbrain is covering the wall of the brain, and the form of this plexus indicates the form of the brain. The vascular arch which surrounds the large peduncle of the optic vesicle (see Evans's figure 395) is incompletely injected in

plate 1, figure 1. It shows the relative size of the optic vesicle and the forebrain at this stage. The side of the thalamus and the midbrain is nearly covered by a plexus extending toward the dorsal wall of the neural tube. Along the cephalic part of the hindbrain is a wide vessel connected with the aorta by two arteries. It already shows sprouts along its dorsal border, two of which bound the otic vesicle. This deep single channel becomes a plexus along the side of the neural tube at a point just in front of the first myotome. This is the point where the cephalic end of the anterior cardinal vein joins the neural vessels, and, in terms of the neural tube, it is at the cephalic end of the origin of the roots of the vagus nerve. The transverse vessel of the first interspace which is so prominent in the chick is but a small vein in the pig like the other intersegmental veins, and does not become an important vessel, as in the chick. As is well known, the upper myotomes are occipital myotomes, so that it is clear that the point of transition between the deep vessel of the hindbrain and the primitive plexus, as shown in plate 1, figure 1, is not between the hindbrain and cord, but is near the upper part of the medulla. The lateral plexus along the cord is injected in the specimen of plate 1, figure 1, down to the fourteenth somite, which is opposite the lowest transverse artery injected, and the spinal arteries are injected down to the twentieth interspace. These lower vessels are omitted in the drawing.

In plate 1, figure 1, can be traced very clearly the origin of the cephalic part of the primary head-vein; that is, the primitive cerebral vein. Extending from the groove between the telencephalon and the diencephalon as Evans showed in his figure 395 in 1912), is a superficial capillary plexus, indicated in blue, which receives its blood from the deep plexus of the forebrain and midbrain and drains into the deep vessel of the hindbrain. In this plexus will develop the primitive cerebral vein; at this stage it is entirely a plexus without any definite longitudinal channels. The specimen is just at the stage of the second vascular arch, which is probably present and uninjected, as shown in Evans's figure 394 from an earlier stage. Opposite the lower end of the primitive vessel of the hindbrain is a plexus of exceedingly tiny vessels spanning the gap between the deep vessel of the hindbrain and the anterior cardinal vein on the one hand, and reaching toward the second aortic arch on the other. These tiny capillaries form the origin of the lateral vein of the region, that is, the middle segment of the primary head-vein, just as has been shown for the chick. This plexus will span the gap between the second and third aortic arches as they form, and the cephalic end of the primary head-vein, until there is a double vascular channel from the head, as shown on plate 4, figure 1.

Figure 1 of plate 4 is from a specimen of the same litter as that of figure 2 of plate 5, and is given because of the extravasation in the head region in the latter figure. At the stage of three aortic arches the primary head-vein is complete. The primitive veins which pass ventral to the eye are not injected in the specimen of plate 4, figure 1, except just where they join the primary head-vein in front of the ganglion of the trigeminus. The primary head-vein starts opposite the thalamus and extends in a double curve down to the anterior cardinal vein. It

lies mesial to the Gasserian ganglion and lateral to the otic vesicle. The pattern of the deep and the superficial vessels in plate 4, figure 1, deserves careful study. The place of origin of the roots of the Gasserian ganglion is marked by a plexus of the deep vessels which are growing around it, leaving a non-vascular area where the nerves emerge. The deep plexus is also forming a dorsal arch around the otic vesicle which now lies between the deep and the superficial vessels. The pattern of the vessels also indicates the position of the acoustic complex of ganglia and the glosso-pharyngeal ganglion, both of which lie between the deep capillaries and the superficial veins, one cephalic to the otic vesicle and the other just caudal to it. It is clear that the relations of the primary head-vein to the Gasserian ganglion and to the acoustic complex are the same in the pig as in the chick, and are due to the fact that this vessel forms while the ganglia are attached to the skin in their respective placodes. The primary head-vein develops mesial to the placode of the Gasserian ganglion, but curves dorsalward opposite the acoustic ganglia and opposite the ganglion of the glosso-pharyngeus. Sections of an embryo slightly older than that of plate 4, figure 1, cut so that a long stretch of the primitive head-vein is included in one section, show that the lateral border of the acoustic ganglion is in a straight line with the mesial border of the Gasserian ganglion, so that the superficial vein takes the shortest course in passing mesial to the ganglion of the trigeminus and lateral to the ganglia of the acoustic complex.

The relations of the branches of the vena capitis prima are very important at the stage of plate 4, figure 1. The branches from the aortic arches are not injected, nor are the primitive maxillary veins. The lateral veins from the cerebrum have hardly begun. The superficial veins opposite the midbrain have a very characteristic pattern; they are, as it were, creeping along on the deep plexus toward the mid-dorsal line. It will be noted that the deep plexus itself has not yet reached the mid-dorsal line at this stage, but it is in advance of the superficial veins. This gradual extension of the branches of the vena capitis prima to the mid-dorsal line characterizes the branches of this vein over the entire brain. When the superficial veins meet in the mid-dorsal line they will give rise to all of the sinuses and veins of this line, as has been shown by Mall and Streeter.

Opposite the hindbrain the branches of the vena capitis prima have the same fundamental relation to the deep plexus. It is true that caudal to the otic capsule there are a few veins from the deep plexus draining into the ventral border of the vena capitis prima, which may be forerunners of the small ventral veins of the medulla in the adult, but almost all of the veins of the hindbrain drain into the dorsal border of the primary head-vein. These veins have the same characteristics as the rest of the neural veins; that is, they gradually creep dorsalward on the deep plexus. Over the hindbrain, however, the pattern of the veins is not as simple as over the midbrain, because here they are profoundly affected by the ganglia of the hindbrain and by the otic capsule.

It has been shown that the deep plexus makes an arch of capillaries around the roots of the nerves, as seen around the root of the trigeminus in plate 4, figure 1. The superficial veins also curve around the roots of the nerves. Their

beginning is shown in plate 4, figure 1. Here it is clear that branches of the primary head-vein are tapping the deep neural plexus around the root of the trigeminus.

Lateral to the acoustic complex and to the ganglion of the glosso-pharyngeus, the branches of the primary head-vein make a very extensive plexus. The superficial venous arch around the otic vesicle is just beginning in plate 4, figure 1. The veins around the trigeminus and around the otic capsule are exceedingly important, because of their ultimate relations to the basal sinuses of the dura. These vessels are shown in plate 4, figure 1, at the stage when they are scarcely more than capillary sprouts. They will be traced farther in the next figure.

The specimen of plate 7, from a pig which measures 6.5 mm. in oil, is given to emphasize the fate of the primitive vein of the hindbrain, to bring out the ventral artery that now extends the full length of the nervous system from the base of the optic cup to the tip of the tail, and to show the characteristic relations of the veins to the neural tube and its ganglia.

The injection of the specimen of plate 4, figure 1, did not bring out the ascending neural arteries as did a corresponding injection of the chick (plate 6), but the specimen of plate 7 shows that there is now a longitudinal artery which extends from the primary aortic branch to the brain opposite the subthalamus, along the ventral or ventro-lateral border of the neural tube to its caudal tip. This artery is an anastomosis between all of the neural arteries, both cerebral and spinal. As can be seen in plate 7, the carotid artery leads to an arterial plexus which covers the lateral surface of the subthalamus and gives rise to a cerebral artery passing dorsal to the eye. The plexus on the subthalamus anastomoses with the plexus of the opposite side in the mid-ventral line; it is tapped by a vein leading to the primary head-vein just cephalic to the maxillary vein. Opposite the groove between the thalamus and the midbrain the two plexuses on either side of the subthalamus give rise to a single ventral artery which curves along the ventral border of the neural tube down to the level of the third occipital interspace, where the single median artery becomes an arterial plexus. From this point to the caudal end of the spinal cord there is a double line of capillaries, such as was shown by Evans in his figure 440 (1912). As Evans showed, this double capillary chain will give rise to the anterior spinal artery. The importance of this longitudinal neural artery, which gives rise to the circle of Willis, the basilar artery, and the anterior spinal artery, is obvious. The anastomosis of the arterial plexus of the subthalamus and the ventral surface of the cerebrum with the corresponding plexus of the other side across the mid-ventral line accounts for the anterior communicating artery of the circle of Willis. At the stage of plate 7 the longitudinal neural artery is supplied by the two carotid arteries, by direct arteries opposite the hindbrain, of which two are shown on the right side of plate 7, and by all the intersegmental arteries on either side. This artery is not supplied as yet by the vertebral arteries, which form later as an anastomosis between the upper intersegmental arteries.

The arterial plexus over the subthalamus leads into a finely meshed plexus which covers the entire cerebrum except a small area in the mid-dorsal line near the thalamus. This plexus is not shown in the drawing, but it has the same character as the plexus over the midbrain. The cerebral plexus completely surrounds the optic stalk; in this plexus the only vessel larger than the rest is the cerebral artery, which is seen dorsal to the eye in plate 7. The longitudinal neural artery along the ventral border of the midbrain and the hindbrain gives off a series of nearly equal, regular, small arteries which lead into the capillary plexus on either side of the neural tube.

The capillary plexus on the neural tube is very characteristic. As has been said, it is finely meshed over the cerebrum, the thalamus, and the midbrain; it is more coarsely meshed over the hindbrain, where the plexus has developed later, especially around the roof of the fourth ventricle, which has not yet been invaded by the vessels. The plexus on the hindbrain in plate 7 demonstrates the fate of the primitive vessel of the hindbrain, the beginning of this plexus as coming from the primitive vessel of the hindbrain having been seen in the living chick. The primitive vessel of the hindbrain disappears only in giving rise to the capillary plexus of the hindbrain. If the pattern of the neural plexus in plate 7 is observed carefully it will be seen that there is just a suggestion of transverse lines in the plexus, indicating that the direction of the flow of the blood is from the ventral to the dorsal border of the neural tube. In this plexus will ultimately come transverse arteries. Opposite the first somite will be noticed the beginning of three layers of vessels, a deep layer of very fine capillaries, a second layer of larger vessels also shown in gray, and a third layer of more superficial veins. This is the very beginning of the next stage in the development of the neural vessels.

The most important point about the form of the deep plexus on the neural tube is the way it conforms exactly to the neural tube and its nerves. Over the midbrain the plexus is very uniform, but over the hindbrain the character of the plexus indicates very clearly the position of the nerves. At the stage of plate 7 there are bare spots, that is, places with no blood-vessels, on the hindbrain corresponding to each nerve root; in later stages the vessels penetrate between the small bundles of the fibers of each root and then an injection of the deep plexus does not show the position of the nerves so clearly. As seen in plate 7, the positions of the roots of the trigeminus nerve and of the acoustic group of nerves are very clear. The otic capsule now lies just lateral to the deep capillary plexus, and thus its position is indicated only by the superficial veins. Opposite the ganglion of the glosso-pharyngeus is a bare spot in the deep plexus, which is nearly hidden by a very extensive group of superficial veins. The position of the roots of the vagus and the spinal accessory roots along the line of the posterior cerebral vein is very important. It is clear that the deep plexus outlines this long line of nerve roots, and the same is true along the more ventral line of the medulla, where the pattern of the vessels indicates the position of the roots of the hypoglossal nerves.

Along the spinal cord the pattern of the capillary plexus shows the position of the ventral nerve roots in the same manner.

The veins which form the branches of the vena capitis prima must now be followed. The veins from the visceral arches, still largely in the form of capillaries, are completely injected in the specimen, but are indicated in the drawing only at the point where they join the middle segment of the primitive head-vein. In plate 7 the cerebral and the cardinal segments of the vena capitis prima are shown in plastic form, but the middle segment is shown merely in outline in order to make more plain the relations of the neural artery beneath. Beginning with the maxillary vein, the entire maxilla is filled with a capillary plexus which leads to the maxillary vein. This capillary plexus anastomoses with the plexus of the mandibular arch. Besides these capillaries the vein receives a large group of tiny superficial veins which arise in the deep plexus that covers the entire olfactory area of the cerebrum, together with primitive ophthalmic veins which arise in the marginal vein of the optic cup, as in plate 6. One of these subophthalmic veins runs in the groove of the optic stalk. These cerebral veins from the rhinencephalon and from the inferior part of the eye are very important in the early drainage of the brain, but it is well known that the main permanent ophthalmic veins develop dorsal to the eye.

In the zone dorsal to the eye at the stage of plate 7 is a group of tiny superficial veins opposite the cerebrum which are like the small veins over the mid-brain. They were omitted in plate 7, but are adequately shown for the chick in plate 6, and they are alike in both forms. These are the primitive cerebral veins. Over the mid-brain the veins are characteristic. It is plain that they are lifted off from the surface of the neural tube, that they are all superficial to the deep plexus; they spread out like a fan from the primary head-vein and clearly extend along the deep plexus, which they tap at their tips, and approach the mid-dorsal line.

Opposite the hind-brain the veins are exceedingly interesting; they follow exactly the same general course of development as the rest of the neural veins; that is, they lie superficial to the deep plexus, are transverse to the long axis of the neural tube, and gradually extend toward the mid-dorsal line, constantly tapping the deep plexus at their tips. On the other hand, they are profoundly modified in their development of the ganglia of the hind-brain and by the otic vesicle, so that their pattern is much more complex than the pattern of those opposite the mid-brain.

The vessels around the ganglion of the trigeminus deserve careful study. At this stage the entire lateral surface of the Gasserian ganglion is covered by a capillary plexus which was omitted in the drawing. This capillary plexus extends along the second and third divisions of the nerve and becomes continuous with the capillary plexus of the maxillary and mandibular processes. Besides this sheet of capillaries which covers the lateral surface of the ganglion, there are two transverse veins above and below the ganglion which outline the root of the trigeminus nerve. These veins are very characteristic, and mark the position

of the Gasserian ganglion in any injected specimen up to the stage measuring 20 mm., when the transformation of the veins into the dural sinuses is well advanced, as can be seen in Streeter's figure 3 (*Amer. Journ. of Anat.*, 1915, XVIII, page 156). The superficial vessels around the ganglia of the eighth nerve are still in the form of capillaries in plate 7. Opposite the otic vesicle the deep plexus has completely covered the surface of the hindbrain; there are a few superficial veins across the lateral surface of the vesicle, which are shown cut off in the drawing close to the primary head-vein. Two of these transverse veins make a border for the otic vesicle exactly as do those above and below the Gasserian ganglion. In other words, the veins of the hindbrain can be most simply described as a series of transverse vessels, some of which are forced to curve by the Gasserian ganglion and the otic vesicle. Opposite the ganglion of the glossopharyngeal nerve is a series of transverse veins draining into the primary head-vein.

The veins opposite the vagus nerve are also very interesting. It is clear that the largest vein of the medulla at this stage is one which in a general way follows the roots of the vagus nerve. This vein was called the posterior cerebral vein by Mall. In general, the place where it joins the vena capitis prima marks the cephalic end of the anterior cardinal vein; it may be a single vein at its roots or a group of veins. In the pig the vagus nerve curves around its cephalic border, passing in the angle between this vein and the primary head-vein. Some of the injections show the nerve passing through a venous loop in this angle. Stracher describes the vagus nerve just caudal to the vein in the chick. The relations of the vagus nerve to the primary head-vein formed the basis of Kastchenko's original study of the primitive veins of the head.

As will be seen in plate 7, the main vein of the medulla primarily follows the course of the roots of the vagus nerve. It arches caudalward along the dorso-lateral surface of the medulla in the line of the spinal accessory nerve and roots of the vagus. The line of the vein on the medulla can be well seen by following the vagus roots in Streeter's plate 11 (*Amer. Jour. of Anat.*, 1905, IV).

While it is clear that this vein and its tributaries originally follow the path of the vagus nerve, if its development is followed it will be seen that it becomes a very important vein of the embryo, not even limited to the drainage of the neural tube. At the stage of plate 7 it anastomoses with the lateral venous plexus of the lower medulla, and the first and second occipital veins are correspondingly small. Subsequently it gives rise to an extensive group of dorsal branches that grow over the caudal part of the roof of the fourth ventricle and largely drain the developing choroid plexus. The posterior cerebral vein next develops an exceedingly interesting relation to the vascular system of the occipital myotomes. This relation was illustrated in two figures from injected embryo pigs in my article on the origin and development of the lymphatic system (1913, figs. 4 and 5). Opposite the entire zone of the myotomes a plexus of capillaries develops, forming the third vascular sheet of this region. Primarily there is a plexus of capillaries on the surface of the neural tube; secondly, a more lateral plexus of capillaries and veins especially related to the ganglia; thirdly, this sheet of capillaries lateral to

the myotomes. Opposite the occipital myotomes the capillary plexus drains, by a series of veins on the one hand into the main vein of the medulla, on the other hand into the anterior cardinal vein. The history of the neural branches of this vein of the medulla involves the entire subject of the circulation of the medulla. The relation of the branches from the occipital myotomes involves the subject of the development of the external jugular vein and its branches. The main stem of the vein was shown by Mall, in 1905, to become a part of the great transverse sinus. For this vein I am using the term *primitive posterior cerebral vein*. It might also be termed the primitive vein of the medulla.

The stage of plate 7 shows the beginning of the veins of the hindbrain. It will be seen that the primitive branches of the primary head-vein draining the hindbrain are greatly modified by the ganglia of the hindbrain and the otic capsule. Opposite the midbrain these veins are regular and nearly equidistant; opposite the hindbrain they are grouped according to the ganglia. Of these veins of the hindbrain, the group caudal to the Gasserian ganglion and the stem of the posterior cerebral vein bear the most important relations to the future cerebral sinuses at the base of the brain.

In this account of the early blood-vessels of the neural tube three facts have been brought out which are essential to an understanding of the development of the neural vessels. First, there forms a ventral neural artery, originally paired, which extends along the ventral surface of the entire neural tube from the base of the optic cup to the caudal end of the spinal cord, which is an anastomosis of all the direct neural arteries from the aorta; second, this artery leads to a capillary plexus which completely invests the neural tube and all its ganglia; third, the primary veins of the neural tube are all transverse vessels superficial to this primary plexus, and they gradually extend toward the mid-dorsal line and are profoundly modified by the ganglia, both cerebral and spinal. All of the veins of the brain drain into the primary head-vein. As has been shown by Mall and Streeter, the only segment of the vena capitis prima which remains as a part of the dural sinuses becomes the cavernous sinus, which is that portion of the primary head-vein medial to the Gasserian ganglion. All other dural sinuses develop from the branches of the vena capitis prima.

It has been shown that the middle segment of the vena capitis prima develops in the pig, as in the chick, as a chain of capillaries between the aortic arches and the anterior cardinal vein; it becomes very large, because it makes a more direct outlet for the primitive cerebral vein. The vena capitis prima develops from three segments and is the first true vein for the head; the primitive vessel of the hindbrain serves temporarily as a vein for the brain and then gives rise to the capillary plexus of the upper part of the hindbrain.

CARDINAL VEINS IN THE PIG.

It was shown in the chick that the cardinal veins begin from dorsal diverticula of the aorta which project into the interspaces and dilate just opposite the dorsal border of the nephrotome. In the line of the nephrotome these separate dilatations become connected, making a common cardinal vein which, at the stage of 12 somites, is opposite every interspace. I have not the corresponding early stages of the cardinal veins in the pig. In my earliest stage in the pig, the cardinal veins are related to the aorta and to the spinal veins, as is shown for the chick in the section on plate 3, figure 2; that is, there are direct spinal arteries from the aorta to the cord and spinal veins leading to the cardinal vein. At the stage of plate 4, figure 3, the posterior cardinal vein is injected, extending from the zone of the ninth intersegmental artery almost to the duct of Cuvier. The anterior cardinal vein is not injected, but must be present in the specimen. The pig embryo shown in figure 1, plate 1, gives the best view of the cardinal veins in my series. In this specimen it is clear that the anterior cardinal vein joins the neural plexus cephalic to the first somite, so that the vein of the first interspace which was so important in the chick is like all of the rest of the intersegmental veins in the pig. Opposite the first nine somites in the pig, as shown in plate 1, figure 1, the cardinal veins appear to be an accompanying vein to the aorta. Just below the ninth intersegmental artery in the pig there are the lateral arteries to the nephrotomes, and over all of the rest of the course of the posterior cardinal veins the lateral cardinal vein must also be considered. Opposite the first nine somites I have not been able to find any direct connections between the cardinal veins and the aorta, such as were shown for the earlier stages in the chick. In other words, the cardinal veins are well formed rather than just beginning in all of my specimens. One embryo, of the same litter as the one in plate 1, figure 1, showed some tiny sprouts of the anterior cardinal vein opposite the second somite extending toward the aorta; sections, however, did not demonstrate any connections, and I could not prove that they were not the beginning of tiny veins that soon drain the pharynx.

The series of the pig embryos also does not show the origin of the duct of Cuvier, but the fact that it is made up of an extensive plexus is well shown in plate 1, figure 1, as well as its relation to the umbilical veins. Below the zone of the ninth somite the cardinal veins will be considered with relation to the vessels of the pronephros.

NEPHRITIC VESSELS IN THE PIG.

The nephritic tubules in the pig receive an early and characteristic blood-supply. For the limit for the chick between the pronephros and the mesonephros I have followed Lilly, who regards the tubules as belonging to the pronephros down to the fifteenth or sixteenth somite (page 190). For the pig I have arbitrarily followed Felix's estimation for the human embryo (1912, page 762). He places the limit of the pronephros at the fourteenth somite. It will be seen in figure 3, plate 4, that just below the ninth intersegmental artery a series of lateral

arteries gives rise to a plexus which is ventral to the posterior cardinal vein, but which connects with it. The Wolffian duct intervenes between this plexus and the posterior cardinal vein. In figure 1 of plate 5, and figure 1 of plate 1, are given very characteristic views of these lateral arteries to the pronephros, and in figure 5, plate 3, is shown a ventral view of the arteries of the pronephros from a specimen of the same litter as that of plate 5, figure 1, but a little farther developed.

Figures 1 of plate 5 and 1 of plate 1 show a series of tiny lateral arteries beginning just below the ninth dorsal segmental artery. These arteries are about four to a somite, corresponding to the number of the nephritic tubules, and are connected by a tiny longitudinal artery close to the aorta and by a tiny lateral vein. In text-figure 4 is shown a section from a specimen of the litter of plate 1, figure 1, passing through about the fourteenth somite, showing an injection of one of these lateral arteries. Its exact position with reference to the developing tubule is, I think, important. This is most clearly recognized from the diagram given by Felix of the development of the nephritic tubules (fig. 561, Keibel and Mall, *Manual of Human Embryology*, page 804). The stage corresponds with diagram *d* of Felix's figure, and the artery passes directly across the curved bowl which makes the neck of the future Malpighian corpuscle. This is the earliest stage of the vessels of the nephritic tubules I have injected. As is seen in text-figure 4, the lateral vein, the vena cardinalis lateralis, lies ventral to the Wolffian duct, while the vena cardinalis posterior lies directly dorsal to the duct. The posterior cardinal vein is plainly shown in text-figure 4, but was not injected so far caudalward in any of my series.

Text-figure 5 gives a very interesting section from the same series as text-figure 4. The level of the section is shown in plate 1, figure 1; it is about halfway between the level of the lowest transverse artery injected and the allantoic arteries. At this level the nephritic tubule is in the stage of Felix's figure 561*b*, consisting of a Wolffian duct and a mass of nephrogenic epithelium. Here, instead of an artery which can be injected, the section shows a chain of angioblasts running ventral to the nephritic tissue to the lateral cardinal vein, and other sections show similar chains of angioblasts connecting the aorta and the posterior cardinal vein.

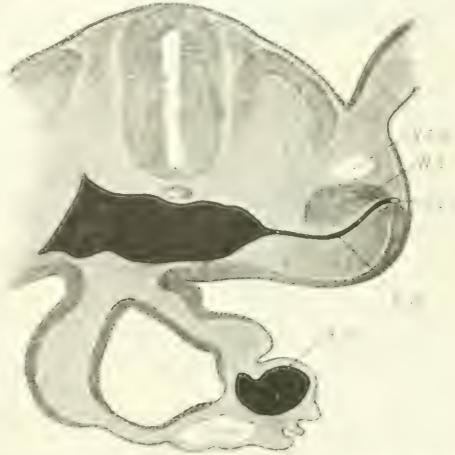


FIG. 4.—Transverse section of an embryo pig of 23 somites, passing through about the lateral interspace of the pronephros. The section is from a specimen of the same litter as the one shown on plate 1, figure 1, and from the same series as figures 5 and 6. The level of the section is shown by a line on plate 1, figure 1. The section is 20 μ thick and is stained with hematoxylin and counterstained with orange G, eosin, and aurantia. $\times 115$. *A. om.*, a. omphalo-mesenterica; *A. pr.*, a. of the pronephros which was injected from the aorta; *V. om.*, v. omphalo-mesenterica; *V. c. l.*, v. cardinalis lateralis; *V. c. p.*, v. cardinalis posterior; *W. d.*, Wolffian duct.

These angioblasts are mesial to the nephritic tissue. This section is, I think, similar to the section in Evans's figure 416 from a human embryo of the same stage, namely, with 23 somites, which shows the posterior cardinal vein dorsal to the Wolffian duct and the lateral cardinal vein ventral to the duct.

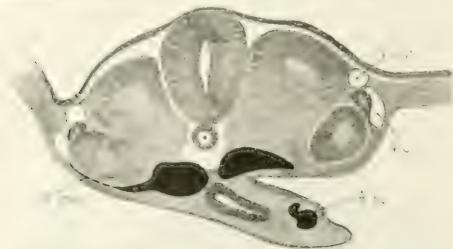


FIG. 5.—Transverse section of an embryo pig of 23 somites, passing through one of the lower myotomes and the mesonephros, to show the position of the subintestinal artery and a chain of angioblasts which will form an artery of the mesonephros. The section is from a specimen of the same litter as the one shown on plate 1, figure 1, and from the same series as figures 4 and 6. The level of the section is shown by a line on plate 1, figure 1. The section is $20\ \mu$ thick and is stained with hematoxylin and counterstained with orange G, eosin, and aurantia. $\times 115$. *A. mes.*, a chain of angioblasts which connect the aorta with the v. cardinalis lateralis and which will form an artery of the mesencephalon but were not injected because they are still solid; *A. si.*, a. subintestinalis; *V. c. l.*, v. cardinalis lateralis; *V. c. p.*, v. cardinalis posterior.

of text-figures 4 and 5), seems to me to indicate that the posterior and lateral cardinal vessels extend caudalward in connection with chains of angioblasts from the aorta which pass dorsal and ventral to the nephritic tubules in lines which are very plain in figure 5.

In figure 5 of plate 3 is shown a ventral view of the pronephritic vessels in a pig of 20 somites, in which it is clear that there is a tendency toward a grouping of the transverse arteries of the pronephritic tubules around segmental lateral arteries. For example, between the ninth and tenth spinal arteries there is one lateral artery giving off four branches; between the tenth and eleventh spinal arteries are two lateral arteries from the aorta, with three transverse branches.

The longitudinal artery shown in plate 3, figure 5, persists for some time in the pig and connects the glomerular arteries even after the arterial tufts of the glomeruli are well formed. As seen in plate 3, figure 5, the transverse arteries lead directly to a lateral vein, which in turn connects with the posterior cardinal vein. Moreover, as is shown opposite the tenth somite, the posterior cardinal vein has many direct connections with the aorta.

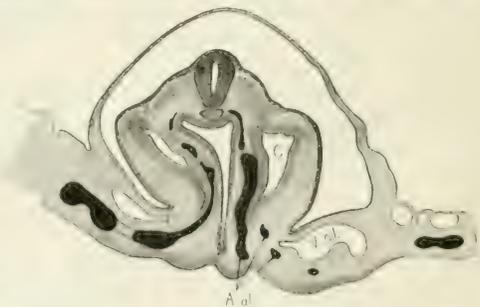


FIG. 6.—Transverse section of an embryo pig of 23 somites, passing through the allantoic arteries to show that the primitive allantoic arteries are in the splanchnopleure. The section is from a specimen of the same litter as the one shown on plate 1, figure 1, and from the same series as figures 4 and 5. The level of the figure is shown by a line on plate 1, figure 1. The section is $20\ \mu$ thick and is stained with hematoxylin and counterstained with orange G, eosin, and aurantia. $\times 53$. *A. al.*, artery of the allantois; *C.*, celom; *V. al.*, vein of the allantois in the zone where the splanchnopleure, the somatopleure, and the amnion are fused.

The next stage in the development of the circulation of the Wolffian bodies is the formation of the mesial cardinal vein. I have illustrated the position of this vein in two sections, one from an injected pig embryo of 30 somites, measuring 7 mm. before the caudal flexure has formed, and the other from an injected chick of 69 hours' incubation (text-figs. 7 and 8). The mesial cardinal vein lies ventral to the nephritic arteries, close to the aorta, in the angle between the root of the mesentery and the Wolffian ridge. The course of the mesial cardinal vein can be readily imagined in plate 3, figure 5, wherein it is noted that opposite the tenth and eleventh somites the posterior cardinal vein is in the form of a plexus, dorsal



FIG. 7.—Transverse section of an injected embryo pig of 30 somites, to show a typical cross-section of the vessels of the pronephros of the pig after the v. cardinalis mesialis has formed—that is, to show the pronephros with a central artery and three peripheral veins. The embryo measured 7 mm. after fixation and dehydration; it had no caudal flexure and was a little farther developed than the one on plate 5, figure 2. All the vessels shown were injected. The arteries are represented in black, the veins in white. The section is 50μ thick and is unstained. $\times 53$. A, *pr.*, artery of the pronephros which gives off capillaries to the tubules and extends to the v. cardinalis lateralis; V, *c. l.*, v. cardinalis lateralis; V, *c. m.*, v. cardinalis mesialis; V, *c. p.*, v. cardinalis posterior; W, *d.*, Wolffian duct.



FIG. 8.—Transverse section of an injected chick of 35 somites, after 69 hours of incubation, passing through the fifteenth somite. The section shows a typical cross-section of the vessels of the pronephros in the chick after the v. cardinalis mesialis has formed—that is, it shows the pronephros with a central artery and three peripheral veins. All the vessels were injected. The aorta is shown with a black rim, the artery is black, and the veins are white. The section is 50μ thick and is unstained. $\times 53$. A, *p.*, artery of the pronephros; V, *c. l.*, v. cardinalis lateralis; V, *c. m.*, v. cardinalis mesialis; V, *c. p.*, v. cardinalis posterior; W, *d.*, Wolffian duct.

to the nephritic tubules (text-fig. 7). At the stage of 30 somites a vein from this plexus passes ventral to the nephritic artery opposite the eleventh somite and grows caudalward just ventral to the nephritic arteries, between the aorta and the longitudinal artery of plate 3, figure 5. This is the medial cardinal vein, the subcardinal vein of F. T. Lewis. There is thus formed the primitive pattern of the circulation of the Wolffian body, as shown in text-figures 7 and 8, consisting of a central artery and three longitudinal superficial veins—the posterior cardinal vein dorsal to the Wolffian duct, the lateral cardinal vein just ventral to the duct, and finally the mesial cardinal vein near the root of the mesentery. The mesial cardinal forms the connection with the vessels of the liver and—as shown by Hochstetter—also anastomoses with branches of the omphalo-mesenteric vein along the mesentery.

I emphasize the lateral cardinal vein because it has not been adequately recognized in the literature. In the pig it is very obvious in total preparations.

such as are shown in plate 3, figure 5. It develops early and is very straight. In the chick it is not straight and therefore is much less striking in total preparations. Its primary connections with the posterior cardinal vein are lateral to the Wolffian duct, as seen in text-figure 5 for the pig. That this is also true for the chick is shown by Graefe's figure 6 (1906), which shows the pronephros of the chick at the stage of 2 days and 15 hours. Later, in both the pig and chick, these two veins are connected by branches which are mesial to the duct, as shown in text-figures 7 and 8.

The failure to take into account the lateral cardinal veins has led to some confusion in the literature; for example, in the study of the pronephros, Graefe (in his figure 11) has labeled the lateral vein close to the Wolffian duct the subcardinal, while in figure 13 he has labeled the true subcardinal vein ventral to the nephritic artery the subcardinal, but has not labeled the lateral vein at all, though it is shown in the section.

In Keibel and Mall's Embryology, Felix gives some extremely interesting sections from the R. Meyer human embryo No. 300. This embryo had 23 somites and was 2.5 mm. long. It is to be compared with my plate 1, figure 1. In figure 532*a* Felix shows solid angioblasts, both dorsal and ventral to the Wolffian duct; he does not label the dorsal angioblasts which represent the posterior cardinal vein, but on the other hand calls the ventral angioblasts the posterior cardinal vein. Again, in figure 559 he calls angioblasts which are ventral to the duct the posterior cardinal vein. These sections show that in the human embryo there are angioblasts both dorsal and ventral to the duct and bring out the value of the two names for the veins, the posterior and lateral cardinal veins. They also show that the posterior and lateral cardinal veins extend as solid angioblasts and so bring up the question as to whether these veins may not differentiate as chains of angioblasts connected with the aorta by chains of angioblasts.

CONCLUSION.

In this study it seems clear to me that the chick affords very valuable material for the study of the most fundamental point in connection with the vascular system that is still at issue, namely, how long in the life of the embryo do new angioblasts continue to differentiate from mesenchyme and join the blood-vessels? The answer to this question involves more extensive observations on the living blastoderm than I have yet made. It has been shown that blood-vessels first arise not only in the membranes but also in the embryo by a differentiation of cells into angioblasts, by the process which His had described, and not from a dilatation of spaces in the mesenchyme and a flattening-out of cells to form their border.

It has been proved that the aorta at least in part differentiates *in situ*. Evidence has been given that a part at least of the neural vessels and their connections with the aorta differentiate *in situ*. On the other hand, the cardinal veins begin as a growth from the wall of the aorta. They are a longitudinal anastomosis between direct branches of the aorta. A more detailed study of the later stages of the cardinal veins is necessary to determine if any part of them differentiates *in situ*.

I think that it is important to emphasize the extent of the development of the blood-vessels both of the membranes and of the embryo at the time when the circulation begins. This has been done for the chick, and it would be of great value to obtain the same observations for the mammal.

This study gives a more complete account of the primitive vessel of the hind-brain than is to be found in the literature. I have followed its origin, its relations, and its fate. The fate of this vessel is a very important point. This primitive vessel of the hindbrain differentiates early, opposite the first part of the neural tube to develop. It has been shown why it remains so long a single channel, namely, because it serves temporarily as a vein for the forebrain and midbrain before it takes the characteristic form of a plexus like the other early vessels on the surface of the neural tube. As the vena capitis prima becomes complete, so that the blood of the forebrain and midbrain is shunted out of the primitive channel of the hindbrain, this channel receives new arterial connections and breaks down into the very important capillary plexus of the rhombencephalon.

It has been shown that the first true vein of the head, the vena capitis prima, as contrasted with veins which drain only the brain, develops in three segments. The anterior segment is a purely cerebral vein which drains the forebrain and midbrain and originally empties into the primitive vessel of the hindbrain; the posterior segment is the anterior cardinal vein; the middle segment develops last, as a capillary chain between the capillaries of the maxillary, the mandibular and the other visceral arches, and the anterior cardinal vein. This middle segment anastomoses with the primitive cerebral vein from the forebrain and midbrain and forms a much more direct and favorable channel for draining the brain, and so rapidly supplants the more indirect channel along the hindbrain. It drains the other structures of the head in addition to the neural tube. The embryonic vein extending from the region of the thalamus to the duct of Cuvier is the first true vein of the head, in the sense of draining the entire head, that is, the brain and the visceral arches, and may thus be termed the vena capitis prima.

In connection with the vascular system of the nervous system, it has been shown that the early pattern of the blood-vessels is very uniform for the entire tube. There is a capillary plexus which completely invests the tube and all of its ganglia. It is fed by bilateral longitudinal arteries, which form as an anastomosis between all of the neural arteries from the aorta and extends from the carotid arteries at the base of the optic stalk to the tip of the spinal cord. The bilateral character of these arteries persists only around the subthalamus, where the circle of Willis is formed; elsewhere the two arteries become a single ventral artery—the basilar artery and its primary continuation, the anterior spinal artery. I have thus brought out the origin and the significance of the basilar and anterior spinal arteries and have shown that they precede the vertebral arteries.

The first neural veins are all transverse superficial vessels, which tap the deep plexus and gradually extend dorsalward on the deep plexus. They are profoundly modified by the eye, the ear, and by all the sensory ganglia. Opposite the brain they all drain into the primary head-vein; all the rest of the neural veins

are intersegmental branches of the cardinal veins. It is thus clear that the general direction of the blood to the neural tube is from the ventral to the dorsal border and that the direction of the flow of blood from the neural tube is the reverse.

In connection with the pig it has been shown that the large branches of the aorta near the caudal end of the embryo are primary allantoic arteries which run in the splanchnopleure, and that the umbilical arteries in the somatopleure develop later and anastomose with the primary allantoic arteries, exactly as in the chick. I have also given an analysis of the subintestinal vein of the chick and of the corresponding artery in the pig, and have shown that the fact that the vessel is an artery in the pig means that the primitive type of circulation of the yolk-sac persists longer in that form than in the chick. It has been shown that both the primitive allantoic arteries and the subintestinal arteries arise in a capillary plexus and end in a capillary plexus, so that in the case of these two vessels the blood must pass through two capillary plexuses in its return to the heart.

The study of the circulation of early embryos by means of injecting living embryos and watching the flow of the ink in them or by watching the circulation of the blood in the living specimen brings out some remarkable changes in the direction of the circulation; for example, the change in the direction of the circulation in the vessels of the area vasculosa in the chick when the veins invade a plexus which had been arterial. Again, in connection with the development of the primitive vessel of the hindbrain into a capillary plexus, the direction of the circulation is entirely changed. In the original vessel the blood flowed from the cephalic to the caudal border of the hindbrain, while when the new arterial connections bring blood to the entire ventral border of the vein the blood begins to flow from the ventral to the dorsal border of the hindbrain. In the case of the subintestinal artery is a third example of a profound change in the direction of the circulation. The blood originally runs through this artery out to the yolk-sac, but when the vessel becomes a capillary plexus in the wall of the gut, the blood flows toward the heart within the embryo in the new mesenteric veins.

From these studies it is clear that it is important to consider each vessel of the embryo from the standpoint of the function it performs throughout its development and that the effort toward a precise usage of the terms *artery*, *capillary plexus*, and especially of the term *vein*, is an effort to understand the circulation of the embryo.

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EXPLANATION OF PLATES.

PLATE 1.

1. Injection of the vascular system of an embryo pig of 23 somites, which measured 7 mm. when fresh and 6.2 mm. after fixation and dehydration. The injection is nearly complete and shows especially the primitive relations of the vasa primitiva rhombencephali at the stage when it serves as a vein for the forebrain and midbrain and as a capillary plexus for the hindbrain. $\times 38$. *A. l.*, artery of the first interspace; *al.*, allantois; *a. si.*, a. subintestinalis; *b. c.*, bulbus cordis; *l.*, liver; *pl.*, plexus on the spinal cord; *s. v.*, sinus venosus; *t. a.*, truncus arteriosus; *v. c. a.*, v. cardinalis anterior; *v. c. l.*, v. cardinalis lateralis; *v. c. p.*, v. cardinalis posterior; *v. u.*, v. umbilicalis; *va. p. r.*, vasa primitiva rhombencephali; *ven. c.*, ventriculus cordis.
2. Partial injection of the vessels of a chick of 12 somites. The needle was introduced into one of the omphalo-mesenteric veins near the heart. The transverse lines show the position of the interspaces. The sections shown in text-figures 1, 2, and 3 are from a chick of the same stage which was completely injected. $\times 54$. *Me.*, mesencephalon at the level of the section shown in figure 1; *v. om.*, v. omphalo-mesenterica.
3. Injection of the heart and the cephalic aorta, both dorsal and ventral, in a chick of 9 somites. The needle was introduced into the dorsal aorta opposite the somites. $\times 94$. *Ao. d. c.*, aorta dorsalis cephalica; *ao. v. c.*, aorta ventralis cephalica; *h.*, heart; *me.*, mesencephalon; *v. om.*, v. omphalo-mesenterica.

PLATE 2.

1. Partial injection of the vessels of a chick of 14 somites. The needle was introduced into the dorsal aorta opposite the somites. The vascular plexus on the mesencephalon is not injected, though it is present at this stage, as is shown in text-figure 1. $\times 100$. *A. so.*, artery of the somatopleure; *d. C.*, duct of Cuvier just before it has connected with the omphalo-mesenteric vein; *me.*, mesencephalon; *v. c.*, v. cardinalis communis, that is, before it has an anterior and a posterior division; *v. so.*, vein of the somatopleure; *v. l.*, v. transversa of the first interspace; *va. p. r.*, vasa primitiva rhombencephali.
2. Injection of the blood-vessels of a chick of 16 somites, to show the relation of the primitive vessel of the rhombencephalon to the primitive cerebral vein, on the one hand, and to the anterior cardinal vein, on the other. This is the stage before the vena capitis prima is completed. $\times 58$. *D. C.*, ductus Cuvieri; *v. c. a.*, v. cardinalis anterior; *v. c. p.*, v. cardinalis posterior; *v. ce. p.*, v. cerebralis primitiva, which will become the cephalic division of the v. capitis prima; *v. l.*, v. transversa of the first interspace; *va. p. r.*, vasa primitiva rhombencephali.
3. Transverse section of an injected chick of 30 somites, after 52½ hours of incubation, passing through the twenty-first interspace. The section is from the same series as figure 4 on the same plate, figures 2 and 3 on plate 3, and figure 2 on plate 4. Figure 4 on plate 3 is from another series. This section is to show the beginning of the spinal arteries as they show in the tenth to the seventeenth interspaces on plate 3, figure 1. The section is below the level of the omphalo-mesenteric arteries. It is 50 μ thick and is unstained. $\times 140$. *Ao.*, aorta; *v. c. p.*, v. cardinalis posterior; *v. om. p.*, v. omphalo-mesenterica posterior; *W. d.*, Wolffian duct.
4. Transverse section of an injected chick of 30 somites, after 52½ hours of incubation, passing through the twenty-seventh interspace. The section is to show the relative position of the direct arteries to the posterior cardinal vein and the arteries of the somatopleure. The section is below the level of the omphalo-mesenteric arteries and is in the region of the posterior limb-bud. It is 50 μ thick and is unstained. $\times 140$. *Ao.*, aorta; *v. c. p.*, v. cardinalis posterior; *v. om. p.*, v. omphalo-mesenterica posterior; *W. d.*, Wolffian duct.

PLATE 3.

1. The cardinal veins from an injected chick of 25 somites, to show the method of origin of the spinal arteries. $\times 106$. *A. 3* and *a. 18*, arteries of the third and eighteenth interspaces; *d. C.*, ductus Cuvieri; *pl.*, plexus on the spinal cord; *v. c. a.*, v. cardinalis anterior; *v. c. p.*, v. cardinalis posterior; *v. l.*, v. transversa of the first interspace; *va. p. r.*, vasa primitiva rhombencephali.
2. Transverse section of an injected chick of 30 somites, after 52½ hours of incubation, passing through the fifteenth interspace. The section is to show a spinal artery like that of the seventh interspace of plate 3, figure 1; it is above the level of the omphalo-mesenteric arteries and shows the posterior omphalo-mesenteric veins on either side. The section is 50 μ thick and is unstained. $\times 140$. *Ao.*, aorta; *v. c. p.*, v. cardinalis posterior; *v. om. p.*, v. omphalo-mesenterica posterior; *W. d.*, Wolffian duct.
3. Transverse section of an injected chick of 30 somites, after 52½ hours of incubation, passing through the sixteenth somite. This is the next section in the series below that of plate 3, figure 2. It shows a direct dorso-lateral artery to the posterior cardinal vein. The section is 50 μ thick and is unstained. $\times 140$. *Ao.*, aorta; *v. c. p.*, v. cardinalis posterior; *v. om. p.*, v. omphalo-mesenterica posterior; *W. d.*, Wolffian duct.

PLATE 3—Continued.

4. Transverse section of an injected chick of 25 somites after 52 hours of incubation, passing through the seventeenth interspace. The section is to show the transition between the stage of figure 3 of plate 2 and figure 2 of plate 3, in the formation of a spinal artery. It is 50μ thick and is unstained. $\times 140$. *Ao.*, aorta; *v. c. p.*, v. cardinalis posterior; *W. d.*, Wolffian duct.
5. Injection of the aorta, the arteries of the pronephros, and the lateral and posterior cardinal veins in an embryo pig of 20 somites, from a specimen of the same litter as the one on plate 5, figure 1. The specimen is shown for the ventral aspect. $\times 140$. *A. 9* to *A. 12*, arteries to the spinal cord in the ninth to the twelfth interspaces; *ao.*, aorta; *v. c. l.*, v. cardinalis lateralis; *v. c. p.*, v. cardinalis posterior.

PLATE 4.

1. Injection of the vessels of the head of an embryo pig of 27 somites, measuring 7.1 mm. after fixation and dehydration. The specimen is from the same litter as the one on plate 5, figure 2, and is to show the completion of the vena capitis prima and its relation to the vasa primitiva rhombencephali. It shows that the primitive vessel of the hindbrain does not atrophy when the vena capitis prima is completed, but rather develops into a plexus on the hindbrain. $\times 94$. *At.*, atrium; *b. c.*, bulbus cordis; *f. H.*, fretum Halleri; *t. a.*, truncus arteriosus; *v. cap. p. 1*, v. capitis prima, first or cerebral segment, which drains the forebrain and midbrain; *v. cap. p. 2*, v. capitis prima, second segment, which drains the forebrain, the midbrain, and the visceral arches; *v. cap. p. 3*, v. capitis prima, third segment, which is the anterior cardinal vein; *va. p. r.*, vasa primitiva rhombencephali; *ven. c.*, ventriculus cordis; *ves. a.*, vesicula auditiva; *V*, position of the root of the n. trigeminus; *VIII*, position of the roots of the nn. cochlearis et vestibularis; *IX*, position of the root of the n. glossopharyngeus.
2. Transverse section of an injected chick of 30 somites, after 52½ hours of incubation, passing through the twenty-fifth interspace. The section is to show the diverticula of the dorsal aorta like those of the first and second interspaces in plate 1, figure 2, which give rise to the cardinal veins. The section is 50μ thick and is unstained. $\times 140$. *Ao.*, aorta; *W. d.*, Wolffian duct.
3. Partial injection of the vessels of an embryo pig of 14 somites, measuring 4 mm. after fixation and dehydration. The specimen was injected through the dorsal aorta opposite the somites. $\times 56$. *A. 9*, artery to the spinal cord in the ninth interspace; *at.*, atrium; *b. c.*, bulbus cordis; *f. H.*, fretum Halleri; *s. 1*, first somite; *s. v.*, sinus venosus; *t. a.*, truncus arteriosus; *v. c. p.*, v. cardinalis posterior; *v. om.*, v. omphalo-mesenterica; *ven. c.*, ventriculus cordis; *ves. a.*, vesicula auditiva.

PLATE 5.

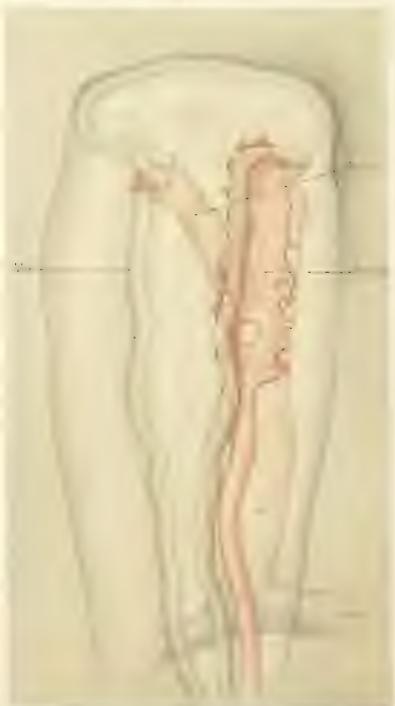
1. Partial injection of the vessels of an embryo pig of 20 somites, measuring 6 mm. after fixation and dehydration. It shows the omphalo-mesenteric arteries, the subintestinal artery and the arteries of the pronephros; $\times 41$. *A. 9*, artery to the spinal cord in the ninth interspace; *a. om.*, a. omphalo-mesenterica; *a. si.*, a. subintestinalis; *al.*, allantois; *b. c.*, bulbus cordis; *s. al.*, stalk of the allantois; *t. a.*, truncus arteriosus; *v. c. l.*, cardinalis lateralis; *ven. c.*, ventriculus cordis.
2. Partial injection of the vessels of an embryo pig of 27 somites, measuring 7.1 mm. after fixation and dehydration. It shows the general development of the vascular system at the stage when the vena capitis prima is completed. The vessels opposite the hindbrain, both deep and superficial, are extravasated in this embryo and hence they are shown on plate 4, figure 1, from an embryo of the same litter. $\times 51$. *A. om. d.*, a. omphalo-mesenterica dextra, the other two omphalo-mesenteric arteries in the figure being on the left side; *at.*, atrium; *b. c.*, bulbus cordis; *d. C.*, ductus Cuvieri; *l.*, liver; *t. a.*, truncus arteriosus; *v. cap. p. 1*, v. capitis prima, first or cerebral segment, which drains the forebrain and midbrain; *v. cap. p. 3*, v. capitis prima, third segment, which is the anterior cardinal vein; *v. om.*, v. omphalo-mesenterica; *v. u.*, v. umbilicalis; *ven. c.*, ventriculus cordis; *r.*, extravasation involving both the vasa primitiva rhombencephali and the vena capitis prima, as can be seen on plate 4, figure 1.

PLATE 6.

Injection of the vessels of the head of a chick of 29 somites, to show the origin of the vena capitis prima. The vein extends from the region of the dienecephalon to the duct of Cuvier. The injection shows that the vein arises in three segments; the first segment is a true primitive cerebral vein, which drains the forebrain and will soon drain the midbrain; the second segment is an anastomosis between the maxillary, the mandibular, and the other visceral arches and the anterior cardinal vein, and it drains the forebrain, the midbrain, and the visceral arches; the third segment is the anterior cardinal vein, which drains the brain and the visceral arches. $\times 128$. *A. b.*, artery on the rhombencephalon, which at this stage is bilateral and is part of a plexus which will give rise to the basilar artery; *a. 3*, artery to the medulla in the third interspace; *d. C.*, ductus Cuvieri; *v. c. p.*, v. cardinalis posterior; *v. cap. p. 1*, v. capitis prima, first or cerebral segment which drain the forebrain and midbrain; *v. cap. p. 2*, v. capitis prima, second segment which drains the forebrain, the midbrain, and the visceral arches; *v. cap. p. 3*, v. capitis prima, third segment, which is the anterior cardinal vein; *r. m. p.*, v. maxillaris primitiva; *v. om.*, v. omphalo-mesenterica; *v. l.*, v. transversa of the first interspace; *v. u.*, plexus in which the v. umbilicalis will arise; *va. p. r.*, vasa primitiva rhombencephali; *ves. a.*, vesicula auditiva; *V*, position of the root of the n. trigeminus; *VIII*, position of the roots of the nn. cochlearis et vestibularis.

PLATE 7.

Injection of the vessels of the brain of an embryo pig measuring 6.5 mm. in length after fixation and dehydration. The injection is a complete one, but the vessels of the visceral arches and most of the vessels of the cerebrum have been omitted in the drawing. The figure shows, first, the longitudinal artery of the central nervous system, which extends from the tip of the carotid artery to the caudal tip of the spinal cord; this artery is a plexus opposite the subthalamus, a single vessel down to the lower part of the medulla, and again a plexus on the cord; second, a part of the capillary plexus which invests the entire neural tube; third, the relation of the primitive veins of the forebrain, the midbrain, and the hindbrain to the vena capitis prima. $\times 73$. *A. b.*, a. basilaris; *a. c. 1*, a. carotis interna; *a. 1*, artery to the medulla in the first interspace; *a. m. p.*, a. maxillaris primitiva; *v. cap. p. 1*, v. capitis prima, first or cerebral segment, which drains the forebrain and midbrain; *v. cap. p. 2*, v. capitis prima, second segment, which is shown only in outline, and which drains the forebrain, the midbrain, the hindbrain, and the visceral arches; *v. cap. p. 3*, v. capitis prima, third segment, which is the anterior cardinal vein and which drains the brain and the visceral arches; *v. m. p.*, v. maxillaris primitiva; *ves. a.*, vesicula auditiva; *3, 4, and 6*, third, fourth, and sixth aortic arches, which are coming from the heart and are leading to the descending aorta, which is concealed by the cardinal segment of the vena capitis prima; *V*, position of the root of the n. trigeminus; *VIII*, position of the roots of the nn. cochlearis et vestibularis; *IX*, position of the root of the n. glosso-pharyngeus; *X*, position of the root of the n. vagus; *XII*, position of the root of the n. hypoglossus.



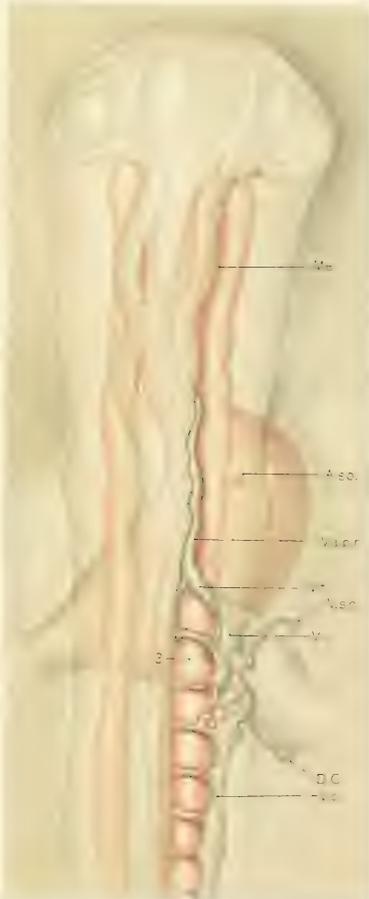


Fig. 1



Fig. 2



Fig. 3

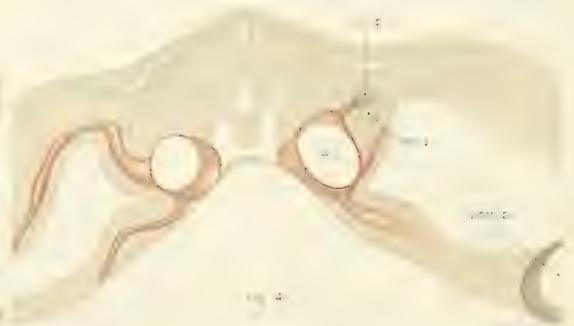


Fig. 4

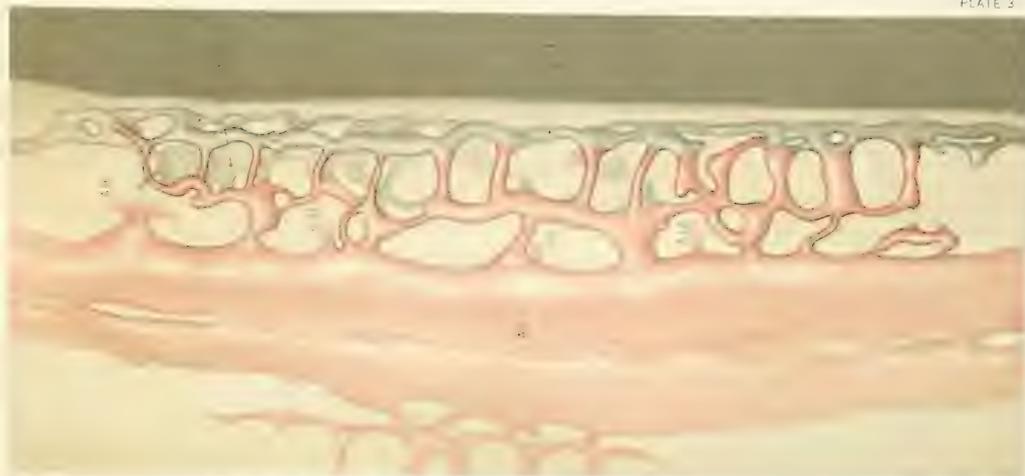


FIG. 5



FIG. 4

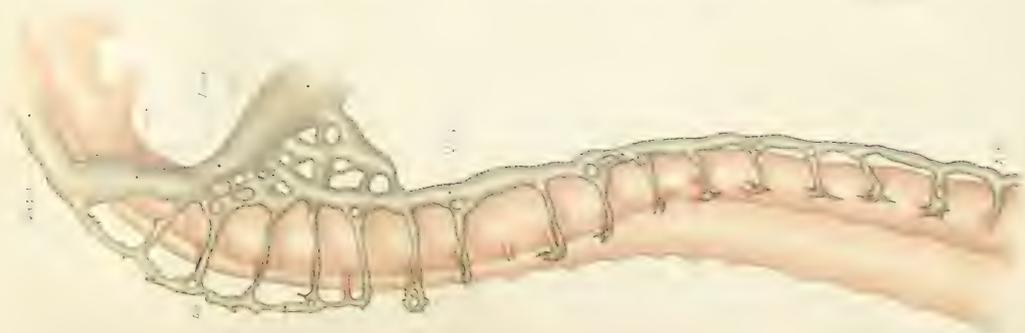


FIG. 1



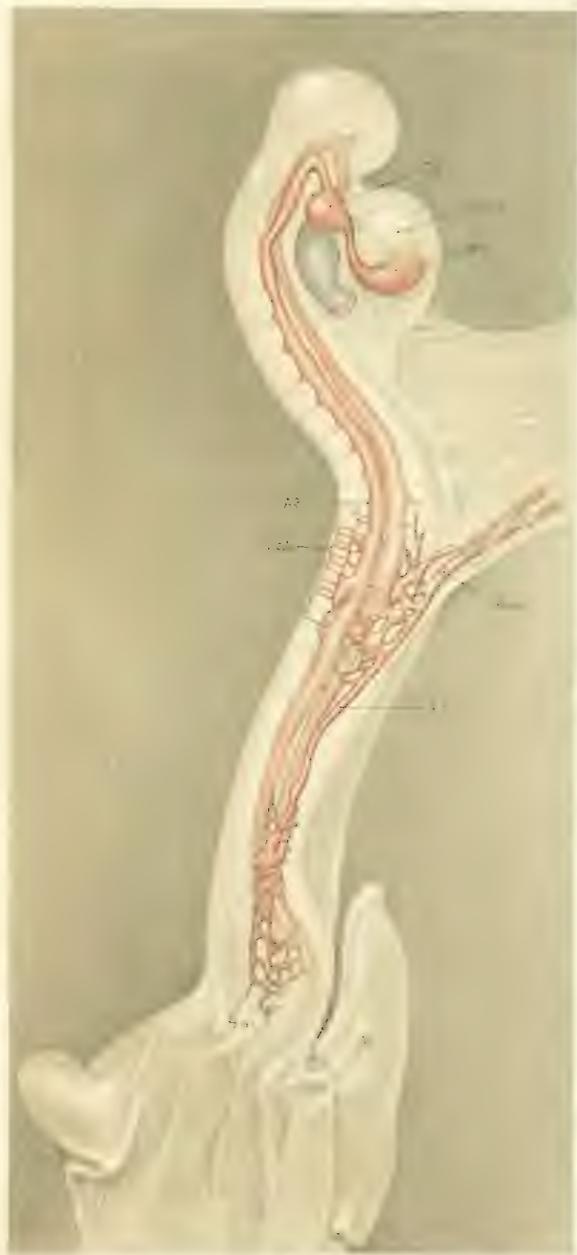


FIG. 1



FIG. 2



J. F. Didusch fecit



CONTRIBUTIONS TO EMBRYOLOGY. No. 19.

A HUMAN EMBRYO OF TWENTY-FOUR PAIRS OF SOMITES.

BY FRANKLIN PARADISE JOHNSON.

Of the University of Missouri.

Eight plates, nine text-figures.

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A HUMAN EMBRYO OF TWENTY-FOUR PAIRS OF SOMITES.

BY FRANKLIN PARADISE JOHNSON.

INTRODUCTION.

The embryo herein described was received February 9, 1914, from Dr. W. L. Allee, of Eldon, Missouri. Accompanying the specimen was a letter with the following information, but no further data regarding its history are obtainable:

The patient menstruated January 20 to January 25. Menstrual flow recommenced February 2, but it was freer and brighter in color than usual. On February 7 the specimen was aborted. Less than five minutes after abortion it was placed in 10 per cent formalin, in which fluid it was sent to me.



FIG. 1.—Drawing showing position of embryo in chorionic vesicle. Section 95 (of embryo). $\times 15$ diameters.

The chorionic vesicle appeared as an elongated rounded body, of a cream color and a very delicate texture. It measured 15 by 9 by 8 mm. Extending from end to end of the vesicle, a flat fold with a slit in it was visible. It was covered with villi everywhere except along the fold. For fear of ruining a valuable embryo no attempt was made to open the sac. The whole vesicle was run

through the graded alcohols, cleared in chloroform, and embedded in paraffin. It was cut in sections 8 microns thick and stained with alum hemotoxylin and eosin. An idea of the plane of sectioning can be obtained from text-figure 1.

The age of the specimen is uncertain from the data obtained. As determined from a model of the embryo, its greatest length is about 2.4 mm. This measurement and those of the chorionic vesicle make it correspond very closely to an embryo of 28 days, as estimated by Mall²², p. 199). If such is its age the specimen falls into that group of embryos which continue to develop in the uterus during a menstrual flow.

Comparison with descriptions of other embryos shows that my specimen is near the age of Robert Meyer's embryo No. 300, described by Thompson⁴⁵; it resembles also Janosik's specimen²⁰ and His's embryo "Lg."¹⁸. In most respects, however, it is younger than the above-named specimens and also younger than the embryos described by Mall²⁹, Gage¹², Bremer², and Tandler⁴⁴. It is apparently older than the twin specimens of 17-19 segments described by Watt⁴⁸.

A glance at the figure (plate 1, fig. 1) of the embryo is sufficient to show that there is a marked ventral bend in its back, such as has been found in many specimens of comparable age. Since this ventral flexure is not invariably present, and varies in degree, it is regarded by Keibel²² as essentially abnormal. In my embryo it occurs without rupturing the underlying structures; if an abnormality, it is the only one which seems to be present.

I am indebted to my former students, Messrs. W. L. Brosius, L. B. Hohman, H. L. Houchins, L. H. Rutledge, Florian Vaughn, and T. F. Wheeldon, for certain reconstructions which have aided me greatly in my study of the embryo. To these men and also to Mr. G. T. Kline, who has made many of the illustrations, I wish to express my sincere thanks. I wish also to express my gratitude to Professor Frederic T. Lewis, who has read my manuscript and offered many valuable criticisms.

EXTERNAL FORM.

The external form of the embryo has been studied from a wax reconstruction which was made at a magnification of 120 diameters. As already stated, the embryo was cut up without being previously drawn. It was necessary, therefore, to use the walls of the chorionic sac as guide-lines in making the model.

As seen from the left side (plate 1, fig. 1), the embryo is roughly the shape of a reversed S. Its back presents two well-developed curves. The upper of these is convex dorsally; it is large and rounded. The lower curve is convex ventrally, since the caudal portion of the embryo is bent sharply backward at about right angles to its longitudinal axis. Not only is the caudal end of the embryo bent backward upon itself, but at the point of bending it is twisted through an angle of 90° to the right. Thus the dorsal wall of this portion of the embryo is turned to the right and the ventral wall to the left; also, the caudal end of the embryo is directed to the left, so that its tip lies to the left of the axis of the body of the embryo.

The head of the embryo is large and rounded. When viewed from in front (plate 1, fig. 2), it appears somewhat egg-shaped. Toward its middle, on either side, are distinct outward bulgings, beneath which lie the optic vesicles. The mouth is a well-defined opening, limited on each side and below by the large mandibular arches.

Behind each mandibular arch there are three distinct gill-clefts. The first two of these are long and narrow and are directed obliquely to the longitudinal axis of the body, but the second bends dorsalward in its upper portion at almost right angles; the third is more shallow and rounded. The gill-clefts are bounded by four distinct arches. The first of these, the mandibular, is large and rounded, the second is similarly shaped but smaller, the third and fourth appear merely as rounded eminences.

The pericardium and heart lie just ventral to the arches. They form a large bulging which is more prominent on the right than on the left.

Beginning some little distance caudal to the last arch and placed at regular intervals throughout the remainder of the embryo, the mesodermic somites can be seen bulging through the skin. About 15 of these are perceptible from the surface, 10 in front and 5 behind the sharp flexure of the back.

The amnion is reflected from the embryo at the lower end of the pericardial cavity. The line of reflection here is curving and follows the lower curvature of the pericardial wall. At the side of the embryo, about halfway between the dorsal and ventral mid-lines, the line of reflection turns caudally, passes along the sides of the yolk-stalk, along the sides of the embryo, and finally on the body-stalk. Thus all the head, pericardial wall, and most of the caudal extremity and the dorsal portion of the remainder of the body of the embryo are within the amnionic sac.

Ventral to the line of reflection of the amnion is the yolk-sac. This is a large, irregular vesicle, broken through in places and flattened laterally. The yolk-stalk, which proceeds from the embryo just beneath the pericardial sac, is also flattened, but from above downward.

The outer surface of the hind gut, *i. e.*, its mesothelial surface, is distinctly seen turning backward to follow the curvature of the caudal extremity. At its bend there are two projections of the body-cavity, which likewise pass backward into the tail.

The body-stalk is attached to the embryo near its lower end, ventral to the caudal extremity. It turns sharply backward. The amnion is reflected from its upper surface for a short distance beyond the embryo. As a whole the body-stalk is short and broad, gradually becoming broader as the chorion is neared.

No indications of either fore or hind limb-buds can be found on the body of the embryo.

In comparing this embryo with Janosik's, it is seen that the back of the latter presents a more even curvature, which extends to the tail of the embryo. The angle formed at the top of the head (the midbrain bend) is almost identical in both specimens. The head of Janosik's embryo, however, is pointed, while in my specimen it is more rounded. In marked contrast to both of these specimens is the Robert Meyer embryo No. 300, as modeled by Thompson. Here the head

bend is very gradual, and the head itself much narrower and more pointed than in either Jamesik's specimen or mine. Although somewhat similar in appearance to His's¹⁸ embryos "Lg," "Sch," and "BB," my specimen differs from them in regard to the position of the ventral flexure of the back. In His's specimens the much-discussed bend in the back is always placed opposite the attachment of the yolk sac and stalk. In my specimen, however, the bend is placed further caudally, and the portion of the body which is bent backward is relatively shorter. The twin specimens which Watt⁴⁸ describes show definite ventral curvatures of the back, but these also are placed relatively higher up and are not as sharp as the bend in my specimen.

INTEGUMENT.

In general, it may be said that the integument of the embryo is made up of one or two layers of ectodermal cells. The thickness of this layer and the shape and size of its cells, however, vary considerably in different regions of the body.

Over the sides of the head the ectoderm is thin, being composed for the most part of two layers of flattened cells with rounded nuclei. On the dorsum and front of the head the epithelium is still thinner, there being but one layer of flattened cells. Over the optic vesicles, where the lens placodes will later develop, there is as yet no indication of thickening, but in the region of the gill-arches the epithelium is considerable thicker, its cells being either cubical or columnar in shape.

In the region of the hindbrain there is seen from the surface a minute aperture (plate 2, fig. 1). Here the integument dips in and expands to form a sac, the auditory vesicle. This is flattened laterally, and approximately triangular in external view. It is closely applied to the brain, overlying the fifth and a part of the sixth neuromeres. With the exception of its form it is quite closely in accord with the more spherical vesicle of Thompson's embryo. The walls of the auditory vesicle are much thicker than the overlying ectoderm, and exhibit two or three layers of rounded or oval nuclei. Mitotic figures in the auditory vesicle are numerous.

The integument in the region of the mouth shows no especial thickenings. A few clusters of cells (the remains of the oral plate) are attached to it; anteriorly, one such cluster is found at about the level of the cephalic end of the notochord; other clusters are found on the sides and ventral wall of the oral cavity. The epithelium of the roof of the mouth is placed in close apposition to the floor of the forebrain, being separated from it by a few strands of mesenchyma only. There is no doubt that this portion of the oral integument is destined to become the anterior lobe of the hypophysis, but as yet there is no definite differentiation of this organ.

The integument of the body-wall of the embryo overlying the pericardial cavity is very thin, suggesting a stretching-out of the epithelium. It is also thin over the dorsum of the trunk and over the mesodermic somites. The integument which overlies the body-wall in the region of the umbilical vein is somewhat thicker and its nuclei are more closely packed together. It gradually thins out again as it is reflected to form the amnion.

THE NERVOUS SYSTEM.

The nervous system is represented by the brain, with its two optic vesicles and the beginnings of the trigeminal, acustico-facial, glosso-pharyngeal, and vagus nerves, and the medullary tube with its ganglionic crest. The dorsal wall of the nervous system is placed just beneath the integument of the mid-dorsal line and conforms to all its curvatures. The cavity of the tube is entirely closed off from the outside, disregarding a longitudinal slit in the mid-dorsal line, which is clearly artificial, and in this respect differs from that of Janosik's embryo, which showed a small anterior neuropore.

THE BRAIN.

The three primary vesicles of the brain are easily recognizable in plate 2, figure 1. The prosencephalon is large and bulbous, and is marked off from the mesencephalon by a deep groove. Broad in front and in the region of the optic vesicles, it gradually becomes narrower behind. Its cephalic end is rounded and lies in close contact with the ectoderm; an actual fusion is at one place apparent, marking probably the position of the closed anterior neuropore. There is no indication of a hemispherical division. The optic vesicles are attached to this portion of the brain slightly ventral and anterior to its middle; they extend outward, backward, and slightly dorsalward. There is as yet no definite indication of a division of the prosencephalon into diencephalon and telencephalon. A slight rounded protuberance of the ventral wall behind the points of attachment of the optic vesicles probably marks the beginning of the infundibulum.

The mesencephalon is a small wedge-shaped portion of the brain-tube. Of the grooves which mark it off from the prosencephalon in front and the rhombencephalon behind, the anterior is deeper; the posterior groove is faint dorsally, but ventrally it ends in a deep notch. The mesencephalon is much narrower from side to side than the prosencephalon. Its antero-posterior dimension is only a trifle greater than those of the neuromeres of the rhombencephalon immediately behind it. In marked contrast to this is the much longer and larger mesencephalon of the Robert Meyer embryo, as modeled by Thompson⁴⁵. Somewhat similar to it, however, is that of Ingalls's embryo.

The rhombencephalon is elongated and flattened laterally. As seen from the side it is slightly curving, its dorsal wall being convex.

MEDULLARY TUBE.

The spinal part of the medullary tube extends from the rhombencephalon to the tip of the tail, gradually tapering from above downward. It is ovoid in section, the lateral walls being thicker than the dorsal and ventral walls. The dorsal wall is thinnest and lies almost in contact with the covering ectoderm.

NEUROMERES.

Both the rhombencephalon and spinal portion of the medullary tube are marked off by transverse grooves into a series of segments, the so-called "neuromeres." These begin at the cephalic end of the rhombencephalon and continue downward through the medullary tube. The first six neuromeres are narrow,

Lying next to the second neuromere is the ganglion of the trigeminal nerve; next to the fourth neuromere is the ganglion of the acustico-facial. The auditory vesicle lies opposite the fifth neuromere and partly overlaps the sixth; the latter is in process of giving off the cells of the glosso-pharyngeal ganglion. Beginning with the seventh, the remaining neuromeres are longer, being equal in length to the body segments. They do not lie within the segments themselves, however, but are arranged intersegmentally, the crest of each neuromere being placed opposite an intersegmental cleft. This arrangement begins with the first body segment and continues throughout the embryo. Above the first segment are $8\frac{1}{2}$ neuromeres.

The number of neuromeres belonging to the rhombencephalon can not be ascertained from this specimen alone. To determine this point, the author undertook a separate study of neuromeres based upon young human, pig, sheep, and cat embryos. Although this study is yet incomplete, it is evident that the last rhombic neuromeres stand out more clearly in certain specimens than in others; in some their presence is extremely doubtful. Just what this may be attributed to I am not able to state; it may be due to the stage of the specimen examined, or their presence on the one hand or absence on the other may be regarded as artificial. In those specimens which show distinctly a complete series of neuromeres, I have found that the first cervical ganglion is constantly related to the tenth neuromere. It appears evident, therefore, that the first 9 neuromeres belong to the rhombencephalon. It is to be noted that the first pair of somites in the embryo under discussion begins approximately opposite the crest of the eighth neuromere.

Of the previous description of neuromeres in young human embryos, those which appear to accord most closely with my own are the ones of Gage¹² and Watt¹⁸. In a description of an embryo of 28-29 pairs of somites, Mrs. Gage finds 9 folds or neuromeres in the rhombencephalon; of these the second is associated with the trigeminal nerve; the fourth with the auditory and facial nerves; the fifth is opposite the auditory vesicle; the sixth is in relation to the glosso-pharyngeal nerve; the seventh to the vagus; and the eighth and ninth to the accessory nerve. In regard to the spinal cord she states:

"Beyond the clearly formed folds, above discussed, there occur several others, each corresponding with an enlarged part of the ganglionic cord. As this cord has no further indication of dorsal nerve roots, the exact relations can not be determined. Moreover, the following total folds in the myel (spinal cord) are not strongly marked, and in other specimens it is only in favorable sections that they can be seen at all." (pp. 435-436.)

Watt¹⁸, in describing twin human embryos of 17-19 pairs of somites, similarly shows 9 neuromeres in the rhombencephalon. The tenth neuromere, he states, is opposite the first cervical ganglion. The results I have obtained with other mammalian embryos confirm this observation. Watt also shows spinal neuromeres extending along the medullary tube as far as the eleventh spinal ganglion.

In 1892, Minot³⁶ reviewed the earlier literature on this subject and made the general statement that "the entire medullary tube undergoes a segmentation by a series of alternating slight enlargements and constrictions." He adds:

"They appear first in the hind-brain and cervical region, and from there they appear progressively toward the fore-brain and the tail The medullary tube becomes slightly constricted between each pair of segments and slightly enlarged opposite each intersegmental space. Each intersegmental dilation is a neuromere Each neuromere produces a pair of nerves, but when the first trace of roots appears, they are seen to spring from the constriction between the neuromeres, but later from the neuromere."

Minot believes, therefore, that the so-called neuromeres of early stages represent, not true neuromeres, but the caudal and cephalic halves of two adjacent neuromeres.

An internal view of the brain is shown in plate 7, figure 5. The cavity of the prosencephalon is large and deep. Towards its anterior end the cavity of the optic vesicle is seen extending outward and backward. Posteriorly this is marked off from the forebrain cavity by a sharp indented ridge. The slight eminence of the ventral wall which will probably give rise to the infundibulum is again seen, being placed at the anterior end of the notochord. The walls of the rhombencephalon, when viewed from within, show the negative impression of the neuromeres, the "rhombic grooves" of Streeter⁴².

The question of neuromeres in mammalian embryos is still an open one. Those of the spinal cord are undoubtedly related to the metameres, *i. e.*, representing true or parts of true morphological units of the medullary tube. Similar evidence concerning the "neuromeres" of the rhombencephalon is lacking. Although the arrangement of cerebral nerves is not contradictory to this view, *i. e.*, each neuromere, with the exception of the first, receiving afferent fibers and sending out efferent fibers (Johnson)²¹, the muscles which the efferent nerves supply, the source of these muscles, and the "neuromeres," from which the efferent nerves spring, have not yet been demonstrated to coincide. In fact, the evidence, so far as gathered, contradicts this arrangement. Streeter⁴² believes that the rhombic folds are not related to the metameric system, but is inclined to the view that "they may be fitted in with and form a part of the branchiomic system."

He adds:

"The one discordant feature is groove *d* (5th neuromere), which has no corresponding branchial arch."

Neal³⁸, after extended observations and studies of head segmentation, in embryos of lower vertebrates, concludes that neuromeres offer no criterion for the determination of segmentation of the vertebrate head.

CEREBRAL NERVES.

As in the Janosik and Thompson embryos, the ganglia of the trigeminal and acustico-facial nerves are clearly present. Of the two, the trigeminal arises a little higher dorsally than the acustico-facial. Each is made up of a cluster of nuclei which are more compact and deeply staining than the mesenchymal cells

which surround them. They are connected with the wall of the brain by strands of cells: definite fibers are apparently just beginning to form. Distally, ganglion cells and fibers can be traced for some little distance, the trigeminal nerve passing toward the maxillary and mandibular arches and the acustico-facial into the dorsal portion of the second arch.

In addition to the trigeminal and acustico-facial nerves, there is found just behind the auditory vesicle and near the brain-wall a small cluster of undifferentiated cells. These are found in the region of the sixth neuromere and very probably represent the ganglion of the glosso-pharyngeal nerve. Opposite the seventh neuromere is another similar group of cells, the probable beginning of the vagus nerve.

GANGLIONIC CREST.

Indistinctly connected to the vagus ganglion and extending down beyond the ventral bend in the back of the embryo, a ganglionic crest is discernible. It is not distinct, however, for its cells so closely resemble those of other adjacent tissues that they can not always be identified with certainty. It is largely due to the position of its cells, *i. e.*, between the medullary tube and the somites, and to the arrangement of its nuclei, that the presence of the ganglionic crest can be detected.

Streeter⁴⁰ has described the neural crest of a 4 mm. embryo as follows:

"This structure (the ganglionic crest) can be seen in the 4 mm. embryo as a flattened cellular band which extends caudward from the auditory vesicle along the lateral wall of the neural tube to its extreme tip That part of the crest which corresponds to the spinal cord is characterized at this time by segmental incisures along its ventral border. The dorsal border of the crest remains intact until the appearance of the dorsal rootlets, in the meantime constituting a cellular bridge connecting the more ventral ganglionic clumps."

In my specimen the ganglionic crest likewise forms a cellular band. Segmental incisures along the ventral border are indicated in certain regions. The crest, however, is apparently not so far developed as shown by Streeter. Caudally its development is not so far advanced as toward the head. So indistinct and uncertain are its outlines, as seen in sections, that I have made no attempt to represent it graphically.

Lenhossek²⁵ has shown the ganglionic crest in a formative stage in a human embryo of 13 segments. Its cells are described as arising from the roof of the medullary tube. Such pictures as Lenhossek has shown I have been able to find in my specimen only in the caudal portion of the body, where the roof of the medullary tube is relatively thick; there, as seen in cross-section, the developing ganglionic crest appears as a cap lying on the roof of the medullary tube; it is composed of closely packed cells with nuclei of about the same size as those of the remainder of the tube.

DIGESTIVE SYSTEM.

MOUTH.

The mouth has been partly described in connection with the integument. It is directly continuous with the pharynx, the line of former separation being represented above by a few scattered clusters of cells, and below by a thin ridge of epithelium, remnants of the oral membrane. The oral cavity is broad transversely but narrow dorso-ventrally; there is no indication of the anterior lobe of the hypophysis. The scattered cells which Janosik²³ has designated as the hypophysis are undoubtedly remnants of the oral membrane.

I shall here mention His's¹⁸ description of his embryo "Lg" (2.15 mm.), in which he recognizes both Rathke's and Seessel's pockets, while the oral membrane is still intact. Concerning these he says:

"Of the two peaked recesses between which it (oral membrane) passes, the anterior becomes Rathke's pocket, while posterior becomes Seessel's pouch."

In an embryo of 2.6 mm. in Keibel and Elze's Normentafel²³, in which the pharyngeal membrane is still present, the hypophysis is "just indicated." The Robert Meyer embryo of 23 segments, according to Thompson, shows no hypophysis, although the Normentafel states its beginning is "doubtful."

FOREGUT.

PHARYNX.

The cavity of the pharynx is broader and deeper than that of the mouth. In the median plane its dorsal wall lies ventral to the notochord and follows closely the curvatures of that structure, being fused with it posteriorly (text-fig. 7). Ventrally the floor of the pharynx is more irregular. It possesses, toward its anterior end, a short, rounded diverticulum, the beginning of the thyroid gland (plate 2, figs. 2, 3, 4). This is in close relation ventrally to the ventral aorta, with which it lies in contact. Thompson describes a thyroid gland which is apparently in a similar stage of development, but Janosik and His (in his embryo Lg), fail to show this organ.

Behind the thyroid diverticulum the ventral wall of the pharynx shows two shallow depressions which cross the midline (plate 2, fig. 4). These are the cut sections of the transverse grooves (the "ventral pharyngeal grooves" of Grosser) which extend from side to side and connect the pouches of one side with those of the other. As described by Grosser¹⁵, I find that the ventral groove of the first pouch before reaching the midline divides into two limbs which surround a median elevation. This he identifies as the "tuberculum impar." It is to be noted that the thyro-glossal duct proceeds from the summit of this elevation. This relation is noted by Grosser, who describes it as follows:

"The opening of the thyroglossal duct is situated at first upon the summit of the tubercle, but later it becomes shifted into the furrow bounding the tubercle posteriorly or, according to Ingalls, in an embryo of 4.9 mm., into 'the region of the second arch, immediately aboral to the tuberculum impar.'"

In a study of two embryos of 3 mm., Hammar¹⁷ reports the tuberculum impar present in only one.

Between the ventral pharyngeal grooves of the second and third pouches is a second medial elevation in the form of a transverse ridge. From its position I believe it to be the so-called "copula" which, according to His and others, helps to form the tongue.

A third prominent elevation is found behind the ventral pharyngeal groove of the third pouch. It marks the point where the pharynx turns sharply caudalward. This probably is the area which Grosser had denoted the "cardiac swelling" and which he states goes into the formation of the larynx.

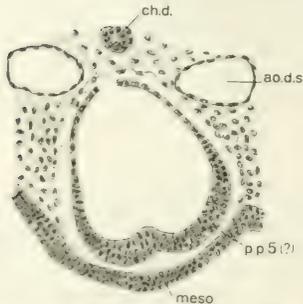


FIG. 2.—Portion of section 70. $\times 166$ diameters.

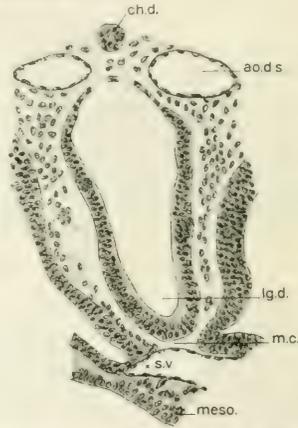


FIG. 3.—Portion of section 84. $\times 166$ diameters.

The lateral borders or wall of the pharynx extend outward into three distinct pharyngeal pouches. Of these, only the first and second reach the ectoderm. The first pair of pouches is situated only a short distance behind the oral membrane. They are flattened from before backward, the left being somewhat broader than the right. In studying the first pouch of young embryos, Grosser¹⁵ describes an invagination of its epithelium into the pharyngeal cavity as follows:

"In the region of the first pouch there projects ventrally or caudally from the closing membrane into the pharyngeal lumen an irregularly knobbed process filled with mesoderm It disappears quite early and may perhaps be interpreted as a rudimentary internal gill."

I have looked carefully in the region designated by Grosser for the structure which he describes, but have been unable to find any definite indication of it. A slight irregularity of the epithelium, however (more distinct on the left side than on the right), corresponds with it in position.

The second pouch is somewhat larger than the first and is flattened dorso-ventrally. A distinct ventral diverticulum can be seen. The third pouch is more rounded in form and ends bluntly in the mesenchyma, falling somewhat

short of reaching the ectoderm. A fourth pouch is seen extending from the ventro-lateral surface of the pharynx. It arises to a certain extent in common with the third, and shares with it the third ventral pharyngeal groove. It is slightly pointed and is directed outward, backward, and downward.

The remaining portion of the foregut, that is, that part between the fourth pharyngeal pouch and the yolk-stalk, is shown in plate 3, figures 3 and 4. It presents, above, a definite swelling which is apparent when seen in either front or side view. Looked at from in front, the swelling appears double, a median longitudinal groove separating two rather elongated protuberances. A cross-section of this region is shown in text-figure 2. A short distance below this swelling there is another which, when viewed from the side, is seen to be rather pointed. It is just dorsal to the sinus venosus (text-fig. 3). Still farther caudally is seen the hepatic diverticulum (text-fig. 5).

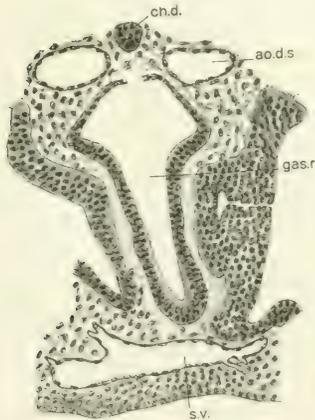


FIG. 4.—Portion of section 92. $\times 166$ diameters.

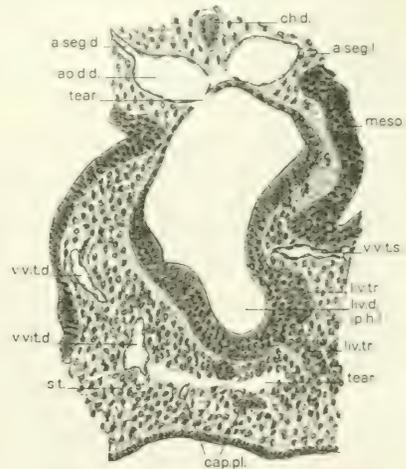


FIG. 5.—Portion of section 112. $\times 166$ diameters.

PULMONARY DIVERTICULUM.

The significance of the two upper swellings I am unable to determine definitely from this specimen alone. Thompson¹⁵ describes a somewhat similar condition in the Robert Meyer embryo of 23 segments. The upper swelling he interpreted as a bilateral pair of lung-buds; the lower as the beginning of the stomach. Grosser¹⁵, however, from a study of this region of the same embryo, concluded that Thompson's bifid swelling represents probably a fifth pair of branchial pouches, and that the lower swelling is the lung-bud.

Regarding Thompson's description he states:

"Thompson (1907) asserts that in this embryo the lungs have a paired origin, but he does not figure it, and this statement has been transferred to the *Normentafel*. But he is clearly in error as to the place where the lungs develop, as shown by his own descrip-

tion and a figure which he published later (1908). In 1908 he wrongly identified what is actually the lung-bud as the stomach, and 1907 he placed the lung-bud in the region of the diverticulum, which I have identified as a questionable fifth pharyngeal pouch. In fact, the embryo has as yet no indication of the stomach. Moreover, his model was probably made on too small a scale."

The longitudinal groove which Grosser¹⁵ describes extending from the region of the last pouch down to the lung-bud is not present in my specimen.

According to Lewis²⁸, the Bremer embryo possesses a definite pyriform lung-bud, which is directed ventrally and caudally. The Broman embryo of 4.25 mm., as figured by Grosser¹⁵, shows a similar lung-bud.

GASTRIC REGION.

For the following unpublished statement concerning the gastric region of the Bremer embryo I am indebted to Professor Lewis:

"In the Bremer embryo a gastric region may be referred to, but it is not marked off in any way from the esophagus or duodenum. It may be located only by its relation to the liver and body cavities. It is a flattened or laterally compressed tube, having a cleft-like lumen."

From the condition found in the Bremer embryo, which is undoubtedly older than mine (having limb-buds), it would seem that the presence of a stomach in my specimen is improbable. A pulmonary diverticulum is to be expected. It seems to me, therefore, more reasonable to interpret the lower swelling, from its relation to the hepatic diverticulum, lying above the transverse septum and consequently in the pleuro-pericardial portion of the coelom, as the lung-bud. The divided swelling situated above the pulmonary diverticulum probably corresponds to Grosser's doubtfully identified fifth pouches, but I am unable, from my specimen, to confirm Grosser's interpretation of its significance. That portion of the foregut between the lung-bud and hepatic diverticulum presumably corresponds to what Lewis²⁸ has designated the gastric region. A cross-section of this region is shown in text-figure 4.

HEPATIC DIVERTICULUM.

A short distance below the gastric region the gut widens out considerably, forming a third diverticulum, the liver (text-fig. 5, and plate 3, figs. 3 and 4). It is directed ventrally and orally. It presents at about the junction of its lower and middle thirds a broad, shallow, transverse groove which divides the diverticulum into two portions, both entirely embedded in the septum transversum. Extending ventrally and laterally from the upper portion are a number of buds, the beginnings of hepatic trabeculae; no such buds are found in connection with the lower part. The buds are composed of proliferating epithelial cells which, as indefinite cords, have invaded the mesenchyma of the septum transversum. Most of them are indistinct and their extent is doubtful, for their cells closely resemble those of the mesenchyma and there is no definite line of separation between the two, such as a basement membrane. They are readily overlooked, and it is only with an oil-immersion lens that they are made out with any degree of certainty.

Felix⁹ notes the same difficulty in tracing hepatic trabeculae in a slightly older embryo. Careful study shows that the nuclei of the entodermal cells are slightly larger than those of the mesenchyma, a point that is helpful in determining which cells belong to the trabeculae.

The Bremer embryo (4 mm.) shows a somewhat more differentiated stage in the development of hepatic trabeculae. Here they form anastomosing cords (Lewis)²⁸. As shown by Ingalls¹⁹ in a 4.9 mm. embryo, the trabeculae are very extensive and form a large mass of anastomosing cords. Thompson⁴⁵, however, found no evidence of hepatic trabeculae. In a later note⁴⁶ he states that "the transverse septum is seen before the cells of the liver bud have invaded the vessels which lie in it." He shows in sections, however, a transverse septum which, like the one in my embryo, is quite thick, and he apparently considers that all its cells (excluding endothelial and blood cells) are mesenchymal. Judging from the definiteness and size of the hepatic trabeculae of the Bremer² embryo, which is evidently only a trifle older than Thompson's or my own, one would naturally expect to find some evidence of the trabeculae in the latter two. I believe that, owing to their indistinctness, it is possible that they were overlooked by Thompson.

Regarding the development of hepatic trabeculae, I believe it safe to draw the following conclusions: that they arise as indefinitely outlined buds of proliferating entodermal cells from the upper portion of the hepatic diverticulum; that while they grow in the mesenchyma and anastomose with one another, their cells undergo further differentiation and they become more distinctly differentiated from the mesenchyma.

I must mention briefly at this point the relation of the hepatic trabeculae to the veins of the transverse septum. Janosik²⁰ has noted that the early hepatic diverticulum in the human embryo is not related to the vitelline veins in the same way as in birds. Bremer² states:

"The liver cords are found growing into the 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."

I find with Janosik and Bremer that the hepatic diverticulum and trabeculae are not in close relation to the vitelline veins. These lie dorsally and laterally to the diverticulum. Somewhat ventrally and anteriorly is found the sinus venosus. In the region of the hepatic trabeculae can here and there be made out minute spaces which contain one or two red blood-corpuscles, apparently blood-vessels; but in my specimen I have been unable to make out a definite plexus as found by Bremer.

The lower knob-like portion of the hepatic diverticulum presents no special feature other than a very thick ventral wall. Brachet¹, in a careful study of the development of the liver in several different vertebrates, shows that in the rabbit the hepatic diverticulum is an elongated outpocketing of the foregut.

extending from the region of the sinus venosus to the yolk-stalk, and divisible into cranial and caudal portions. The upper of these he states goes into the formation of the liver proper and the hepatic duct; the lower into the formation of the gall-bladder and the cystic duct. The two portions are designated by Maurer³⁵ the "pars hepatica" and "pars cystica" respectively. The twin embryos which Watt described are apparently too young to show these divisions of the liver--in fact, the liver forms merely a slight swelling on the ventral wall of the foregut where the latter joins the yolk-stalk. It is called by Watt the "liver bay." Thompson, however, recognizes the hepatic and cystic portions of the liver diverticulum in the Robert Meyer embryo No 300. Somewhat similar divisions are described by Ingalls in his embryo of 4.9 mm., but his specimen is considerably older than the above-mentioned ones. Evidence of a division into two portions is apparently altogether lacking in the Bremer embryo of 4 mm., the liver diverticulum of which has been modeled by Bremer and more recently by Lewis.

YOLK STALK AND SAC.

Just below the hepatic diverticulum the gut becomes narrow, but a little more caudally it again gradually broadens. This broadening leads out into the cavity of the yolk stalk and sac. The yolk-stalk is short, being in fact merely the constriction between the yolk-sac and the gut. It is flattened antero-posteriorly, but is broad transversely (plate 1, fig. 2). It measures roughly 0.5 mm. from side to side and 0.16 mm. antero-posteriorly.

The yolk-sac is a flattened vesicle, rather irregular in form, and with a number of folds of various shapes and sizes on its surface. It fills up practically the entire space between the embryo and the wall of the chorion, and extends into the artificially made fold of the chorionic wall as described above. Its dimensions are roughly as follows: length, measured parallel to long axis of embryo, 3.3 mm.; width, measured parallel to dorso-ventral axis of embryo, 2.7 mm.; thickness, measured transverse to embryo, 1.1 mm. Its histological structure will be considered later.

HIND-GUT AND CLOACA.

Caudal to the place at which the yolk-stalk passes out is the beginning of the hind-gut. It has a funnel-shaped opening which tapers as it passes toward the tail into a small rounded tubule. It is surrounded by loose mesenchyma, the whole being attached to the dorsal body-wall by a short, thick mesentery. The hind-gut occupies a position slightly to the left of the median plane of the embryo. It bends backward with the body of the embryo at the ventral bend in the back. It passes without sharp demarcation into the cloaca (text-figs. 8 and 9). The cloaca is the cephalic portion of the spindle-shaped termination of the hind-gut. Its cephalic limit is not definitely indicated, but its caudal extent is marked by the cloacal membrane.

ALLANTOIC DUCT.

The allantoic duct is a very long, slender, hollow tube which proceeds from the cephalic end of the cloaca and extends into the body-stalk. At its origin it is funnel-shaped, and its lumen is distinct. It tapers rapidly as it enters the body-stalk. For a few sections it becomes almost lost from view, owing to the indistinctness of cell boundaries and scattered nuclei. Although continuity of the allantoic cells can be made out, its lumen is lost. A few sections farther distally the allantoic duct again becomes distinct and a trifle larger. Lying between the two umbilical arteries, it follows the ventral bending of these vessels. Still lower down the arteries fuse and then split apart again, thus forming an arterial fork. The small allantoic duct passes in front of the fused part, and then, turning dorsally, passes through the above-described fork. Crossing the umbilical stalk obliquely, it terminates in a small bulb, the allantoic vesicle, which is situated close to the fused umbilical veins.

CLOACAL MEMBRANE.

A short distance from the end of the gut is a very slight outward bulging of the ventral wall. This portion of the cloacal wall is in contact with the ectoderm of the proctodeal invagination, and together these layers of epithelium form the cloacal membrane (text-figs. 8 and 9). The entodermal portion is slightly thicker than the ectodermic.

CAUDAL INTESTINE.

The portion of the gut beyond the cloacal membrane ends bluntly in the extreme end of the tail, separated from the ectoderm by only a small amount of mesenchymal tissue. This portion of the gut represents the post-anal or caudal intestine (text-figs. 8 and 9).

HISTOLOGICAL STRUCTURE OF THE DIGESTIVE TUBE.

Histologically considered, the digestive tract may be described as an epithelial tube surrounded by mesenchyma. Only the former shows signs of differentiation as yet, the mesenchyma being everywhere of the same character. The epithelium takes on widely different appearances in different regions. In general it may be said that throughout the whole of the digestive tube, with the exception of the lower end, the dorsal wall is much thinner than the ventral. The former is made up of a single layer of cubical or flattened cells. On either side of the mid-dorsal line the epithelium gradually becomes thicker and the nuclei more crowded. The side-walls and floor of the pharynx show from two to three layers of nuclei. At the places where the entodermal epithelium of the pharyngeal pouches comes in contact with the ectoderm, it is thin and fused to the ectoderm. The membrane closing the second gill-cleft on the left side has broken through to the outside; this is undoubtedly a mechanical tear. The wall of the thyroid diverticulum is not different from that of the floor of the pharynx, being composed of an epithelium of two to three cell-layers. The epithelium of the ventral wall of the remainder of the fore-gut is thicker, the change from the thin dorsal wall to the

thick ventral wall taking place gradually on the sides of the tube. The pulmonary diverticulum is two to three cell-layers thick, the hepatic diverticulum three to four.

In the region of the yolk-stalk the epithelium is made up of one layer of cubical or somewhat flattened cells. In the yolk-sac the epithelium is not everywhere the same. In some places the cells are large and cubical; in other places flattened and less distinct. Often is it impossible to determine whether or not an epithelium is present, for in such places the epithelial cells can not be distinguished from those of the mesenchyma. The mesenchyma surrounding the yolk-sac also varies in thickness. It contains numerous blood-vessels and is covered by the mesothelium of the body-cavity.

The hind-gut, down as far as the cloaca, is composed of but a single layer of cuboidal cells, which is of equal thickness all around. In the cloaca and caudal intestine the epithelium of the side and ventral walls is thickened, and is composed of two to three layers of cells. The entodermal epithelium of the cloacal membrane shows no distinguishing characteristics. It abuts against the ectodermal epithelium, but both layers can be made out distinctly.

SOMITES.

In all, 24 pairs of somites are present; they extend from the lower end of the hindbrain to a little beyond the point where the allantoic duct passes out from the cloaca. The first somite is found in the region of the eighth and ninth rhombic neuromeres. According to studies on a slightly older human embryo and on young embryos of the pig, sheep, and cat, I have found that this position is normally occupied by the second somite. Watt¹⁸ likewise shows the second somite in this position, the first somite being in relation to the seventh and eighth neuromeres. I have looked repeatedly in my embryo, however, for evidence of another somite in front of the one which I have designated as the first, but have been unable to find any definite indication of such.

As in other young embryos which have been described, the different pairs of somites are found in different stages of development, those nearer the head end always being more advanced than those behind them. In the caudal end of the embryo are found somites in the process of formation; in the head they are already partially broken up. Following Ingalls's¹⁹ plan, I shall begin my description with the somites of the tail and proceed forwards.

The caudal end of the vertebral plates of mesoderm fill up entirely the tail of the embryo around the neural tube, notochord, and tail gut. In the region of the cloacal membrane they appear as solid masses or cords of mesoderm with closely packed cells. At their cephalic ends a small cavity is apparent. Another pair of somites, the twenty-fifth, are partially formed by an incomplete transverse furrow. Numerous mitotic figures are present in the vertebral plates.

The twenty-fourth somite (second lumbar) is in a very early stage of development. It is somewhat cubical in shape and in its center is a distinct cavity, the myocoele. The walls of the somite, which may be described as dorsal, ventral,

medial, and lateral, are all of about equal thickness. They are epithelial in character and contain one to two layers of cells. In the myocœle are found a few scattered stellate cells not unlike mesenchymal cells; later these will enter into the formation of the sclerotome. Mitotic figures are numerous among the cells of the walls and the myocœle, but those of the walls are always at the upper ends of the cells, *i. e.*, the ends bordering on the myocœle. This somite corresponds quite closely with the first coezygeal somite of the Ingalls embryo, except that it probably has fewer cells in its cavity.

The nineteenth somite (ninth thoracic) shows a somewhat more advanced condition. The myocœle in its lower portion is entirely filled with cells. The ventral half of the medial and all of the ventral wall are breaking up. The cells of these walls, together with those on the inside of the myocœle, form the sclerotome. These cells have pushed out slightly toward the chorda dorsalis, forming the notochordal process. The somite corresponds with the sacral somites of Ingalls's embryo.

The fourteenth somite (fourth thoracic) is more distally located from the median plane than the previously described somite. Its ventral and medial walls have both broken down and lost their epithelial character and appear as a mass of mesenchyma between the remainder of the somite laterally, the medullary tube and chorda medially, and the dorsal aorta, coelomic epithelium, and posterior cardinal vein ventrally. The notochordal and aortic processes of the sclerotome, lying dorsally and laterally to the dorsal aorta respectively, are easily recognized. The lateral wall is somewhat thicker than that of the above-described somite, being composed of apparently two layers of distinct columnar cells. The dorsal edge of this wall is bent first medially, then ventrally, and comes to lie near the median surface of the lateral wall. It is, however, separated from the lateral wall by a cleft-like portion of the myocœle. The dorsal border of this cleft—that is, the groove formed by the rolling over of the medial wall—has been termed by Williams⁴⁹ the “upper myotomic groove.” At the place where the bent-over portion of the dorsal border of the median wall is in contact with the sclerotome it has left a groove on the medial surface of the somite. This has been called the “lower myotomic groove” by Williams and others. The ventral edge of the lateral wall is also turned in medially but to a lesser degree. The myocœle, which as stated before is cleft-like at its dorsal part, is larger and broader ventrally. Owing to the breaking-down of the medial wall, a wide opening is left in it, the so-called intervertebral cleft. This somite, on the whole, is quite similar to Ingalls's lumbar somites.

The twelfth somite (second thoracic, plate 4, fig. 2), has its sclerotomic cells scattered between the remainder of the somite laterally and the medullary tube and chorda medially. The dorsal edge of its lateral wall has folded over and grown ventrally along the medial surface of this wall, and has united with the turned-up ventral edge except at one place. The lateral wall can now be described as being composed of an outer lamella (cutis plate, dermatome) and an inner lamella (muscular plate or myotome). The intervertebral cleft which lies at the caudal

end of the medial wall is again distinct. This somite is similar in appearance to Kollman's²¹ plate 1, figure 1, the myotome of a human embryo of three weeks, but its dermatome contains fewer layers of cells than pictured by Kollman.

In the sixth somite (fourth cervical) the inner lamella is thicker, particularly at its anterior end. The dermatome is also slightly thicker and larger. Its cells, of which there are from one to two layers, are distinctly columnar. Mitotic figures are numerous and again confined to the upper ends of the cells. The myocoele is reduced to a small cleft between the medial and lateral lamellæ. Caudally and ventrally the intervertebral cleft is again seen distinctly. This somite is probably similar to those of the lower thoracic region of Ingalls's embryo.

The fourth somite (second cervical) is not so far developed as the first thoracic as described by Ingalls. It is interesting to note, however, that it is quite similar in structure to the second somite of a 25-segment chick as described by Williams⁴⁹. The dermo-myotome is a flattened quadrilateral body lying just beneath the ectoderm. Its lateral and medial lamellæ are closely approximated, there being no evidence of a myocoele. The breaking-up of the dermatome, as described by many writers, is now beginning, as is indicated by the sending out of a few protoplasmic processes of the outer portion of the dermatome to the covering ectoderm.

The third somite (first cervical) is similar to the fourth, but shows a somewhat more broken-up condition. Its dermatome lies almost in contact with the outer ectoderm and its cells are beginning to send out processes. The cells of the myotome are also beginning to undergo further differentiation, for their spindle-like forms can be made out.

The second and first somites (occipital) are not definitely marked off from each other. The second shows a slightly more advanced condition than the third. The first is small. Its dermo-myotome is distinct, but the outlines of the sclerotome are lost. It is also not definitely separated from the mesenchyma in front of it.

CHORDA DORSALIS.

Throughout its whole extent, the chorda dorsalis lies just ventral to the medullary tube, the curvatures of which it closely follows. Its anterior end, which begins opposite the point at which the remnants of the oral membrane are attached to the roof of the mouth, is flattened dorso-ventrally and makes a slight bend to the right. Caudal to this flattened portion, the chorda assumes in general a cylindrical shape, although in some places it is flattened either dorso-ventrally or laterally, while in other places it is triangular in cross-section. It terminates equidially in the tail by joining the undifferentiated cells of the primitive-streak region (text-fig. 9).

An examination of the chorda dorsalis shows that it is not everywhere of uniform size, but that it is alternately expanded and constricted. In order to determine whether the expanded portions are arranged in any way with reference to the body segments, a wax reconstruction of a portion of the chorda was made; but owing to its irregular shape it is difficult to determine from the reconstruction

which portions are actually expanded and which constricted. A segment which appears expanded in side view may appear constricted from the front or back. Relative measurements of cross-sectional (oblique) areas of the chorda dorsalis between the second and thirteenth segments seem to indicate that the expanded portions lie within the body segments, but the evidence for this is not altogether convincing.

That segmental flexures of the chorda dorsalis exist is indicated by the fact that for long distances, including extents of a number of segments, the chorda lies approximately equidistant from the medullary tube. The ventral surface of the medullary tube, as shown in plate 2, figure 1, presents dorsal and ventral curvatures; consequently the chorda must also follow these curvatures. This would make the ventral curvatures of the chorda segmental. Minot³⁷ has described, under the term "segmental flexures of the notochord," a series of dorsal and ventral curves which he found to be constant in a number of mammals, man included; these, he states, are so placed that their dorsal curvatures are segmentally arranged, but he states further that in young pig embryos the ventral curves are segmental and that a shifting of the flexures takes place in embryos of about 12 mm. The flexures which are apparently present in my embryo, therefore, accord with those which Minot describes for pig embryos younger than 12 mm.

The chorda is fused with the entoderm of the digestive tube in one region only, namely, in the posterior part of the pharynx at about the level of the third to fourth body segments. Here it is attached in two places, each extending through but a few sections and the two fusions separated from one another by but three sections. The more anterior fusion is shown in text-figure 7. It is of interest to note that Gage¹² and Thompson¹¹ both found a fusion of the chorda to the entoderm in the same location and that Watt⁴⁸ shows the chordæ in the twin embryos he describes to be least developed in this region. Watt believes, therefore, that that section of the chorda opposite the posterior part of the pharynx is the last in point of time to develop, and that embryos showing a fusion of chorda and entoderm in this region are evidence in favor of this view.

Throughout its whole course the chorda dorsalis is invested on either side with mesenchyma. Dorsally and ventrally, however, this investment is not complete. Ventrally it is incomplete not only at the points of fusion with the entoderm, but both anteriorly and posteriorly to them. Anteriorly it lies almost in contact with the roof of the greater part of the pharynx (text-fig. 6). Posteriorly it soon becomes separated from the pharyngeal wall by intervening mesenchyma. From the seventh body segment caudally the chorda lies almost in contact dorsally with the medullary tube, there being from this point caudally no mesenchyma on the dorsum of the chorda. Elsewhere the chorda is completely surrounded by mesenchyma. The younger chordæ described by Watt showed mesenchyma ventrally in one small region only, namely, at the level of the eighth segment, while dorsally mesenchyma passed between the chorda and medullary tube in two places—at the anterior end and again opposite the first body segment. The rapidity with which the notochordal processes of the sclerotomes separate

The chorda from the medullary tube dorsally and the digestive tube ventrally can be noted by a comparison of the specimens. Its dorsal separation seems to begin anteriorly and to proceed caudalward. Ventrally separation apparently begins in the middle region of the body and progresses both anteriorly and posteriorly. Another point of ventral separation begins anteriorly and progresses caudally, the last portion to become separated undoubtedly being that which is fused to the pharynx.

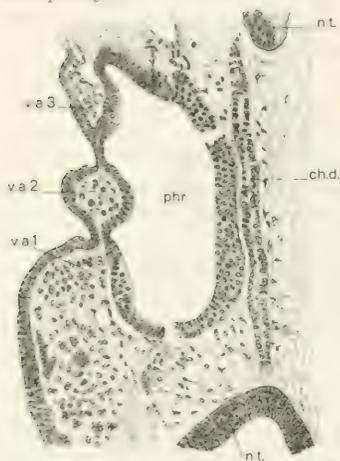


FIG. 6.—Portion of section 42. $\times 125$ diameters.

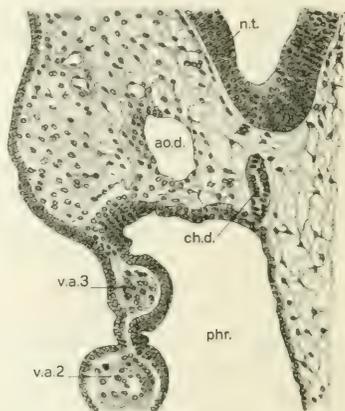


FIG. 7.—Portion of section 46. $\times 125$ diameters.

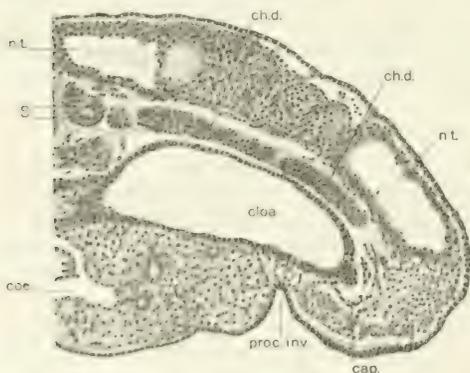


FIG. 8.—Portion of section 218. $\times 125$ diameters.

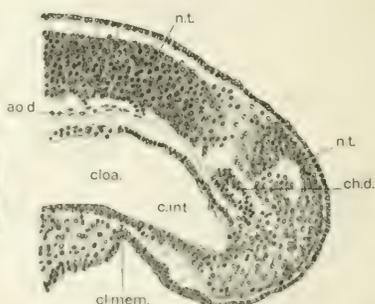


FIG. 9.—Portion of section 222. $\times 125$ diameters.

Watt describes what he considers to be neurenteric canals in his twin embryos. They are situated just dorsal to the cloaca and consist of a connecting rod of cells joining the chorda with the medullary tube dorsally and with the entoderm ventrally. This region in my specimen is cut through sagittally, but as seen in text-figures 8 and 9, no evidence of such a cord of cells is present.

The chorda dorsalis consists of polygonal and wedge-shaped cells, with large rounded nuclei and considerable granular cytoplasm. In places the cells are arranged radially about the center of the chord, while in other regions this arrangement is less distinct. Mitotic figures are numerous among the cells.

In the center of the chorda a fine lumen can be made out in certain sections (plate 4, fig. 3). This lumen is not continuous throughout, but is present in numerous places, each extending through but a few sections. In size it varies from 2 to 4 microns. That this discontinuous lumen of the chorda is normal in certain young stages of the human embryo seems to be well established, since it has been found by His¹⁸ in his embryo L1 of 2.4 mm., by Eternod⁹ in three embryos of 1.3 mm., 2.11 mm., the third somewhat larger, and by Watt¹⁸ in twin embryos of 17-19 paired somites.

An ill-defined cuticular membrane surrounding the chorda dorsalis, such as has been described by Van den Broeck⁴⁷, is present, except where the chorda is fused to the entoderm of the pharynx.

NEPHRIC SYSTEM.

In describing the Robert Meyer embryo of 23 somites (Thompson's embryo) Felix¹⁰ states:

"The pronephros is almost completely developed, so far at least as one may speak of its completion. It consists of a number of tubules and the primary excretory duct. There are in all seven tubules present, the most anterior of which is not united with the succeeding tubule The tubules 2-5 have fused so as to form a collecting duct. The tubules 5, 6, and 7 are not yet united, but their union is imminent."

In my specimen I likewise find a series of tubules representing the nephric system. Although conditions on both sides of the embryo are not identical, for the most part they are quite similar. The following description is based on their arrangement of the left side and the terminology used is the same as employed by Felix.

On the seven pronephric tubules which are present, the first two are rudimentary (plate 4, fig. 4). The first is represented by two or three small clusters of cells at the level of the ninth body-segment. It is indistinct and indefinite and its identity is determinable only by the position which the clusters of cells occupy and by the fact that the cells are more or less isolated from the mesenchyma.

The second tubule is larger and more distinct. It is composed of a single cluster of cells which form a spherical mass. Its cells are isolated from the mesenchymal cells by a clear space. It also lies in the seventh body-segment. The remaining pronephric tubules are elongated gland-like tubes of epithelium, rather bulbous at their cephalic extremities and tapering caudally. Each in its course crosses the body-wall obliquely in a ventro-dorsal direction; thus in its upper part each lies close to the mesodermal lining of the coelomic cavity, while below it comes in close relation with the ectoderm (plate 4, fig. 4). According to the description of Felix, each of these tubules represents two different portions of the

pronephros. The bulbous portion is the inner pronephric chamber. It is connected to the mesothelium of the coelomic cavity by a slender strand of cells, the nephrostome, which joins it ventrally. The caudal portion of the tubule Felix terms the principal collecting tubule.

The third pronephric tubule is the longest. It is rounded in cross-section and more regular in form than any of the succeeding tubules. The inner pronephric chamber is small cephalically, but is not connected to the mesodermal lining of the body-cavity by means of a nephrostome. A pit on the mesodermal lining of the body-cavity opposite the tubule, however, probably represents a broken-down nephrostome. The principal collecting tubule extends caudally close to the ectoderm. It terminates in relation with the principal collecting tubule of the fourth pronephric tubule. Whether an actual fusion exists between the two collecting tubules or whether they merely touch, I am unable to determine. The third pronephric tubule possesses a distinct lumen, which extends throughout most of its length. It lies in the tenth body segment, its collecting tubule extending into the eleventh segment.

The fourth tubule is almost the same shape and size as the third and is less regular in form. Again a connecting nephrostome is lacking, although its position is marked on the free surface of the mesothelium by a depression. The principal collecting tubule ends in connection with that of the fifth tubule. The joined distal ends of the collecting tubules give rise to the primary excretory duct. The fourth tubule possesses a discontinuous lumen divided into two portions.

The fifth (plate 4, fig. 2), sixth, and seventh tubules differ from the third and fourth in possessing connecting nephrostomes. Each nephrostome begins as a funnel-shaped opening on the coelomic wall. In no case, however, can a lumen be traced into the tubule. Each nephrostome is joined to its inner pronephric chamber by a strand of cells. Of these, that of the fifth tubule is extremely long and band-like. The inner pronephric chambers are definite swellings situated near the middle of the tubule. That of the seventh is the largest. The principal collecting tubules are joined by the preceding ones as shown in plate 4, figure 4. Lumens are present only in the inner pronephric chambers. The fifth and sixth pronephric tubules are situated in the twelfth body-segment, while the seventh is found in the thirteenth.

Histologically the tubules in all parts are made up of polygonal or columnar cells, with rounded or elongated nuclei. Where a lumen is present the cells are arranged radially about it, but in other places there seems to be no definite arrangement.

Beyond the seventh pronephric tubule and apparently continuous with it is a large rounded cord of cells which extends through the remainder of the embryo. This cord possesses a number of irregular swellings, the mesonephric vesicles. These vary in size and shape and are not so definitely marked off from one another as shown by Felix¹⁰ and Watt¹⁶; 16 to 18 may be counted (plate 4, fig. 4); they possess nephrostomes connecting them with the coelomic mesothelium similar to those of the pronephric tubules.

The primary excretory duct occupies a position just dorsal to the nephrogenic cord. In its course caudally it lies close to the ectoderm. Felix was unable to determine whether it developed from the ectoderm or whether it arose independently in the mesoderm, but he doubts that the ectoderm has any participation in its development. Watt⁸ also was unable to determine definitely its mode of formation. The caudal end of the primary excretory duct, the point at which its formation is supposed to be taking place, is in my embryo cut sagittally. It is, however, indefinite. Just before its termination the duct is seen lying close to the ectoderm in the mesenchyma. The mesenchyma possesses several mitotic figures in the region in which it terminates. While I am inclined to favor Felix's view of a mesenchymal origin, the evidence found is not convincing.

VASCULAR SYSTEM.

HEART.

The heart lies in that portion of the body-cavity which is bounded by the pharynx above, the fore-gut behind, the anterior body-wall in front, and the transverse septum below. It is still a simple tube and viewed from in front is roughly the shape of the letter U. It is placed so that the loop of the U is directed toward and lies in the right side of the pericardial cavity. The limbs of the U are turned to the left, but upon reaching the body-wall of the left side turn dorsalward at an angle of almost 90 degrees. The upper limb then bends sharply cephalad, joins immediately the pericardial wall, and passes into the ventral aorta. The lower limb bends medially and joins the pericardial wall as it passes into the sinus venosus. The ends of the heart-tube are therefore fixed to the body-wall, but the remainder of the heart lies free within the cavity.

An examination of the heart-tube shows that it is not everywhere of the same caliber, but presents certain expanded portions separated by more or less definite constrictions. Beginning with the venous end of the heart, the sinus venosus passes into the atrium with but a slight constriction. Figure 2, plate 1, shows that portion of the heart which I regard as the atrium. As seen from behind (plate 3, fig. 1), it presents a V-shaped bend, the apex of which is pointed toward the left, the limbs lying in a horizontal plane. On its upper surface is a distinct irregular projection, as to the significance of which I am in doubt.

The atrial portion of the heart passes into the ventricular portion with only a slight constriction, the atrio-ventricular canal. The ventricle is much enlarged, having a transverse diameter which is greater than that of any other portion of the heart. It fills up the entire lower right-hand portion of the pericardial cavity. Its cephalic end is bounded by a shallow constriction, in front of which is the bulbus cordis.

The bulbus cordis is large where it is attached to the ventricle, but gradually becomes narrower towards its cephalic end. It is directed downwards and towards the left. As described by Watt and others, it reaches farther cephalad than any other portion of the heart. It becomes continuous with the truncus

arteriosus, a short, narrow portion of the heart-tube which is directed dorsally and towards the left. The truncus arteriosus bends sharply upward and joins the ventral aorta.

The cavity of the heart approximately follows the center of the heart-tube. Like the outside of the heart, it also is not of uniform diameter, but presents swellings and constrictions, as shown in plate 3, figure 2. The sinus venosus passes to the left as an irregular tube. It is marked off from the atrium by a slight constriction. At the apex of the atrial bend the cavity enlarges considerably. On the superior surface of this enlargement is a crescentic projection, the concavity of which is directed medially. This extends into the above-described projection of the atrium as seen from the surface.

Between the atrium and ventricle is a long, narrow portion of heart-cavity, the atrio-ventricular canal. The difference in size between this narrow portion and that of the adjacent swellings is greater than that between the same portions of the heart as seen from its surface. The cavities of the ventricle and bulbus cordis are again enlarged and separated from one another by a rather long constriction portion. This is again much narrower proportionally than the same constriction seen from the surface of the heart. In cross-section the cavities of the ventricle and bulb, particularly the former, are triangular. In the truncus arteriosus the cavity is again small and irregular. Cephalad it gradually widens out as it becomes the ventral aorta.

The endothelium of the heart is everywhere a syncytium of one layer of cells. Its nuclei are oval in shape and coarsely granular, while the cytoplasm stands out sharply in contrast to the coagulum without. In the bulb and ventricle, at the angles of the cavity as seen in cross-section, the endothelium sends out plate-like processes of cells. These give to the various portions of the endothelial heart a stellate appearance. In some instances these processes extend to the myocardial layer and apparently fuse with it. One such process is shown in plate 4, figure 1. It passes off from the endothelial tube of the heart as a solid cord of cells. It extends entirely across the space between the endothelial and myocardial layers and terminates in a bulbous expansion in contact with the myocardium. In the expanded end is seen a distinct mitotic figure. The significance of processes of this kind can not be doubted. They represent the earliest beginnings of the so-called "heart sinusoids," which in embryos a little older are numerous. By their growth and anastomoses they give rise to the trabeculae of the heart.

In the atrium, with the exception of the above-described atrial projection, the endothelium lies in contact with the myocardium, there being no intervening space such as is found in the remainder of the heart-tube. This relation of the endothelium to the myocardium in the atrium has been noted by His¹⁸. The same condition has been described by Mall²², who states:

"This arrangement is so pronounced in the early heart that it affords a way by which we may determine with precision the exact portion of the heart tube from which the atrium arises."

No mention, however, is made by Mall, or by Watt¹⁸, who describes a similar arrangement, of that portion of the atrium which projects upward and in which the endothelium is not closely applied to the myocardium.

The outer layer of the heart-tube, the so-called myo-epicardial layer, is composed of several layers of cells, the thickness varying in different portions of the heart. As Tandler¹⁴ has shown in an embryo of 3.5 mm. (embryo Hal. Institute of Anatomy, Vienna), these cells form a distinct synectium. The myo-epicardium is directly continuous at the venous and aortic ends of the heart with the transverse septum and lining of the body-cavity, respectively, and it is by means of these attachments that the heart is held in place.

In that portion of the atrium where the myo-epicardium lies in contact with the endothelium, its cells are closely applied to one another. Nuclei are crowded and intercellular spaces are small. The myo-epicardium of the atrio-ventricular canal is somewhat thicker than that of the atrium, and its cells are more widely separated. Numerous small protoplasmic processes of the cells form a network, the meshes of which are filled with delicate fibrils. In the ventricle and bulbus cordis the myo-epicardium is still thicker, but in the truncus arteriosus it again becomes thin.

In describing the 3.5 mm. embryo, Tandler¹⁴ says:

"The myo-epicardial mantle differentiates to the extent that in the region of the ventricular loop and in the bulbus its superficial layer is formed by a continuous row of cells, the epicardium, while on the atrium and sinus, so far as the latter has a free surface, no such differentiation can be said to exist."

In my embryo I find that the outside layer of cells of the myo-epicardium have not yet become flattened and detached from the underlying cells, as shown by Tandler in his figure 377, but over the entire surface of the heart they are arranged in a distinct layer (plate 4, fig. 1). As seen in cross-section, they are in places cubical and closely packed, while in other places they are more rounded and farther spread apart. I am also unable to find any differentiation of the myocardium proper into an inner spongy portion and an outer cortical portion, as Tandler describes for the 3.5 mm. specimen.

The broad space which exists between the myo-epicardial and endothelial layers, according to Tandler, is filled during life with serous fluid, since it is occupied in section by a clot-like fibrous mass which is entirely destitute of cells and stains feebly with the hemotoxylin. In my specimen I find a similar clot-like mass. The small, delicate fibrils form an anastomosing plexus, the meshes of which are empty. For the most part these fibrils extend radially from the endothelium to the myocardium, to both of which they gain attachment. They radiate out particularly from the plate-like processes of the endothelium, making it impossible to determine in every case where the endothelium leaves off and where the fibrin begins. It seems probable that the delicate fibrils found in the myocardium are due to a similar coagulation of serous fluid.

Mitoses within the tissue of the heart are few. Occasionally one may be observed in the endothelium, particularly in the cells of its plate-like processes.

In the myo-epicardium, with the exception of that of the sinus venosus, they are extremely rare. In the sinus venosus, and particularly in those portions of the mesothelium of the body-cavity and transverse septum which are directly continuous with the myo-epicardium, mitotic figures are comparatively numerous. This finding would seem to indicate that the multiplication of cells of the myo-epicardium of the heart takes place at this stage principally in the region of the sinus venosus.

VEINS.

VENA CARDINALIS ANTERIOR.

The vena cardinalis anterior (plate 5, fig. 1) draws its blood principally from the region of the brain. In Ingalls's embryo this vein begins at the junction of two veins which course caudally from the region of the prosencephalon. Ingalls, basing his interpretations upon the work of Mall²¹, regards the dorsal of these as the source of the future sinus sagittalis superior; the ventral one, the vena ophthalmicus. In my specimen the region drained by the two above-mentioned veins is occupied by a venous plexus. One tributary, which extends upward from the region of the optic vesicle, may already be identified with reasonable certainty as the ophthalmic vein. The much more extensive plexus above probably gives rise to the embryonic superior sagittal sinus. The tributaries of the above-described plexus come together medial to and behind the trigeminal ganglion, where they form an enlarged venous sinus, between the trigeminal ganglion in front and the acustico-facial ganglion behind. From this the anterior cardinal vein passes caudally by two main channels, the dorsal of which is situated above the origin of the acustico-facial ganglion, while the ventral one is placed medial to it. Passing the interspace between the acustico-facial ganglion and otocyst, these branches unite again medially to the otocyst, forming another enlarged portion of the vein. Caudal to the otocyst it divides into three smaller veins, which soon come together again. Opposite the first somite the anterior cardinal vein becomes very small in diameter, but gradually becomes larger when traced still farther caudally. From the second segment to its termination, the vena cardinalis anterior is represented by two small veins which are closely related to one another. In several places, however, they unite to form a single vessel. At the level of the fifth body-segment the vena cardinalis anterior enters the vena cardinalis communis (duct of Cuvier).

In its course the vena cardinalis anterior receives tributaries on both its dorsal and ventral walls. On the dorsal, the first is found in the region of the trigeminal ganglion and proceeds from the direction of the mesencephalon to reach the anterior cardinal vein at the posterior border of that ganglion. The second lies just in front of the otocyst close to the rhombencephalon. The position which it occupies (just in front of the otocyst) indicates that it is the same vein which Mall²¹ describes as the vena cerebri media. Behind the otocyst is a small stump of a vessel which could not be traced far dorsally. Owing to its position, just behind the otocyst, it becomes evident that this vein must be identical with the one occupying a similar position in Ingalls's embryo and which Evans⁸ has inter-

puted to be the vena cerebri posterior. Just caudal to this vein is a longer vein, which, arising in the region of the first segment and extending anteriorly and ventrally, joins the anterior cardinal vein at the point where the latter is broken up into two portions. I am in doubt concerning its identity. Several small dorsal tributaries of varying size are received throughout the remainder of the vena cardinalis anterior. They include the lateral loops of the second, third, and fourth dorsal segmental arteries, which are described below.

The ventral tributaries are more numerous than the dorsal. They may be described as belonging to the different visceral arches. One arises in the mandibular arch close to the mouth, passes dorsalwards, and unites with a network of small veins. The blood from this tributary may reach the anterior cardinal vein, either in the region of the trigeminal or of the acustico-facial ganglion.

The venous tributaries of the second arch do not arise as far down as those of the mandibular arch. They form a plexus which lies in close relation to the facial nerve. One tributary passes medially to the ganglion, while the others are laterally situated. There is thus established about the acustico-facial ganglion a venous ring. The plexus anastomoses with that of the first arch.

In the upper part of the third arch are found three small tributaries, which unite and reach the anterior cardinal vein as a single vessel. No others are found in this visceral arch. Farther caudally, opposite the second and third somites, several small veins unite with the anterior cardinal. They probably represent similar tributaries from the fourth arch.

At the point at which the anterior cardinal veins empty into the common cardinal there is received, on the ventral side, a long, slender vein (vena linguofacialis). The smallest tributaries of this vein may be traced as far as the third visceral arch. Uniting, these tributaries form the vein which proceeds caudally and dorsally. In its course it passes in the antero-lateral body-wall over the heart. A similar vein has been described by Ingalls¹⁹, as follows:

"Am Anfang des Ductus Cuvieri münden in ihn auf jeder Seite je ein von der vorderen Bauchwand kommendes Gefäss. Auf der rechten Seite ist dies besonders gross, es lässt sich ventralwärts bis in die Nähe des Ursprungs der ersten Aortenbogen verfolgen und weiter kaudalwärts bis dahin, wo die ersten Kiemenbogen mit der vorderen Körperwand verschmolzen sind, um sich schliesslich in dem ersten Bogen zu verlieren."

The same vein had been found by Salzer⁴⁰ in embryos of the guinea-pig and later by Grosser¹⁶ in bat embryos. Lewis²⁶ describes it in the pig embryo under the term "transverse vein," and later²⁷ discussed its origin and fate in rabbit and human embryos. Apparently the first reference to this vein, which is now recognized as of constant occurrence and fundamental morphological importance, was made by Phisalix, as Dr. Lewis has pointed out to me. Phisalix³⁹, in 1888, showed it clearly in a figure of a 10 mm. human embryo and described it as follows:

"Entre la veine jugulaire et la veine cardinale se trouve une vaste poche dont le sang s'écoule par les canaux de Cuvier En avant et au-dessus, chacune de ces poches reçoit des veinules qui accompagnent le nerf hypoglosse et qui viennent de la base des arcs branchiaux."

In injections of the veins of pig embryos, Smith⁴¹ shows definite anastomoses between the linguo-facial vein and the venous plexuses of the visceral arches. The anastomosis between this vein and the plexus of the first visceral arch is apparently already formed in Ingalls's 4.9 mm. human embryo. In my embryo I have been unable to find an anastomosis, but the direction which the vein takes (plate 5, fig. 1) seems to indicate the possibility that these two sets of tributaries might soon unite.

VENA CARDINALIS POSTERIOR.

The blood from the tail and lower half of the embryo is carried to the vena cardinalis communis by means of the vena cardinalis posterior. This arises in the tail of the embryo in the region of the unsegmented mesodermal plates as a slender irregular vessel. In its course to the bend in the back it passes along the ventro-lateral border of the somites, with which it lies in contact. In this part of its course, owing to the plane of sectioning, it is difficult to trace its capillary connections, but in places there is evidence that connections similar to those above the nineteenth somite exist below it.

For the most part that portion of the posterior cardinal vein from the bend in the back to the common cardinal stands out quite sharply. In general it courses along the ventro-lateral border of the somites, lying between them and the pronephros or mesonephros. Opposite the somites of the seventh to eleventh segments the posterior cardinal vein becomes very difficult to follow. It is represented by a slender and apparently solid cord of endothelial cells. In the intersegmental spaces the vessel becomes larger and usually contains a number of blood-cells (plate 5, fig. 1). Opposite the ninth somite (and again the tenth) the existence of the vessel becomes doubtful, owing to the similarity of endothelial to mesenchymal cells. In the corresponding region on the right the continuity of the posterior cardinal vein is even more doubtful. Whether this is due to a closing-up of a once continuous posterior cardinal vein or whether it represents an incompletely developed vein is impossible to determine from the sections. According to Evans⁸, the posterior cardinal veins first appear in human embryos possessing from 15 to 23 somites. He states:

"It is probable that lateral loops of the dorsal segmental arteries are instrumental in the formation of these veins, as in the case of the anterior cardinals. This method of formation of the posterior cardinal veins appears fundamental. Raffaele (1892) and Hoffman (1893) describe it for selachian embryos and Grafe (1905) and the writer have indicated it in the case of the chick."

It is probable, therefore, that in my embryo the above-mentioned portions of the posterior cardinal veins are still in the formative stage, particularly since the intersegmental portions of the vein, to which are joined the lateral loops of the segmental arteries, are more definitely marked than the segmental portions.

From the interspace between the seventeenth and eighteenth segments to its termination, the posterior cardinal vein receives its blood principally from the small lateral loops of the dorsal segmental arteries, as has been described by Evans⁸.

In addition to these tributaries, connections can be made out in several places between the posterior cardinal vein and the lateral branches of the aorta, but these connections are not so distinct as the branches from the dorsal segmental arteries. In the regions of the pronephros and mesonephric vesicles small tributaries are found arranged in pairs, one lying on either side of these organs; both pass dorsally to join the posterior cardinal vein. The lateral tributaries pass, in the region of the pronephros, between the inner pronephric chambers of one pronephric tubule and the principal collecting tubule of the preceding one. Farther down, in the region of the mesonephros, they pass between the mesonephric vesicles and the primary excretory duct.

Evans⁸ shows similar vessels in a reconstruction of the 23-somite embryo of Robert Meyer. He also indicates a longitudinal vein which connects the peripheral ends of the medial tributaries and another similarly connecting the lateral tributaries; these he terms the medial and lateral subcardinal veins, respectively. I have been unable to make out continuous longitudinal connections in my specimen, but indications of them are apparent on a few of the medial vessels. I find, however, connections between the medial tributary and the lateral segmental arteries, such as Grafe¹³ has shown in the chick and Evans⁸ notes in the Robert Meyer specimen. It is interesting to note that the two above-described tributaries of the posterior cardinal vein are not located with reference to the segments, but (as Evans and others have described for the lateral segmental arteries) they correspond quite closely in number and position with the pronephric tubules, where the latter are present. Below the pronephric tubules they are arranged with reference to the mesonephric vesicles.

VENA CARDINALIS COMMUNIS.

The common cardinal vein (duet of Cuvier) is a short, flattened vessel which lies within the sixth body segment. It receives both the anterior and posterior cardinal veins. It is directed caudally in the lateral body-wall and, breaking up into three portions, joins the vena umbilicalis at the point where this vein enters the transverse septum (plate 5, fig. 1, and plate 6, fig. 1).

VENÆ UMBILICALES.

The umbilical veins begin at the distal end of the body-stalk by the union of several large veins which drain the chorion and its villi. At first they form a single vessel, which soon, however, breaks up into a plexiform arrangement of large veins (plate 5, fig. 1). These reunite to form a single large vein, which again divides to form two smaller vessels, the right and left umbilical veins. Immediately upon separating they pass to the outer border of the umbilical stalk, one on either side, and enter the body-wall.

In the beginning of its course the right vein is very small. Soon, however, it increases in size and in the remainder of its course it is similar to and about as large as the left umbilical vein, which is quite uniform throughout. Each umbilical vein, throughout its entire course from the body-stalk to the septum transversum, lies within the body-wall, situated in this at about the junction of its

middle and distal thirds. Each receives from the body-wall tributaries coming from both dorsal and ventral directions. The tributaries which reach the umbilical vein on its ventral wall arise for the most part within villus-like processes of the body-wall. On the left side, one of these (found at the level of the tenth and eleventh body-segments) is even larger in cross-section than the main stem of the vein itself and forms a venous sinus in the villus (plate 5, fig. 1.) It is drained by a relatively small vessel. Above this are a number of tributaries which drain a longitudinal vessel situated in line with the venous sinus below. Concerning the significance of these vessels I am in doubt, but believe them to be either the remnants of the plexus from which the umbilical vein has developed (Evans⁶) or the beginning of the anterior body-wall plexus (Smith⁴¹).

Opposite the seventh somite the left umbilical vein receives a branch from the vitelline vein, which lies ventral and caudal to the main junction of these vessels. Approximately at the level of the interspace between the sixth and seventh body-segments, the left umbilical vein receives from above the vena cardinalis communis, entering by three distinct tributaries (plate 5, fig. 1), as described above. Turning sharply medially into the septum transversum, it unites with the vitelline vein (plate 6, fig. 1), and with it forms the left vitello-umbilical trunk. This trunk, which is represented by one main vessel and two smaller ones, passes medial to join the sinus venosus.

The right umbilical vein likewise receives tributaries from the body-wall all along its course and at its most cephalic point receives the right common cardinal vein. It is united to only a portion of the vitelline vein and at but one point. The vitello-umbilical trunk is represented on this side by a network of smaller veins which connect it with the sinus venosus.

VENÆ VITELLINÆ.

The vitelline veins arise on the surface of the yolk-sac from the yolk-sac plexus. Two principal veins, the right and left, are formed on the yolk-stalk by the convergence of numerous tributaries. These course cephalad, one on either side, and enter the septum transversum (plate 6, fig. 1). They pass dorsally along the sides of the hepatic diverticulum, lying quite close to it and the wall of the fore-gut. Several small tributaries proceeding from the mesenchyma surrounding the fore-gut are received by them from above. Each breaks up into several branches, which form a plexus within the transverse septum. A minute commissural branch connecting the veins of both sides is found in the notch between the hepatic diverticulum and the fore-gut wall. The two connections of the left vitelline vein with the left umbilical vein have already been described. All the blood carried by the left vein, except that which may cross over to the opposite side in the small commissural branch, joins that of the umbilical vein before reaching the sinus venosus. Near its termination the right vitelline vein breaks up to form a plexus, the branches of which diverge. Some of these pass directly into the sinus venosus, while others join the umbilical vein to form a plexus representing the right vitello-umbilical trunk. Only a part of the blood which is carried by the right vitelline vein, therefore, passes through the right vitello-umbilical trunk.

Over the yolk-stalk the tributaries of the vitelline veins form a network of small vessels. These reach dorsally as far as the middle of what might be outlined as the gut. Extending along the gut-wall, therefore, are a number of small longitudinal coursing veins (plate 5, fig. 1). They can be traced caudally along the hind-gut for some little distance below the hind-gut portal. The significance of this plexus I have not been able to determine definitely, but I judge that it will ultimately give rise to the inferior mesenteric vein.

SINUS VENOSUS.

The sinus venosus is situated within the substance of the septum transversum, ventral to the gastric region of the fore-gut and cephalad to the tip of the hepatic diverticulum. It is a broad, irregular vessel (text-fig. 4 and plate 5, fig. 1), much flattened dorso-ventrally. On its left border it receives three vessels which represent the left vitello-umbilical trunk. On its right it receives three small branches from the right vitello-umbilical trunk and as many more directly from the right vitelline vein. The sinus venosus curves ventrally and to the left and, becoming a more rounded vessel, passes out of the transverse septum into the atrium of the heart.

ARTERIES.

AORTA VENTRALIS.

The ventral aorta (plate 2, fig. 3, and plate 3, figs. 3 and 4), the direct continuation of the truncus arteriosus, is an unpaired median vessel. It is situated just ventral to the thyroid diverticulum, with which it lies in contact. It at once breaks up into the aortic arches.

AORTIC ARCHES.

Three pairs of aortic arches are present (plate 5, fig. 2). Of these the first is by far the largest. Each vessel begins at the anterior extremity of the ventral aorta and extends anteriorly and dorsalward in the first visceral arch, just cephalad to the first pharyngeal pouch. Reaching the upper extremity of the arch, it joins with the dorsal aorta. Both first arches are distinctly patent throughout.

The second and third aortic arches are smaller and less distinct vessels, lying within the second and third arches respectively. The vessels of these arches vary in size and distinctness in different regions. In certain places they become so small that a lumen is no longer discernible, and it becomes impossible to determine whether the vessels are continuous or not. It is very probable, however, that connections do exist at these places, but, owing to the great similarity between the endothelial cells and those of the surrounding mesenchyma, the former can not be traced through with any degree of certainty. On the left side the second arch shows two such doubtful interruptions, one at the point where it leaves the ventral aorta, the other where it joins the dorsal aorta. Between the two breaks a distinct vessel is present. The third arch on the left side arises from the dorsal aorta as a small plexus and extends as a distinct vessel halfway down to the ventral aorta. Here it disappears for several sections, but soon reappears as an apparently solid string of endothelial cells. This cord when traced downward is found to connect with the ventral aorta. On the right side the second and third aortic arches like-

wise can not be traced from dorsal to ventral aorta. The apparently absent portions of both of these arches are near the ventral aorta.

As shown in plate 5, figure 2, there is on the left side a small arterial twig which branches off from the dorsal aorta just behind the third arch. The significance of this is uncertain, but from its position it seems quite probable that it may be the beginning of a fourth aortic arch.

Whether the second and third arches are not yet completely developed or whether their lumens have become secondarily occluded I am unable to determine. It would seem, however, that the former is the more probable, even though in the somewhat younger embryos of Watt and Van den Broeck,⁴⁷ and in Thompson's embryo as described by Felix, the second arch is complete. The beginning third arch (and the probable beginning fourth) indicates that in this respect my specimen is older than any of the above-mentioned embryos. The incomplete second arch may be regarded as having been slightly retarded in its development.

AORTÆ DORSALES.

The dorsal aortæ are two large vessels which extend from about the level of the anterior end of the chorda dorsalis to within a very short distance from the tip of the tail. Between the eighth and nineteenth segments the dorsal aortæ are fused together in the mid-line, forming a single median vessel; elsewhere two distinct vessels are apparent. Where two vessels are present they lie one on either side of and slightly ventral to the notochord; where single it lies directly ventral to the notochord. Throughout their entire courses, whether paired or single, the dorsal aortæ lie just dorsal to the digestive tube.

The dorsal aortæ, when traced from their anterior to their posterior extremities, continually change in shape and size. The median dorsal aorta is smallest in cross-sectional area at about the level of the eleventh segment and largest at the level of the fourteenth. The vascular bed (cross-sectional area) at this level is even larger than that of the paired dorsal aortæ combined. The endothelium of the dorsal aortæ is distinct throughout. In the region of the fourteenth segment it forms an incomplete septum, undoubtedly the remains of the originally fused medial walls of the paired vessels, which have not as yet disappeared.

BRANCHES OF THE DORSAL AORTÆ.

Anterior Branches.—At the point where the dorsal aorta and the first aortic arch join, two small arteries (plate 5, fig. 2) are given off from the dorsal wall of the dorsal aorta. The one situated more caudally is the smaller of the two. It extends dorsally and medially and terminates in the region of the posterior end of the prosencephalon, a short distance behind the anterior end of the notochord. I am unable from the specimen or from other descriptions to identify this vessel with any degree of certainty, but presume that it gives rise to one of the cerebral arteries. The anterior branch is larger. It extends medially and anteriorly and comes to lie close to the side-wall of the prosencephalon. Here it divides into two branches, one of which extends forward on the ventral wall of the prosencephalon, while the other passes medially along its ventral wall. The latter

branch terminates near the mid-line of the embryo not far from its fellow of the opposite side. In addition, the anterior branch gives off along its course two or three small arterial twigs. These pass dorsally along the wall of the brain, and probably represent the beginning of the cerebral arteries.

Dorsal Segmental Arteries.—The dorsal segmental arteries are represented by 24 paired vessels. Although for the most part they are similar in position and distribution, they vary greatly in size and in the distinctness with which they can be traced. The following description refers only to those of the left side.

In his account of these vessels of the Robert Meyer embryo No. 300, Evans states⁸:

“At this stage the dorsal segmental vessels form in the tissue of the intersomitic clefts large, well-marked vascular arches or loops, one limb of which is against the neural tube, while the other joins the cardinal vein.”

Evans⁷ had previously shown similar loops in chick embryos.

In my embryo I find that loops are present or indicated in case of all of the upper 18 dorsal segmental arteries except the first. Two such typical vessels are shown in plate 6, figure 2. Each dorsal segmental artery extends dorsally and laterally from the aorta. When it reaches a point about halfway up the medullary tube it branches, sending a short branch medially and a longer one laterally. The lateral branch extends through the intersomitic cleft and reaches the posterior cardinal vein as described by Evans. The medial one, I find, soon divides into two distinct smaller branches, a dorsal and a ventral. These extend along the wall of the medullary tube and tend, with their fellows of the opposite side, to encircle it.

The first dorsal segmental artery has a relatively extensive origin from the dorsal aorta. It extends dorsalward between the first and second somites, where it breaks up into several branches. One of these extends for a short distance anteriorly, while others extend caudally toward the second dorsal segmental. A connection between the two, however, is apparently not yet formed. I am also unable to trace a connection between this artery and the anterior cardinal vein. In Ingalls's specimen of 4.9 mm. the first dorsal segmental artery (known also as the hypoglossus artery) likewise sends branches both anteriorly and posteriorly. The posterior branch, however, has joined the second dorsal segmental artery, while the anterior is much longer and is easily recognized as the *arteria vertebralis*. In my specimen, therefore, one sees the very beginning of the formation of the vertebral artery.

The second and third dorsal segmental arteries show only lateral and dorsal branches, while for the fourth only pieces of a typical dorsal segmental artery could be identified with certainty in the sections. It seems improbable that this and other similar vessels are actually incomplete, more probable that they are complete and that in places are so small and indistinctly differentiated from the mesenchyma that the connecting portions have been overlooked. The lateral branches of the second, third, and fourth dorsal segmental arteries unite with the anterior cardinal vein; those of the remaining dorsal segmental arteries join the

posterior cardinal vein. The fifth and sixth dorsal segmental arteries are shown in plate 6, figure 2. The seventh and eighth are similar to the fifth and sixth; in addition the eighth presents a bulbous swelling which lies against the lateral wall of the medullary tube. The ninth is almost typical, but its ventral branch is either lacking or indistinguishable. The tenth to seventeenth show bulbous swellings similar to that of the eighth of various size extending longitudinally along the neural tube. At some places these swellings extend toward one another and probably form the longitudinal anastomoses along the neural tube, which Felix¹⁰ has indicated are present in the Robert Meyer embryo No. 300. Evans⁷ has shown that such anastomoses exist in the form of a distinct plexus in the chick embryos. In my specimen anastomoses very probably are present, for what appear to be networks of endothelial cells connecting adjacent segmental arteries are distinguishable in many places.

The eighteenth to twenty-fourth dorsal segmental arteries seem to be less well developed. They can only be followed with difficulty, owing to their smallness and to the plane in which the sections are cut. Their origins from the aorta, however, are very apparent.

Ventral Segmental Arteries.—The ventral segmental arteries are paired vessels, but they are found only in the lower segments of the body and their segmental arrangement is not so definite as that of the dorsal branches. According to Evans⁸ there is originally a ventral artery for each segment, but those of the upper body-segments degenerate very early. The upper ones together constitute a row of vitelline arteries, which later, by fusion of the individuals of certain pairs, give rise to the unpaired median vessels of the adult.

In my specimen there are 19 to 20 pairs of ventral segmental arteries in all, including those which go into the formation of the umbilical arteries and which should undoubtedly be classified as ventral segmentals. They extend from the seventh segment to the tail. The largest ones are placed opposite the seventeenth and eighteenth somites. All of the ventral segmental arteries pass ventrally along the wall of the digestive tract. Anastomoses, such as Felix has shown, can be made out in certain places. Most of the branches above the nineteenth segment can be traced to the yolk stalk and sac, where they become larger and enter into an extensive plexus. Those below the nineteenth segment go into the formation of the umbilical arteries, as described below.

Lateral Segmental Arteries.—Lateral segmental arteries are found opposite the twelfth to nineteenth segments. They are small vessels, directed laterally at right angles to the longitudinal axis of the aorta. They can be traced as far out as the nephric system. In several places connections can be observed between them and the termination of the medial tributary of the posterior cardinal vein (the beginning median subcardinal veins as described by Evans). I have been unable to determine whether or not lateral segmental vessels exist below the nineteenth segment, owing to the plane of section and to their indefiniteness.

Terminal Branches.—The dorsal aortae terminate in the tail of the embryo by breaking up into distinct networks of small vessels. These plexuses probably

represent the caudal arteries in their earliest stage, but as yet they can not be said to exist as definite arteries. I have referred to one of them in plate 5, figure 2, as the "arterial plexus of the tail."

Arterie Umbilicales.—The umbilical arteries are formed by the union of the lower ventral segmental arteries, including all those caudal to the twentieth segment. Apparently one or two small capillary twigs from the tail plexus also enters into its formation. Anastomoses between the individual roots of the artery are apparent, thus giving rise to a network. In plate 5, figure 2, the network is represented diagrammatically, since I found it unprofitable to attempt to plot them with any degree of accuracy. In the first part of their course, where they lie on either side of the allantoic duct, the umbilical arteries are small and indistinct. Soon, however, they rapidly enlarge and fuse together to form a large trunk within the body-stalk. Reaching the chorion, the single umbilical artery breaks up into a number of branches, which, after repeated division, terminate as capillaries in the substance of the villi. The relation of the allantoic duct to the fork formed by the fusing umbilical arteries has been described above.

CÆLOM.

The cœlom is represented by a continuous elongated cavity which is in wide communication with the extra-embryonic cœlom. Already it can be divided into two distinct parts, the pericardial and the pleuro-peritoneal cavities. The first of these surrounds the heart, except where the heart is attached by means of its sinus venosus behind and by its truncus arteriosus above.

PERICARDIAL CAVITY.

The pericardial cavity reaches its highest point in the region of the bulbus cordis. Its form is shown in plate 6, figures 3 and 4. It is bounded dorsally by the pharynx and septum transversum, ventrally and laterally by the thin body-wall. Its floor is formed by the septum transversum. The floor is deficient on either side dorsally where the transverse septum is not yet complete, and in the space between it and the posterior body-wall the pericardial cavity establishes its communication on either side with the pleuro-peritoneal cavity which lies below.

PLEURO-PERITONEAL CAVITY.

The pleuro-peritoneal cavity is divisible into two portions, an upper and a lower. The upper is formed by two narrow limbs which unite below the yolk-stalk to form the single lower portion. The limbs of the upper portion join the pericardial cavity high up on its dorsal surface. At first they are directed dorsally and caudalward, but very soon bend directly caudalward. As viewed from behind, their median borders are not straight, but each presents two curves, the concavities of which are directed medially. Fitting into the spaces formed by these curves, as shown in plate 6, figure 4, is the lung diverticulum above and the hepatic diverticulum below. That portion of the digestive tube which I have considered as the gastric region lies opposite the constricted area between the two enlarged spaces.

Caudally the two limbs terminate by uniting below the yolk-stalk. Slightly above this level the pleuro-peritoneal cavity joins the exocoelomic cavity on either side and in front where the anterior body-wall is deficient; that is, following the line of reflection of the amnion (plate 6, fig. 3). That portion of the exocoelomic cavity which lies in front bridges across the space between the two above-described limbs. The bridge lies just above the yolk-stalk, while lying between it and the pericardial cavity are the amnion, the amniotic cavity, and the anterior pericardial wall.

The lower portion of the pleuro-peritoneal cavity lies in relation to the hind-gut dorsally and the posterior surface of the yolk-sac ventrally. On either side it is in wide communication with the exocoelomic cavity. As seen in plate 6, figure 3, its anterior surface is irregularly pitted, the cast of the irregular surface of the yolk-sac. Viewed from behind (plate 6, fig. 4), is a deep groove which marks the position of the hind-gut and dorsal mesentery. At the dorsal bend in the back of the embryo the coelomic cavity continues caudally by means of two prolongations (plate 1, fig. 2, and plate 6, fig. 4). These lie laterally to the hind-gut and cloaca. They extend about as far caudally as the cloacal membrane. It is to be noted that these are not united together ventrally; consequently there exists in this portion of the embryo a ventral mesentery as well as a dorsal one.

MESOTHELIUM.

The body-cavity is everywhere lined with a mesothelium which varies in character and thickness in different regions. As a rule, however, it may be said that the visceral layer is thicker than the parietal. This is not entirely true regarding that portion of the parietal pericardium which lies next to the fore-gut, for this is thicker, being composed of two to three layers of cubical cells. Since, however, this layer lies next to the fore-gut, it is in reality visceral, so that its being thick is not actually at variance with the general rule. The parietal pericardium elsewhere, *i. e.*, lining the body-wall and the pericardial surface of the septum transversum, is thin, being composed of a single layer of cubical cells. The visceral pericardium, the previously described epicardium, is formed by a single layer of cubical or rounded cells.

The parietal peritoneum, with the exception of that covering the peritoneal surface of the septum transversum, is thin and formed by a single layer of flattened cells. Where this becomes continuous with the visceral peritoneum (that is, on the dorsal mesentery) it gradually becomes thicker. The inferior surface of the septum transversum possesses a comparatively thick epithelium, being composed of three to four layers of cells. The visceral peritoneum is thick over the fore-gut and yolk-stalk, somewhat thinner over the hind-gut, and thinnest over the yolk-sac, where in most places it becomes lost as a distinct layer.

The lining of the extra-embryonic cavity, *i. e.*, the inner lining of the chorionic vesicle, is not in the form of a distinct cell layer, but appears to be made up of uncovered mesenchyma. Mitotic figures are found throughout all parts of the visceral and parietal epithelium, except in the epicardium.

SEPTUM TRANSVERSUM.

The septum transversum is a thick plate of mesenchyma which is lined on either side with the mesothelium of the cœlomic cavity. It divides off the pericardial from the pleuro-peritoneal cavity, except posteriorly and laterally, where it is incomplete. It is placed across the body at the level of the venous end of the heart and liver diverticulum and is directed obliquely from in front dorsally and cephalad. As near as can be determined, it is placed opposite the fourth and fifth body-somites. With reference to Mall's³⁴ schema (Mall's fig. 400), it may be said that in position the transverse septum of my embryo lies dorsally at a corresponding position which he has shown for embryos of 2 and 4 mm., and is directed at an angle which would pass some place between those of the 2 and 4 mm. embryos.

Laterally and in front the transverse septum is attached to the body-wall. Postero-medially it is attached to the posterior body-wall by means of the sinus venosus and fore-gut. Postero-laterally its border is free and covered with mesothelium. On either side, between this border and the posterior body-wall, are the communications between the pericardial and pleuro-peritoneal cavities.

Within the substance of the transverse septum are found the following structures: Laterally, running along its attachments to the body-wall, are the umbilical veins. Extending from below, nearer the median plane, are the vitelline veins. The network formed at the terminal ends of these veins and the sinus venosus formed by their union all lie within the transverse septum. In addition is the relatively large hepatic diverticulum, which occupies a central position within the septum.

Structurally the transverse septum is composed of a dense synectium of closely packed mesenchymal cells. At the time of fixation these cells were undoubtedly in a state of rapid growth, for mitotic figures are very abundant.

EMBRYONIC MEMBRANES.

AMNION.

The amnion forms a closed sac, in which lies the greater part of the embryo. It is composed of two layers of epithelium: an inner, directly continuous with the skin ectoderm, and an outer, directly continuous with the mesothelial lining of the cœlom. Between these layers is a small amount of mesenchyma.

LINE OF REFLECTION.

The amnion is reflected from the body of the embryo as follows: cephalad it gains attachment to the body-wall at the lower border of the pericardium; laterally the line of reflection crosses the yolk-stalk on either side and continues directly caudally along the edge of the body-wall to the body-stalk. Although directly continuous with it, the demarcation between the anterior body-wall and the amnion is clearly distinguishable because of the greater thickness of the former. The body-wall ends abruptly not only by a sudden diminution of its size, but also by giving origin to the villus-like projections described above (plate 1, fig. 2). Upon reaching the body-stalk the two lines of reflection of the amnion

approach each other, so that the dorsal surface of the body-stalk (dorsal with reference to the embryo) lies within the amnionic cavity. Some little distance beyond the tip of the tail of the embryo, at the distal end of the body-stalk, the two lines come together and complete the continuous line of reflection. The entire anterior half, a small portion of the caudal end, and the dorsum of intervening portions of the embryo and dorsum of the body-stalk lie within the amnionic cavity.

HISTOLOGICAL STRUCTURE.

Structurally the inner layer of the amnion is composed of a single layer of cells. These are for the most part cubical or rounded in shape and lie close together, but in places where they are further spread apart they are greatly flattened. The flattening affects only the protoplasm of the cells, the nuclei remaining rounded. The cells of the outer layer are also rounded and contain rounded nuclei. They are so placed that their inner surfaces, *i. e.*, the surface which is directed toward the embryo, are all joined together along the mesenchyma, while the remaining portions of the cells are free from one another. The cells therefore project outward away from the amnion, each forming a rounded protuberance. In many places these cells are columnar or pear-shaped, the nuclei being distally placed. Between the two layers of epithelium is found a thin layer of mesenchymal tissue, which is exceedingly poor in cells.

In the region of the yolk-stalk the amnion is somewhat thicker along its line of reflection. It is greatly pitted by branching depressions from the exocoelomic cavity lined with the typical mesothelium, which, when cut in cross-sections, give the appearance of blood-vessels. Some of these depressions are shown in plate 7, figures 1 and 3. Similar pit-like depressions have also been found over the greater part of the amnion and all along its line of reflection, on the left side particularly, and on the body-stalk, where the amnion is reflected from it (plate 7, fig. 2).

Although the coelomic depressions when seen in cross-section resemble blood-vessels at first glance, closer examination shows that they are easily distinguishable from them. The shapes of the cells of the endothelial tubes are more flattened than those of the mesothelial; the endothelial nuclei are elongated, while those of the mesothelium are rounded; and in the blood-vessels hemoglobin-bearing blood-cells are usually found, while in the coelomic depressions they are always absent.

I shall here mention the recent work of Bremer³ regarding the formation of the earliest blood-vessels in man. This author finds funnel-shaped growths of the surface mesothelium in the yolk-sac and in the body-stalk, which he believes give rise to a network of blood-vessels. That the pit-like depressions which I have just described resemble in a way the funnel-shaped ingrowths of Bremer's description is apparent. Judging from Bremer's figures, the pits in my specimen are undoubtedly larger and more numerous, but it must be noted that my embryo is somewhat older than any of those which Bremer describes. I am, however, unable to make out any evidences of blood-vessel formation taking place from these depressions. No definite connections are apparent anywhere between the depressions and the blood-vessels.

CHORION.

CHORIONIC MEMBRANE.

The chorion forms a large vesicle, within the cavity of which lies the embryo. It is composed of two continuous layers, an inner mesenchymal and an outer epithelial. The outer surface of the chorionic membrane is covered with chorionic villi over which the epithelium is continuous. On the side adjacent to the attachment of the body-stalk the surface of the chorion is free from villi and here both epithelium and mesenchymal layers are reduced in thickness. This side is entirely collapsed and thrown into a large fold, as shown in text-figure 1.

CHORIONIC VILLI.

The chorionic villi are of variable size. The largest ones, which measure from 1.1 mm. to 1.3 mm. in height, belong to the chorion frondosum. At their bases they are ordinarily smaller than farther out. One of this type is shown in plate 7, figure 4. It divides dirotomously into two large stalks. These again divide and extend out almost to the end as large, stout trunks. The terminal branches are short and form either rounded or pointed projections. Smaller villi are found in between the larger ones, but they are few in number.

HISTOLOGICAL STRUCTURE.

Histologically the chorion and its villi are quite similar. The epithelium which covers the underlying mesenchyma is composed of two distinct layers of cells. The outer of these is made up of cuboidal cells without distinct boundaries. The protoplasm of its cells is finely granular and vacuolated and stains deeply with eosin. Its free surface is in most places covered with a prickle-process border as described by Grosser¹⁴, giving very much the appearance of cilia. In no place, however, could definite cilia be demonstrated. The nuclei are of irregular rounded or oval contour and deeply staining. Beneath this layer is the basal layer of cells, the so-called "Langhan's layer," which is visibly separable from the outer layer. Its cells are also cuboidal, but the protoplasm in most cells is clearer, more distinctly vacuolated, and not so deeply stained. The nuclei are of rounded shape and are clearer than those of the outer layer. The basal surface of this layer rests directly upon the mesenchyma, there being no basement membrane present. It is irregular, owing to the varied shapes of the ends of the cells, which in places seem to be drawn out into processes which unite with those of the mesenchymal cells.

In connection with the syncytial layer are to be described the so-called giant cells and cell islands. The former are masses of deeply staining protoplasm containing several nuclei. They vary in size and in the number of nuclei contained. As pointed out by Frassi¹¹ they are merely portions of the syncytium, which in many cases are still attached to the epithelium. In my specimen such attachments are readily observable.

The cell-islands are masses of trophoblastic cells which, as stated by Grosser¹³, are always attached to villi. They represent portions of the trophoblast which have failed to become spread out over the villi. They are made up of large decidual-like cells of polygonal shape and distinct boundaries. Their nuclei are usually shielded and their protoplasm is clear and vacuolated. In the centers of these cell-islands are evidences of degeneration. The cells are much broken up, nuclei are very

small and irregular, and considerable fibrin is present. The so-called "cell columns" (Grosser) are similar masses of cells, by means of which the villi are attached to the decidua. They are numerous at the ends of the villi. The intervillous spaces are remarkably free from maternal blood-corpuseles, only a few being found.

The stroma of the chorion and its villi is made up of a network of loose mesenchyma. Its cells are stellate in shape and finely granular; the nuclei are oval, rich in chromatin, and contain nucleoli. In places are to be found greatly elongated nuclei, much resembling those of smooth muscle-fibers. These are found just beneath the Langhan's layer.

Throughout the interspaces of the mesenchyma are to be found blood-vessels of various sizes, filled in many instances with blood elements; also the so-called "Hofbauer" cells (Grosser¹³) with highly vacuolated protoplasm and large nuclei; these appear in the body-stalk as well and occasionally in blood-vessels.

EXPLANATION OF PLATES.

PLATE 1.

1. Model of whole embryo viewed from left side. The body-wall in the region of the heart and septum transversum has been dissected away. At X, pericardo-pleural passage. $\times 80$ diameters.
2. Same, viewed from in front. $\times 80$ diameters.

PLATE 2.

1. Brain and portion of neural tube. In light numbers are indicated brain and spinal-cord neuromeres, numbered from the mesencephalon backward. The black lines and numbers indicate the position of the body-segments. It is to be noted that the neuromeres are intersegmental in position. $\times 80$ diameters.
2. Pharynx, cut longitudinally and left half viewed from medial surface. $\times 133$ diameters. From a model by Mr. L. H. Rutledge.
3. Same, viewed from right side with integument removed. $\times 133$ diameters.
4. Ventral surface of same. $\times 133$ diameters.

PLATE 3.

1. Heart as seen from dorsal surface. From a model by Mr. Florian Vaughn. $\times 166$ diameters.
2. Cavity of the heart as seen from dorsal surface. From a model by Mr. Florian Vaughn. $\times 166$ diameters.
3. Model of fore-gut viewed from left side. $\times 266$ diameters.
4. Ventral surface of same. $\times 266$ diameters.

PLATE 4.

1. Portion of section 94 showing beginning of heart sinusoid in ventricular portion of heart. $\times 600$ diameters
2. Portion of section 165 passing through twelfth somite. $\times 325$ diameters.
3. Portion of section 116. $\times 600$ diameters.
4. Nephric system of left side viewed from medial surface. From wax reconstruction. $\times 125$ diameters.

PLATE 5.

1. Graphic reconstruction of venous system. $\times 80$ diameters. At X, junction of large veins with sinus venosus.
2. Graphic reconstruction of arterial system. $\times 80$ diameters. Between X and X the dorsal aortae have fused, forming a single vessel.

PLATE 6.

1. The sinus venosus and entering veins. From a wax reconstruction by Mr. T. F. Wheeldon. $\times 120$ diameters.
2. Wax reconstruction showing relations of the dorsal segmental arteries. Viewed from behind forward. $\times 120$ diameters.
3. Wax reconstruction of the coelomic cavity viewed from in front. $\times 63$ diameters.
4. Same, viewed from behind. $\times 63$ diameters.

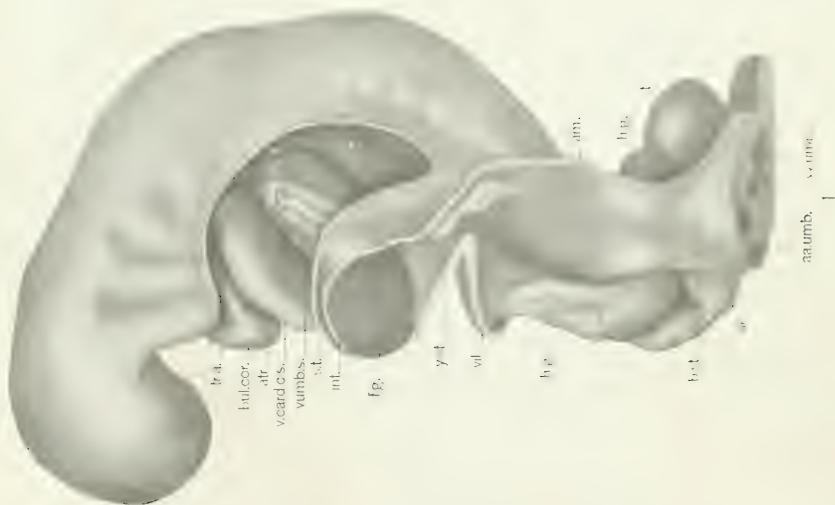
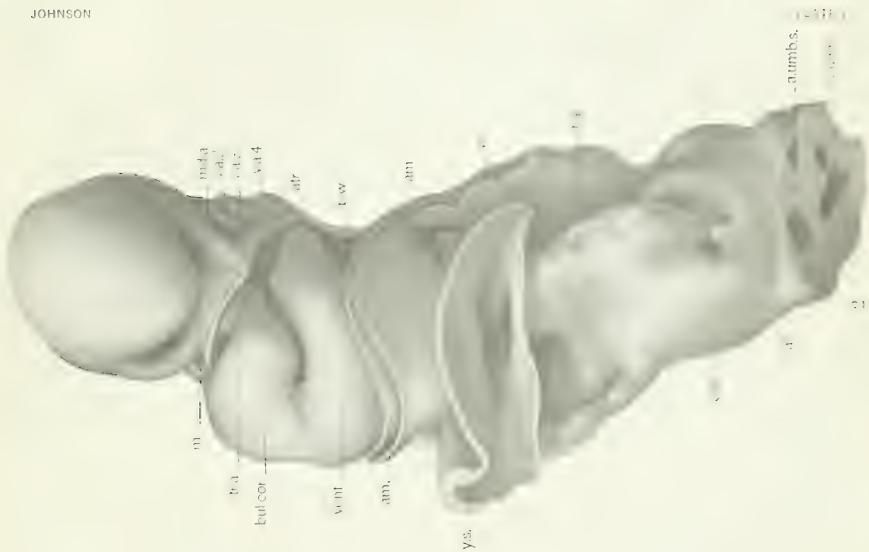
PLATE 7.

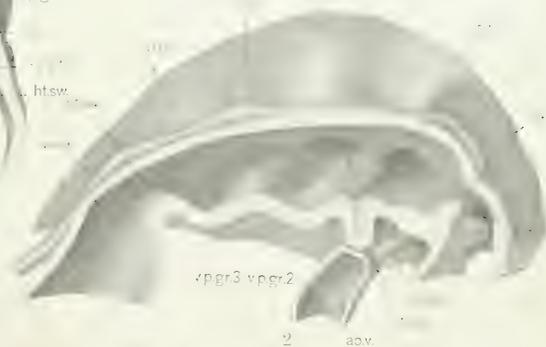
1. Portion of section 127, showing coelomic depressions in region of septum transversum and amnion. $\times 400$ diameters.
2. Portion of section 269, showing coelomic depressions in region of body-stalk. $\times 275$ diameters.
3. Cast of coelomic depressions in region of transverse septum. The irregular depressions are in places branched, as shown on the left side of figure. $\times 215$ diameters.
4. Villus from chorion frondosum. From a wax reconstruction by Mr. H. L. Houchins. $\times 60$ diameters.
5. Graphic reconstruction of left half of embryo, viewed from medial side. Numerous models have been used in making the drawing. $\times 60$ diameters.

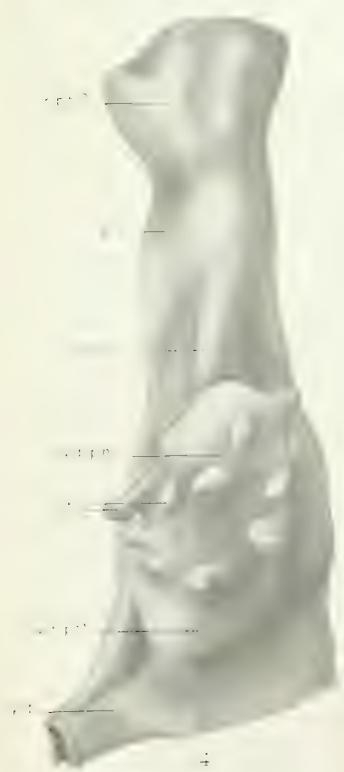
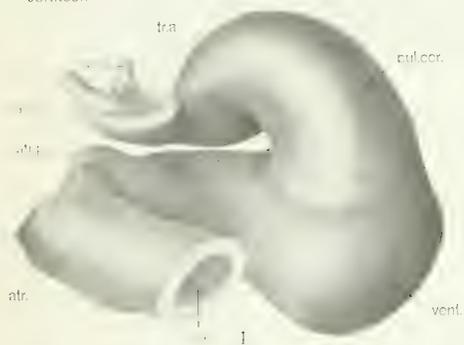
PLATE 8.

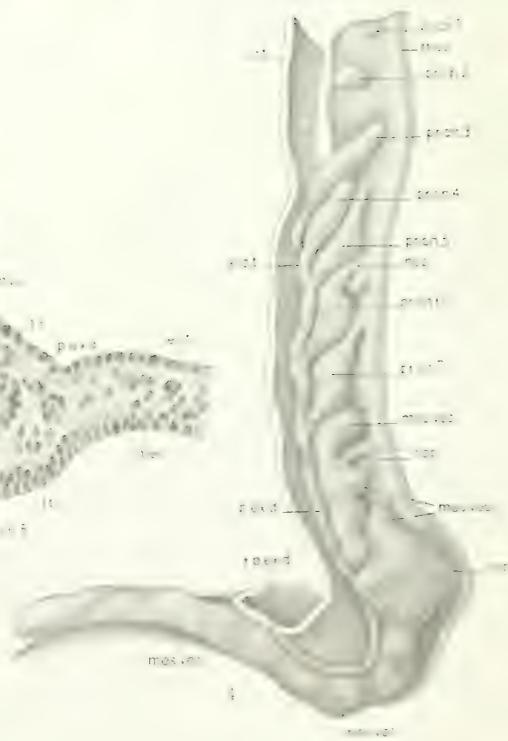
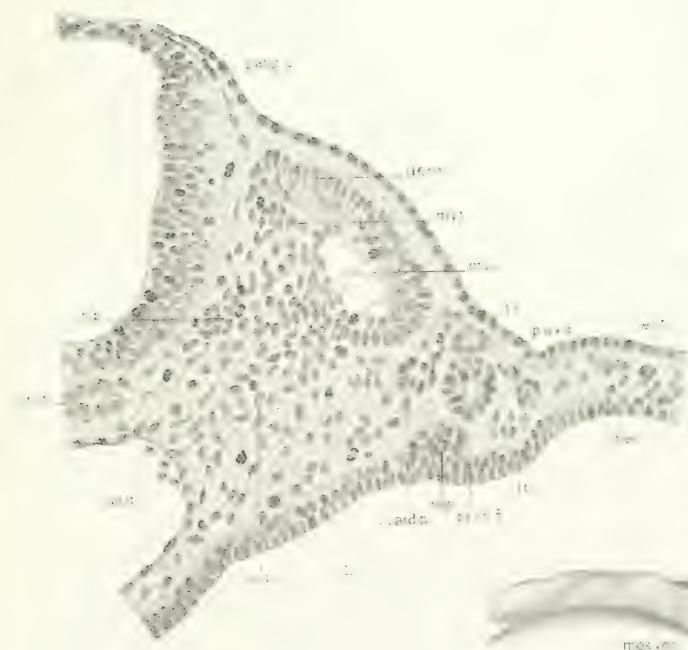
1. 12. Cross-sections of embryo. $\times 40$ diameters.

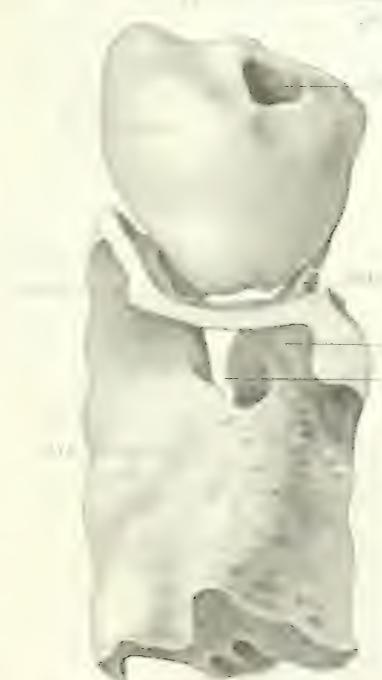
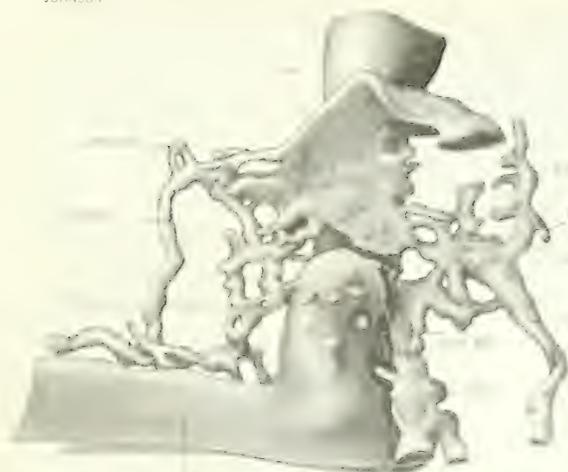
1. Section 31.	4. Section 95.	7. Section 140.	10. Section 211.
2. Section 48.	5. Section 110.	8. Section 162.	11. Section 219.
3. Section 63.	6. Section 123.	9. Section 180.	12. Section 254.

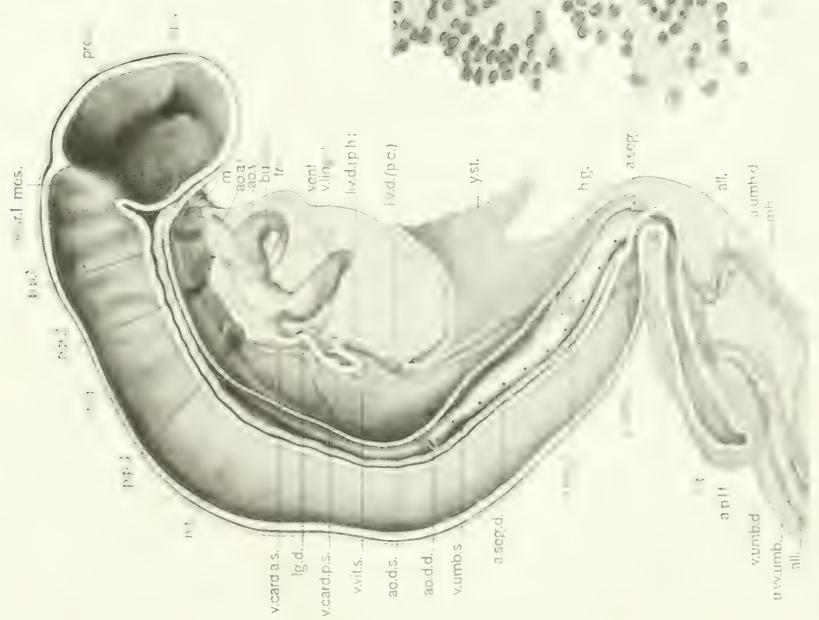
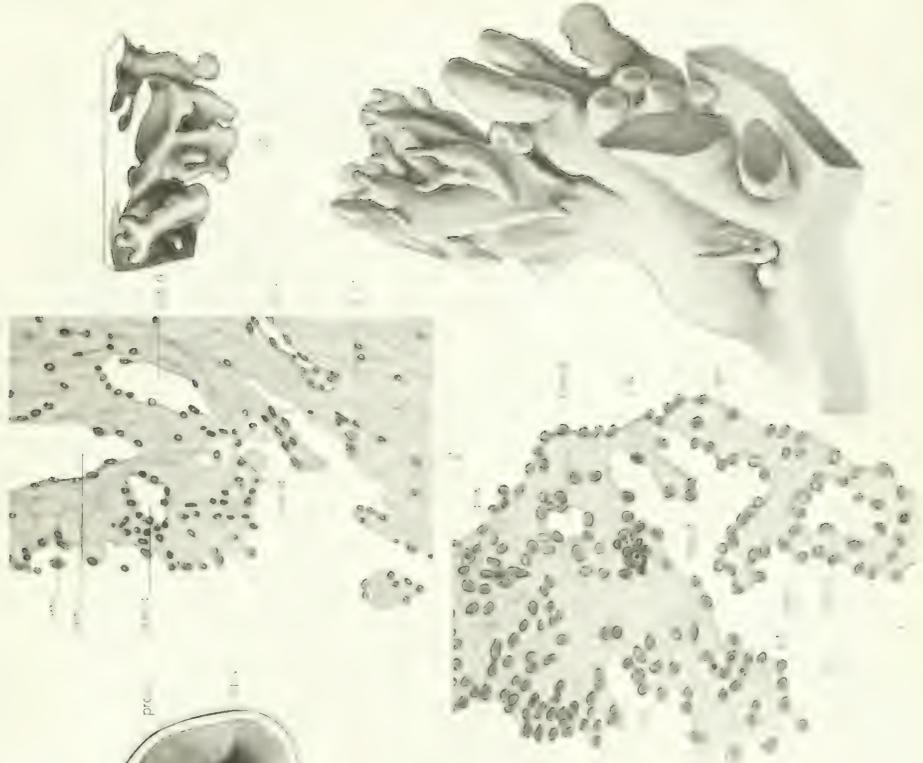


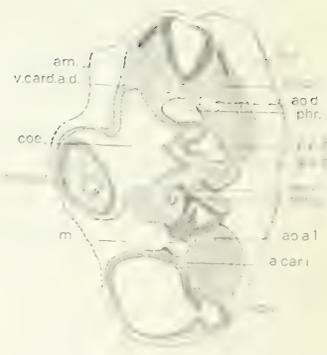
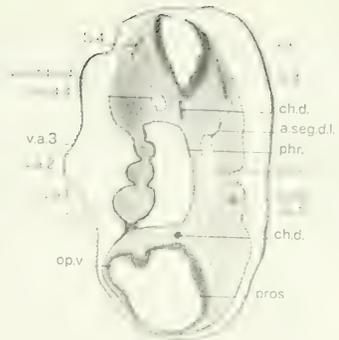
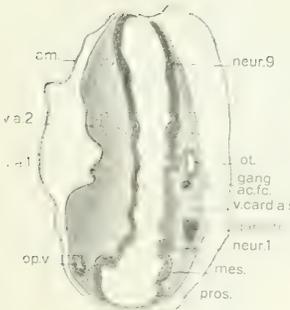












ABBREVIATIONS.

a. bas.,	a. basilaris.	md. a.,	mandibular arch.
a. car. c.,	a. cerebri (?).	m. b.,	medial branch of a. seg. d.
a. car. l.,	a. carotis interna.	m. and l. t.,	medial and lateral tributaries of v. card. p.
a. seg. d.,	a. segmentalis dorsalis.	m. c.,	mesocardium.
a. seg. l.,	a. segmentalis lateralis.	mes.,	mesencephalon.
a. seg. v.,	a. segmentalis ventralis.	mes. vos.,	mesonephric vesicles.
a. umb.,	a. umbilicalis.	meso.,	mesothelium.
a. umb. d.,	a. umbilicalis dextra.	mit.,	mitotic figure.
a. umb. s.,	a. umbilicalis sinistra.	m.,	mouth.
aa. umb.,	aa. umbilicales.	m. c.,	myoepicardium.
all.,	allantois.	myc.,	myocoele.
am.,	amnion.	myt.,	myotome.
am. c.,	amniotic cavity.		
ao. d.,	aorta dorsalis.	nep.,	nephrostome.
ao. d. d.,	aorta dorsalis dextra.	n. t.,	medullary tube.
ao. d. s.,	aorta dorsalis sinistra.	neur.,	neuromere (1-21).
ao. v.,	aorta ventralis.		
ao. v. a.,	aortic arch.	op. v.,	optic vesicle.
ao. p.,	aortic process of sclerotome.	ot.,	otocyst.
a. pl. t.,	arterial plexus of tail.	or. m.,	oral membrane remnants.
atr. p.,	atrial projection.	p. c.,	pericardial cavity.
atr.,	atrium.	p. d. mes.,	position of dorsal mesentery.
aud. p.,	auditory pit.	p. p. d.,	pleuro-pericardial passage.
		p. p.,	pharyngeal pouch.
		p. p. d.,	pharynx.
b. c.,	blood-corpuscles.	p. ex. d.,	primary excretory duct.
b. v.,	blood-vessels.	p. t.,	principal collecting tubule.
b. st.,	body-stalk.	p. lg. d.,	position of lung diverticulum.
b. w.,	body-wall.	pr. ch.,	pronephric chamber.
bul. cor.,	bulbus cordis.	proc. inv.,	proctodeal invagination.
		pros.,	prosencephalon.
cap.,	capillary.	p. v. mes.,	position of ventral mesentery.
cap. pl.,	capillary plexus.	p. v. s.,	position of yolk-sac.
c. int.,	caudal intestine.	p. v. st.,	position of yolk-stalk.
ch. d.,	chorda dorsalis.		
ch. can.,	chordal canal.	scd.,	sclerotome.
ch. p.,	chordal process of sclerotome.	s. t.,	septum transversum.
cloa.,	cloaca.	s. s.,	sinus sagittalis superior.
cl. mem.,	cloacal membrane.	s. v.,	sinus venosus.
c. m.,	cuticular membrane.	s.,	somite (1-22).
coe.,	coelom.		
coe. cloa. d.,	coelom on right side of cloaca and caudal intestine.	t.,	tail.
coe. cloa. s.,	coelom on left side of cloaca and caudal intestine.	tear,	tear in tissue.
coe. d.,	coelomic depressions.	thy. d.,	thyroid diverticulum.
coe. f. g.,	coelom surrounding fore-gut.	tr. a.,	truncus arteriosus.
cop.,	copula.	t. imp.,	tuberculum impar.
		t. p. ex. d.,	termination of primary excretory duct.
d. b.,	dorsal branch of medial limb of a. seg. d.	u. aa. umb.,	union of aa. umbilicales.
		u. vv. umb.,	union of vv. umbilicales.
ect.,	ectoderm.	v. card. a. d.,	v. cardinalis anterior dextra.
end. ht.,	endothelial heart.	v. card. a. s.,	v. cardinalis anterior sinistra.
end. p.,	endothelial process.	v. card. c. d.,	v. cardinalis communis dextra.
end.,	endothelium.	v. card. c. s.,	v. cardinalis communis sinistra.
ex. coc.,	exocoelomic cavity.	v. card. p. d.,	v. cardinalis posterior dextra.
		v. card. p. s.,	v. cardinalis posterior sinistra.
f. g.,	fore-gut.	v. c. a. (?),	v. cerebri anterior (?).
		v. c. m.,	v. cerebri media.
gang. ac. fe.,	ganglion acustico-facialis.	v. c. p.,	v. cerebri posterior.
gang. c.,	ganglion crest.	v. ling-f.,	v. linguo-facialis.
gang. gl. ph.,	ganglion n. glossopharyngei.	v. oph.,	v. ophthalmicus.
gang. tri.,	ganglion n. trigemini.	v. umb. d.,	v. umbilicalis dextra.
gang. va.,	ganglion n. vagi.	v. umb. s.,	v. umbilicalis sinistra.
gas. r.,	gastric region.	v. vit. d.,	v. vitellina dextra.
g. c.,	gill-cleft.	v. vit. s.,	v. vitellina sinistra.
		vv. a.,	veins of visceral arches (I-IV).
ht. sw.,	heart swelling (Grosser).	vv. umb.,	venae umbilicales.
h. g.,	hind-gut.	v. pl. h. w.,	venous plexus of body-wall.
		v. pl. h. g.,	venous plexus of hind-gut.
inf.,	infundibulum.	v. pl. y. st.,	venous plexus of yolk-stalk.
int.,	integument.	v. b.,	ventral branch of medial limb of a. seg. d.
i. e.,	intersegmental enlargement of v. card. p.	v. p. gr.,	ventral pharyngeal groove (1-3).
		v. p. gr. 1 (p. 1.),	ventral pharyngeal groove-posterior limb.
l. b.,	lateral branch of a. seg. d.	vent.,	ventricular portion of heart.
l. t.,	lateral tributary of v. card. p.	vil.,	villus-like projections of body-wall.
liv. d. (p. c.),	liver diverticulum (pars cystica).	v. a.,	visceral arch (1-3).
liv. d. (p. h.),	liver diverticulum (pars hepatica).	y. s.,	yolk-sac.
liv. tr.,	liver trabeculae.	y. st.,	yolk-stalk.
lg. d.,	lung diverticulum.		

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