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CONTENTS OF VOL. IX.

ON SEVERAL ANATOMICAL CHARACTERS OF THE HUMAN BRAIN, SAID TO VARY ACCORDING TO RACE AND SEX, WITH ESPECIAL REFERENCE TO THE WEIGHT OF THE FRONTAL LOBE.

BY

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A survey of the literature on the peculiarities of the brain in men of genius, in women and in the lower races indicates that some anatomists have thought they could determine, almost at a glance, whether or not a given specimen came from a great man, a woman or from a negro. I refer especially to the older works of Huschke and of Parker and to the more recent ones of Spitzka and of Bean.

Huschke[1] cut the frontal lobe from the rest of the brain at the line of the coronal suture, that is he removed that portion of the cerebrum which is covered by the frontal bone and compared it with the rest of the brain. The result showed a *decidedly* greater amount of frontal lobe, fully one per cent (!) in the male than in the female. The fresh brains that were studied by Huschke were simply cut with a knife along the line mentioned above. He further states that the central sulcus is straighter, more perpendicular and nearer the front end in the female brain, the difference in position being about 12½ per cent of the brain length.[2] The latter figures were obtained from wax casts of brains.

Huschke also expresses himself regarding the negro brain as follows: "Aus allem diesen geht hervor, dass das Negerhirn,

[1]Huschke. Schädel, Hirn und Seele. Jena, 1854.

[2]The misprint in Huschke, p. 153, has been copied by Eberstaller, p. 41. The number given is 86.1 per cent, it should be 56.1 per cent.

sowohl das grosse wie das kleine, ja auch das Rückenmark, den Typus des kindlichen und weiblichen Hirns eines Europäers besitzt und ausserdem sich dem Typus des Hirns der höheren Affen nähert," etc.

It is admitted by Huschke that it is extremely difficult to recognize a difference in the convolutions due to sex, but, "es ist aber keine Frage, dass sie existiren." He further generalizes, as has often been quoted, that in the male there is more frontal lobe: "Das Weib ist ein *homo parietalis* und *interparietalis,* der Mann ein *homo frontalis,* und das Weib hat deshalb auch ein runderes Gehirn, als der Mann." According to his measurements it was found that in seven women the frontal lobe, *i. e.* the portion of the brain covered by the frontal bone, contains 23.9 per cent of the brain weight. In fifteen men it contains 24.4 per cent. So it was actually determined by weighing the parts of the brain that the frontal lobe in men is one per cent heavier than in women. This difference he believes corresponds with the differences of the areas of the surface of the brain as well as with that of its volume. It may be noted that the individual frontal lobes given in his tables range from 21.8 per cent to 26.1 per cent, the values being often recorded to the second decimal place (*e. g.,* 24.49 per cent).

Meynert[3] examined 157 brains from insane individuals by separating the mantle from the brain stem which included the basal ganglia and some of the gray substance of the island. He then cut the mantle through the central sulcus with a scissors which gave him the frontal lobe composed of the brain tissue in front of the fissure of Rolando minus the basal ganglia. This portion was then compared with the rest of the brain mantle. He concludes that in men as contrasted with women there is relatively more brain substance in front of the central sulcus than behind it—a conclusion which, it seems to me, is not justified by his own figures. They are as follows. (Note especially the summary in the third table.)

According to Donaldson,[4] Broca divides the cerebrum into three lobes, one of which is the frontal, limited behind by the central

[3]Meynert. Das Gesammtgewicht und die Theilgewichte des Gehirns, etc., Vierteljahrsschrift für Psychiatrie, Bd. 1, 1867.

[4]Donaldson, Growth of Brain, London, 1895.

sulcus and including below its share of basal ganglia. The average weight of Broca's frontal lobe is 43.5 per cent for men and 43.7 per cent for women, thus contradicting what has been asserted by Huschke and by Meynert. When the brain is distorted, due to artificial

MALE.

Age in Years.	Weight of Mantle. Grams.	Weight of Frontal Lobe. Grams.	Per Cent, of Frontal Lobe.	No. of Specimens Examined.
1–19	866	380	43.8	4
20–29	1030	428	41.5	15
30–39	1035	428	41.3	21
40–49	1034	426	41.1	26
50–59	969	402	41.3	23
60–69	1020	424	41.5	12
70–79	948	384	40.5	1
FEMALE.				
20–29	922	390	42.3	10
30–39	910	374	40.1	16
40–49	916	380	41.4	17
50–59	919	378	41.1	8
60–69	917	366	40.0	2
70–79	846	358	42.3	1
80–89	894	390	43.6	1

WEIGHT OF THE FRONTAL LOBE PER 1000.

	Male.	Female.
During development	416	425
" middle age	414	416
" old age	412	410
" all ages	414	415

deformity of the skull, this percentage remains practically unchanged.[5] I have been unable to consult Broca's original papers, but Professor Donaldson has kindly sent me the necessary data which I append in a foot-note.[6]

[5]Ambialet. La Déformation Artificielle de la Tête, etc. Tonlouse, 1893.

[6]In Broca's collected papers, Memoires Anthropologiques, T. V., page 131, under the title, "Sur le poid relatif des deux hemisphere cerebreux et de leur lobes frontaux," he gives a brief statement to the effect that he

It would seem as if the above statements settled the question of the relative size of the frontal lobe in men and women, but the following remarks are of historical interest. It is noted above that Huschke believed he had shown the central sulcus to be more perpendicular and not as far back in the female as in the male, thus making the frontal lobe smaller in the former.

Rüdinger[7] studied the brains of twin fœtuses and believed that he demonstrated that the development in the male is more advanced than in the female and that the frontal lobe is larger in the male. Recently his question has been thoroughly tested by Waldeyer[8] who found that the development of the brain of the male is more advanced in the majority of specimens of twin fœtuses of opposite sexes, but

weighed (1) the entire encephalon, (2) bulb, (3) cerebellum, (4) pons, and then separated each hemisphere by "deux coupes" into three lobes. In this manner he treated 440 cases.

There is every reason to think that he uses the term "hemisphere" in its technical sense, as he knows the difference between that and the mantle. This would involve the basal ganglia in the lobes as he records them.

Further, in the Bulletin Société d'Anthropologie, T. VI, 1871, page 113, in the article entitled "Sur la deformation toulousaine du crane," he gives numerical statements which lead to the same conclusion. The hardened brain in question weighed

	825 grams
Cerebellum	109 grams
Left hemisphere	339 grams
Right hemisphere	351 grams
Total	799 grams

leaving the difference between that and the weight of the entire encephalon, 26 grams for the pons and medulla. These 26 grams are not too much for the weight of the pons and bulb, and on the other hand are not nearly enough to cover the basal ganglia, see "Growth" etc., page 101. It seems probable therefore that his hemispheres included the basal ganglia.

If we take now his analysis of the right hemisphere, weight 351 grams, he gives the frontal lobe 159 grams, occipital lobe 45 grams, and parieto-temporal lobe 147 grams, total 351 grams. Thus his three lobes equal the weight of his hemisphere, and his hemisphere contains the basal ganglia, and I believe that it is by reasoning similar to this that I arrived at the conclusion expressed on page 181 of my book, to which you refer.

[7]Rüdinger. Verhandl. d. Anatom. Gesell., 1894.

[8]Waldeyer. Sitzungsber, d. K. P. Akad., 1907.

that in individual specimens this was not always the case, "so dass wir noch keinesweges in der Lage sind, von einem 'gesetzmässigen Verhalten' wie es Rüdinger tut, sprechen zu können." My own experience confirms Waldeyer's, for while the male of twin pregnancies is often markedly larger than the female it is by no means always so. Of course, this does not mean that the frontal lobe is relatively larger in the male.

More extensive measurements were made by Passet[9] who studied with great care the brains of 17 adult males and 12 females. He found the position of the central sulcus much the same in both sexes, if anything a little further back in the male than in the female. He shows by a diagram (Fig. 6) that there is a great deal of variation of the position of this sulcus in different brains, its angle with the sagittal plane ranging from 46° to 79°. The average is 62° for the male and 64° for the female. He states that the central fissure is shorter and straighter in the female and lies farther forward. Although his work was done with the greatest of care his methods are too crude, the number of specimens studied too small, and the degree of variation so great, that nothing is proved regarding the relative size of the frontal lobe in the two sexes.

Eberstaller[10] in the discussion of the above question in his excellent monograph on the frontal lobe concludes that there are no differences due to sex in the angle that the central sulcus of the brain makes with its sagittal median plane. His measurements included 300 hemispheres and he found that the above mentioned angle varies constantly between 70° and 75°. He further found that the central sulcus when extended intersected the sagittal border of the mantle at 65.4 per cent of the distance from the olfactory trigonum to the occipital pole in men and at 66 per cent in women. If this means anything it indicates that the frontal lobe in the brain of women is relatively larger than it is in men. The objections to the conclusions of Huschke and Passet regarding the percentages of brain in front and behind the central sulcus are fully discussed by Eberstaller, who points out the weaknesses of their observations as well as the objections to their conclusions.

[9]Passet. Arch. f. Anthropologie, XIV, 1883.
[10]Eberstaller. Das Stirnhirn. Wien, 1890.

Cunningham[11] confirms fully the conclusions of Eberstaller in the examination of 86 brains of various ages. "At no period in its growth does the fissure of Rolando exhibit in its position what we might safely regard to be sexual differences." Mingazzini[12] seem to be of different opinion. Regarding his statement, Waldeyer sounds a warning as follows: "Des weiteren möchte ich herzu noch bemerken, dass es mir sehr misslich erscheint, Schlüsse aus Untersuchungen zu ziehen, die auf wenige beobachtete Fälle sich erstrecken." He further remarks that his own experience agrees with the results of Eberstaller and of Cunningham.

It seems to me that it is quite apparent that with the methods used by the above named investigators it cannot be definitely concluded that there is a marked difference between men and women in the relative amount of brain in front of the central sulcus. The variations in various brains are so great that an approximately correct percentage can only be obtained from a very large number of specimens and those have been supplied only by Eberstaller and by Cunningham. Furthermore, the personal equation of the investigator plays a very important rôle in studying a question of this kind, and even if Eberstaller and Cunningham have proved that there is no difference in the position of the central sulcus due to sex, they have not proved that the weight of the frontal lobe does not show such a difference. In fact the methods employed to determine the relative weight of the frontal lobe are so crude that unless the differences found are constant and marked we must challenge the statements of those who assert that differences due to sex exist. I would like to ask them to separate a collection of 100 brains (50 of men and 50 of women) each of the same weight and see how well they can do it. Until their "guesses" prove to be correct in over 50 per cent of the specimens examined we must conclude that the "differences," like those of Huschke, are largely due to the personal equation of the investigator.

While these various attempts, which we consider unsuccessful, have been made to show that there is an unlike distribution of the

[11]Cunningham. Jour. Anat. and Physiol., Vol. 25, 1891.
[12]Mingazzini. Lezione di Anat. clinica dei centri nervosi. Torino, 1905.

brain substance in women and in men, attempts have been made to show that in the brains of negroes as well as in those of men of genius similar distinctions can be found. In general the differences in weight between each of these three classes of brains is fully 100 grams, and if it were shown that the proportion of their parts is different in each class it would be a discovery of great importance. The smaller frontal lobe in women and in negroes, and the larger in men of genius would prove, it is believed, that this portion of the brain is the chief seat of a good mind. It appears, however, that no such unequal distribution of brain substance exists.

A few years ago the startling announcement was made by Spitzka[13] that the area of the cross section of the corpus callosum was larger in eminent than in ordinary men, that of Leidy being 10.6 sq. cm. Since the corpus callosum is associated mainly with the frontal lobe the observation, if correct, would be of great significance. The question was immediately tested[14] by comparing in over 150 white and negro brains the area of the cross section of the corpus callosum with the brain weight and it was found that these characters varied with each other (see Bean, Chart V).[15] Since the average weight of the brain of eminent men is about 100 grams heavier than the average brain weight of ordinary men, and since the average negro's brain is 100 grams lighter, the error of Spitzka is easily explained, for in making his comparison he did not take brain weight into consideration. According to Spitzka the brains of "notable men possessing large capacity for doing and thinking much more than their fellows," "compared with ordinary men, individually and collectively, have larger callosa. The callosum of Joseph Leidy exceeds in cross-section that of any other in this series or recorded in literature. Here again, then, we have an index in somatic terms of how we may distinguish the brain of the genius or talented man from that of

[13]Spitzka. Connecticut Magazine, 1905, and Proc. Amer. Assoc. Anat., Amer. Jour. Anat., 1905.

[14]Bean. Amer. Jour. Anat., Vol. 5, 1906.

[15]Spitzka has not mentioned Bean's observation in his last monograph in the Trans. of the American. Philosoph. Soc., XXI, 1907. Bean compares area of the corpus callosum with the volume of the brain, which is statistically objectionable, but the point made is strong enough to question seriously Spitzka's statement.

persons of only ordinary abilities" (p. 303). What he says regarding the callosum of Leidy is true, but regarding the rest he is in error. All the rest of the callosa of notable men given by Spitzka are not above the average for brains of the same weight, and the callosa given in his group of ordinary men (which are from electrocuted criminals) are very much below the average (compare Spitzka's Tables A and B with Bean's Chart V and with the data given in my table). In fact many negroes of lighter brain weight have larger callosa than most of Spitzka's eminent men. Cope's callosum as measured by Spitzka is far below the average of brains weighing over 1500 grams. Comparing Spitzka's records with Bean's and mine it would be more correct to state that criminals have callosa much smaller than the average.

Furthermore, Bean believed that he had shown that the genu is relatively larger and the splenium is relatively smaller in the negro, an assertion which is even more striking than Spitzka's. From this as well as from other data Bean deduced that the frontal lobe is smaller in negro brains than in white. This is in apparent contradiction to the results he obtained by comparing the position of the central sulcus, which in 126 hemispheres holds about the same position in the two classes of brains. If anything, it lies more posterior in the female negro (Table IV*a*, p. 381) which would indicate that her frontal lobe is relatively the largest of all.

All of Bean's measurements are made from a brain axis which passes in the sagittal plane between the two hemispheres immediately above the anterior commissure and just below the splenium. As a rule this line (the axis) passes parallel with the longest axis of the corpus callosum and just below it. From this line he erected two perpendiculars, one just in front of the genu and one just behind the splenium. The distance between the two perpendiculars was then divided into ten parts, the first three, including the genu, he calls the genu, the second three the body, the next two the isthmus and the last two, including the large rounded splenium, the splenium. He then compared the area of the genu with that of the splenium, using the former as ordinates and the latter as abscissæ in the construction of his Chart VII. It was found by this treatment that

the negro brains separated almost completely from the white brains, in Bean's Chart VII, and this line of separation I have inserted at the proper place in my chart, Fig 1.

I have tabulated as Bean did the area of the genu with that of the splenium in 106 brains and do not find that the symbols for the brains of the two races separate. Most of the negro brains in my chart are intermixed with the white brains above the line which separates them in Bean's chart. My measurements were all made by tracing the outline of the corpus callosum with the very accurate projecting apparatus made by Hermann of Zurich, while Bean's were made with a less precise instrument borrowed from the Smithsonian Institution. The areas of both Bean's and my own were made with a Conradi planimeter whose minimum registration is 10 sq. mm. and its probable error was found to be 10 sq. mm. In order to exclude my own personal equation, which is an item of considerable importance in a study like this, all of the tracings as well as the measurements of all of the areas were made without my knowing the race or sex of any of the individuals from which the brains were taken. The brains were identified from the laboratory records just before the results were tabulated.

Tabulation of the brain weight with the area of the cross section of the corpus callosum confirms what Bean found, that is, the area increases with the brain weight. The same is true when the area of the corpus callosum minus that of the splenium is tabulated with the weight of the frontal lobe. However, there are great individual variations, but they seem to be of like extent in both the white and the negro brains. The female records separate somewhat from the male, but this is due no doubt to the lighter weight of the former.

My figures do not confirm Bean's result that the genu is relatively larger and the splenium relatively smaller in the white than in the negro brain. The specimens I examined include 18 brains which Bean studied, and I find that the measurements I made of the areas of the genu and splenium in them do not agree altogether with his. Ten of the specimens are white and eight negro brains. In making the comparison a deviation of 10 sq. mm. is overlooked, for this error is to be expected from the planimeter we employed. The genu

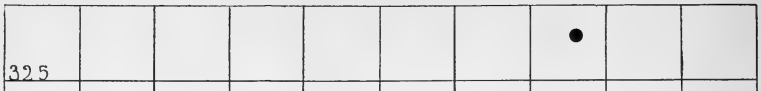

FIG. 1. Showing the relation of the area of the cross section of the genu (ordinates) to that of the splenium (abscissæ). The figures represent square millimeters. The diagonal line is in the position which separated the whites from the negroes in Bean's Chart VII.

is larger in Bean's tables than in mine in 7 white brains and one black brain and smaller in 4 black and 2 white. The splenium is larger in 7 black and 4 white and is not smaller than mine in a single instance in Bean's tables. This discrepancy between our figures is sufficient to account for the racial differences in the corpus callosum found in Bean's tables but not in mine, although the individual deviations in both our charts are very great. I think my chart (Fig. 1) shows conclusively, as far as possible with the method I employed, that there is no variation in either genu or splenium of the corpus callosum due to either race or sex.

In order to determine the relative weight of the frontal lobe in white and in negro brains I made numerous tests in separating this lobe from the rest of the cerebrum to develop first an accurate method. It was found that it is quite easy to break the cerebrum after it has been hardened in formalin through the central sulcus along the motor tract down through the basal ganglia with considerable precision. The real test of the accuracy was made by comparing the results obtained on the right side with those on the left. If the half brains are of equal weight the frontal lobes should be also of equal weight if the method is a reliable one. It was found in over two-thirds of the brains that the two frontal lobes weighed practically alike, *i. e.*, within 5 grams of each other, a variation which could be accounted for by a slight difference in the amount of drainage and evaporation of water from the specimens. In the remaining one-third of the brains the difference between the two sides averaged 10 grams, which in rough equals the weight of half of the precentral gyrus. Expressed differently the probable observational error in the weight of the frontal lobe compared with the whole hemisphere is less than one per cent of its weight, so a deviation in the weight of the frontal lobe due to race or sex would have to be fully two per cent in order to be detected.

Another source of error might be due to the fact that only hardened brains were broken, or could be broken, with precision through the central sulcus. It is well known that formalin causes the brain to swell, and it has been shown by Hrdlicka[16] that there is an unequal

[16]Hrdlicka. Brains and Brain Preservatives. U. S. Nat. Mus., XXX, Washington, 1906.

expansion of the brain, due to both its age and its size. So it is possible for the frontal lobe at first to expand more rapidly than the rest of the brain, and later to shrink more quickly. This, of course, would affect the percentage of the frontal lobe and is a source of error to be reckoned with. The presence of a second preservative like common salt, alum or carbolic acid, which was used in a number of my specimens, is also to be taken into account, for they influence very much the change of volume of the brain.

In order to test this question I weighed the pieces of 5 brains a number of times during a period of nearly a year and found that there was much fluctuation in the brain weight, but the percentage value of the frontal lobe remained very constant, usually within one-half of one per cent.

The figures are as follows. The first weighing was made as soon as the brain was fairly hardened at the end of about a week, so the weights of the parts when fresh were not obtained. Those marked with a star (*) are the weights recorded in the Table and in the Figures.

No.	1907.				1908.	
	March 19.	May 1.	June 4.	Nov. 8.	Jan. 25.	
2861	1110	1190	1120*	1035	1040	gm. of cerebrum.
	44	44	44—	44—	43.5	% value of frontal lobe.
2864	1250	1215*	1190	1150	1150	gm.
	44+	44—	44	44—	44	%
2865	1300	1325	1460*	1210	1235	gm.
	44.5	45	45	44.5	44.5	%
2867	830	870	875*	765	780	gm.
	42	43.5	43—	43	43	%
2878	1170	1240	1205*	1080	1090	gm.
	43—	43.5	43.	43.5	43.	%

No special care was taken to keep the strength of the formalin constant, in fact it was often changed, and this accounts for the fluctuations in the weight of the whole brain. In all cases the parts of each brain were kept together in a single jar in order to subject them to the same strength of formalin from weighing to weighing.

I also weighed the parts of a number of well hardened brains a second time after they had been in formalin for another year. In these the fluctuations of the weight are less marked and the deviation of the percentage value of the frontal lobe is, if anything, less than in the first set. The data are as follows. The figures given in the first column are the ones entered in the charts.

As said above, my personal equation was excluded entirely because all of the breaks and weighings were made without my knowing the race or sex of the individual from which the specimen came.

No.	Jan., 1907.	Jan., 1908.	
1521	1035	1045	weight of cerebrum.
	44.5	45	% value of frontal lobe.
1697	780	775	weight
	45—	45+	%
1720	1030	1025	weight
	43+	43.5	%
1836	950	895	weight.
	45—	45—	%
1840	1140	1130	weight
	44.5+	44.5—	%
2621	1015	1025	weight
	45—	45—	%
2660	960	1020	weight
	42	43	%
2665	830	825	weight
	41—	41+	%
2667	895	885	weight
	44	43.5	%
4x	930	950	weight
	41	42	%

In general I used Broca's method to divide the frontal lobe from the rest of the cerebrum and found as he did that the mean weight of the frontal lobe in both men and women is between 43 and 44 per cent. The same is true for both the negro and the white. This bears out what I have found by measuring the area of the genu and splenium and leads to the conclusion that it is incorrect to state that the frontal lobe of the negro brain is relatively lighter than that of the white.

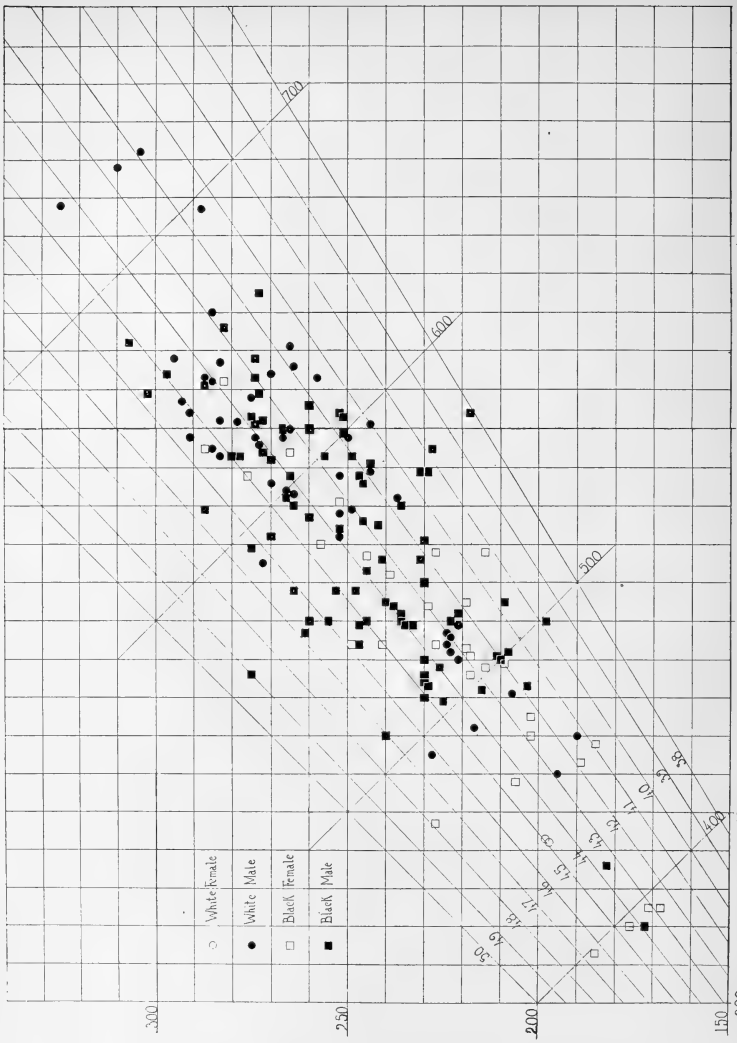

FIG. 2. Showing the relation of the brain substance lying in front (ordinates) of the sulcus centralis to that lying behind it (abscissæ). Each symbol represents a half brain.

The figures are in grams. The long diagonal lines, 38-50, indicate the percentage of the precentral brain weight. The lines marked 400-700 indicate the weights of the hemicerebra. The weights given are reduced to those of the fresh brain.

All of my figures are given in the table at the end of this article and their bearing upon the percentage of the frontal lobe is given in the two charts. In the first chart, Fig. 2, the weight of each hemisphere is treated by itself and the weights are all reduced to their weight in the fresh state. Of course, only those brains in which the weight when fresh is known could be included in this chart. In making the chart the weights of the frontal lobe are given in ordinates and those of the rest of the hemisphere in abscissæ. Thus each symbol gives an individual half brain. The diagonal lines give the percentage of the frontal lobes and the diagonal lines at right angles to them the weight of the hemi-cerebra. The symbols in the first block and to the left represent hemi-cerebra, between 400 and 500 grams, the next block between 500 and 600 grams, etc.

It is noticed that the weights of the hemicerebra range from less than 400 to over 700 grams and that the percentage of the frontal lobes fluctuates from 38 per cent to 49 per cent. The mean is about 43.5 per cent. If in each block the black and the white, and the male and the female are compared it is seen that the distribution is quite even and that on an average the percentage of the frontal lobe is the same in both races and sexes.

In order to give the question another and possibly a better test, I tabulated all the brains in which both halves were weighed, but did not reduce the figures to those of the fresh weight, for in a number of specimens this is not given. Then the combined weight of both sides was divided by two, thus giving the average weight of the frontal lobe of each brain and that of each hemicerebrum behind the central sulcus. In this chart, Fig. 3, each symbol represents a whole cerebrum divided by two, and in it more of the symbols are shifted to the left, for in general there is more shrinkage of the brains due to the long action of formalin and carbolic acid. The individual deviations are not as great as they are in Fig. 2 (39 per cent to 48 per cent) but the mean is about the same (43.5 per cent). Again there is no separation of the brains due to race or sex.

I must therefore conclude that with the methods at our disposal it is impossible to detect a relative difference in the weight or size of the frontal lobe due to either race or sex, and that probably none

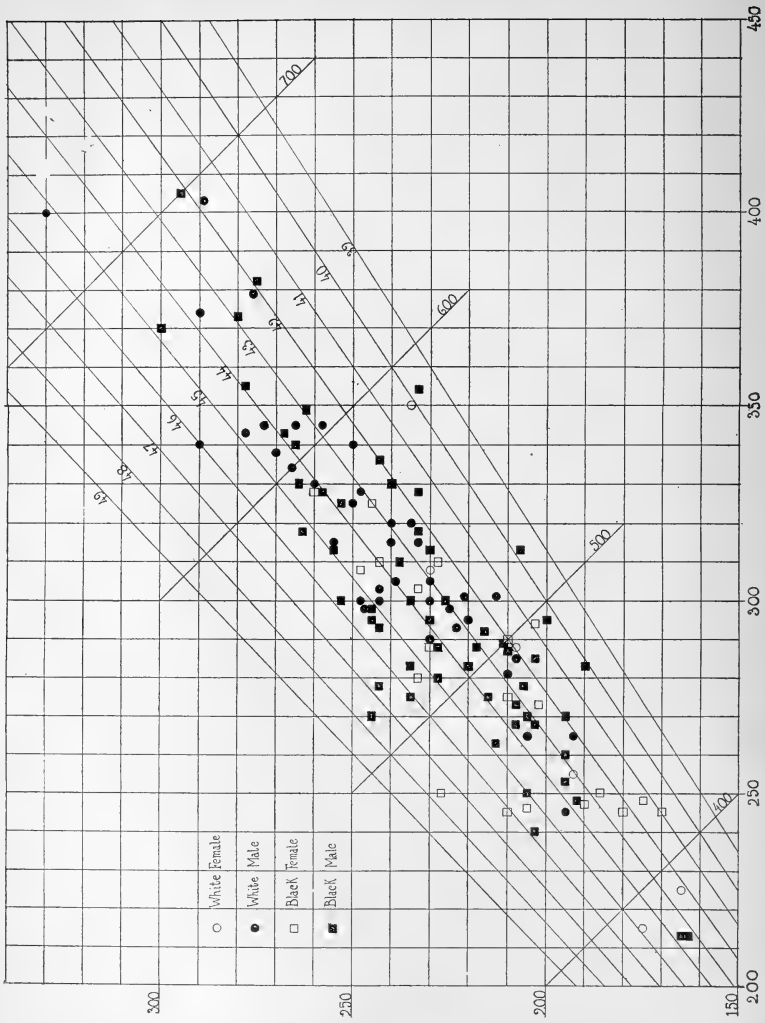

FIG. 3. The same as Fig. 2 with the exception that each symbol represents the average of the two sides of the brain. Each, therefore, represents a whole brain. The weights given are those of hardened brains.

exists. My weighings of the frontal lobe were made in three series and each time I did not know the race or sex of the individual whose brain was being tested until it had been broken and weighed. There were 6 white and 6 negro brains in the first series and the racial difference found in it was very marked,—41 per cent of frontal lobe in negro brains and 44 per cent in white brains. In the next series of the brains, the white and the negro brains came closer together and in the third series of about 10 brains this difference was lost altogether. It is evident, as Schwalbe and Pfitzner[17] have pointed out, that a percentage to be of any significance must not change as the records increase in number.

As it is generally believed that the brains of men of genius are of complex configuration, so it is also believed that the brains of lowly races are of a simple and embryonic type. Thus Parker[18] says that the Sylvian fissure in the negro is 5/8 inches (16 mm.) shorter than in the white and the central sulcus is simpler, straighter and less undulated. He also found a negro brain in which there was a complete connection between the fissures of Sylvius and Rolando. He states that the occipital fissures are ape-like with a well marked perpendicular fissure. The negro brain as it presents itself in this country, he says, bears an unmistakably nearer relation to the ape type than does the white, being also more fœtal in character.

To anyone who is familiar with the negro brain the statements of Parker appear to be careless and superficial. His observations upon the length and form of the fissures of Sylvius and Rolando can not be taken seriously in the light of recent studies of these fissures, and they strike one rather as an opinion supported by a strong personal prejudice, as are so many of the observations upon the gyri of sulci. Furthermore, other students of the negro brain found no such difference and state that they are practically like the white (see Tiedemann, Luschke and Marshall.) Schwalbe,[19] who reviews the work of Parker, states expressly that racial differences in the negro

[17]Schwalbe and Pfitzner. Morph. Arbeiten, Vol. 3.

[18]Parker, A. J. Cerebral convolutions of the negro. Proc. Acad. Nat. Sci., Phila., 1878.

[19]Schwalbe. Neurologie, 1881, p. 575.

brain are in all probability due to similar racial peculiarities of the skull. The same statement is also made by Hrdlicka and has been fully tested by Bean. However, such differences are but slight, for a variation in the shape of the skull influences only the main outlines of the brain and not its gyri. The flattening over the anterior association area, as first observed by Hrdlicka, was fully confirmed by Bean and can be seen in most full-blood negro brains, certainly in more than one-half. One precaution must always be taken in these cases and that is to compare whites and negroes of the same type of form of the skull. The majority of negroes are dolichocephalic and these should be compared only with dolichocephalic whites.

In order to make a preliminary test of this question I attempted to assort a collection of negro and white brains, calling those with the peculiar narrowing and flattening of the upper surface of the frontal lobe, negro, and those in which it was more convex, white brains. The brains tested were a mixed lot which happened to be on one shelf in the brain room. After they had been assorted according to the character above mentioned I found that there were 60 negro and 30 white brains and that their assortment was correct in exactly 75 per cent of the cases. Had all of the brains been dolichocephalic I think the test would have fallen out better, and Dr. Hrdlicka informs me that this is also his opinion.

I then mixed the brains again, added to their number, and assorted them a second time according to the richness of the gyri and sulci, using as a standard the two illustrations given on Plate 54 in Retzius' *Menschenhirn*. In case the configuration was complex, of the Gauss type, it was called stenogyrencephalic, and in case it was simple, of fœtal type, it was called eurygyrencephalic. Doubtful specimens, and there were many of them, were at first set aside and in case it was impossible to render a decision regarding them by a second effort they were excluded altogether.

The results of this test, based upon brains of unknown origin at the time it was made, are given on the opposite page.

The percentage of eurygyrencephaly and stenogyrencephaly is therefore about the same in both races.

In order to make a further comparison the brains pictured in

Retzius' *Menschenhirn* were arranged into two classes to correspond with his types given on Plate 54. This is, of course, more difficult to do and a large number of doubtful ones were necessarily excluded. The classification of the pictures into two groups was made independently by Dr. Mellus, Dr. Sabin and myself, none of us know-

Negro.			
Male.		Female.	
Eurygyrencephaly.	Stenogyrencephaly.	Eurygyrencephaly.	Stenogyrencephaly.
32 brains 68%	15 brains 32 %	12 brains 64 %	7 brains 36%
White.			
19 brains 66%	10 brains 34 %	1 brain 50 %	1 brain 50 %

ing at the time whether the illustrations in question were of the brains of men or of women. Our results are given in the following table:

	Male.		Female.	
	Eurygyrencephaly.	Stenogyrencephaly	Eurygyrencephaly.	Stenogyrencephaly
Dr. Mall.....	29 brains (53 %)	26 brains (47 %)	12 brains (60 %)	8 brains (40 %)
Dr. Sabin.....	29 brains (58 %)	23 brains (42 %)	10 brains (62 %)	6 brains (38 %)
Dr. Mellus....	23 brains (64 %)	14 brains (36 %)	7 brains (54 %)	6 brains (46 %)

Although our results vary considerably they are substantially similar. In general stenogyrencephaly is a little more common in the Swedish brains pictured by Retzius than in the 97 negro and white brains of Baltimore used in constructing the first table. Unless

one attempts to separate brains into complex and fœtal types he does not realize the difficulties in doing it and I think the deviation in a second attempt might be fully ± 10 per cent of the first determination. If the personal equation were added the deviation might be much greater.

The above tables are given to show how unreliable the statements regarding the complexity of the gyri and sulci may be, and that with the present crude methods the statement that the negro brain approaches the fœtal or the simian brain more than does the white is entirely unwarranted.

In this connection the recent statement of Elliott Smith regarding racial peculiarities in the brain should also be considered. It relates to the so-called Affenspalte. Smith[20] says: "It often happens (especially in the brains of lowly human races, such as negroes and aboriginal Australians, and in the anthropoid apes) that the sulcus occipitalis anterior, together with the sulcus occipitalis inferior form a large arc (parallel to the sulcus lunatus) forming the anterior limit of a great tongue of cortex, the tip of which often reaches the upper end of the sulcus temporalis superior in those cases in which there is no temporo-parietalis. The presence of this great arcuate sulcus explains much of the misleading literature relating to the search for an 'Affenspalte' in the human brain."

The "Affenspalte" first described by Rüdinger has caused anatomists much trouble and its presence in all human brains was often questioned. A few years ago Elliott Smith[21] demonstrated that a marked occipital operculum which is identical with that of the gorilla's brain is often present in the brain of the Egyptian fellah. However, the operculum is not always well marked, but it is bounding sulcus, which Smith calls the sulcus lunatus, can be seen in every human brain. Smith's studies are directed rather towards the homology of the Affenspalte which he has fully demonstrated with the aid of the structure of the cortex, *i. e.*, the extent of the stripe of Gennari.[22] At first he showed that the Affenspalte (sulcus lunatus)

[20]E. Smith. Jour. Anat. and Physiol., Vol. 41, 1907.
[21]Smith. Anat. Anz., 24, 1904, p. 74.
[22]Smith. Anat. Anz., XXIV, p. 437.

is present in all Egyptians brains[23] and later he found it present in negro, Syrian, Turkish and Greek brains and with a study of literature he concluded that it is a normal feature of the adult human brain. It would have been easy for Smith to draw a wrong conclusion regarding this sulcus, for he began his study of it with the Egyptian brain; however, he did not end there.

It may also be noted that Parker states that he found a negro brain with a gyrus cunei on the surface as is the case in the simian brain. Since Parker gives no illustrations it is difficult to ascertain whether or not he saw only an annectent gyrus partly on the surface, as described and pictured in Quain's Anatomy.[24] This latter condition I have also observed in both negro and white brains. Until it is thoroughly investigated in a large number of specimens its meaning still remains an open question. Probably it will fall, as do other anatomical peculiarities of the negro when they are fully investigated.

I wish to add a remark regarding the anatomy of the negro. One is often led to believe[25] that there are more anatomical anomalies in the negro than in the European body. I have now had considerable experience in the dissection of the negro and have yet to observe that variations are more common in the negro than in the white. In fact it seems as if excessive development of facial muscles and other variations is more common in the white, but until a large number of statistics are collected no definite statement can be made. However, we have made many thousands of records of nerve variations and find in them no racial peculiarities.[26] The misleading statements are based upon a few dissections of negroes in which the variations found are given as peculiarities of the race. An equal

[23]Smith. Anat. Anz., XXIV, p. 216.

[24]Quain's Anatomy, Tenth Edition, Vol. 3, p. 144 and Fig. 102.

[25]For example, Duckworth. Morphology and Anthropology, 1904.

[26]In tabulating these nerves Bardeen and Elting (Anat. Anz., XIX, 1901, p. 132) say that race seems to play no very marked part as a cause in the number or kind of variations (see also Anat. Anz., XIX, p. 217). In his later and more extensive publication Bardeen does not consider race in the tabulation of nerve variations, presumably because it did not seem to influence them (Amer. Jour. Anat., VI, 1907.)

number of variations will be found in any corresponding series of white cadavers.

The hope has often been expressed that through the study of the brains of men of genius anatomical conditions would be found which may account for their eminence. In fact one of the first studies included the brain of Gauss[27] and showed that this particular brain was unusually rich in gyri and sulci. Since then the brain of Gauss has often been used as a type representing the highest development. But Wagner says that higher intelligence may exist in individuals with brains either rich or poor in gyri, but the normal brain must be of a certain weight, a certain richness of gyri and sulci as well as certain thickness of cortex. Since Wagner's time quite a large number of brains of distinguished persons have been studied and in general the conclusion has gradually been reached that with the methods at our disposal we are unable to detect in their anatomy conditions to account for great mental ability. The recent studies of Retzius[28] all point in this direction, for he was unable to detect anything remarkable in the brains of distinguished individuals, and no one is more competent than this investigator to deal with this subject.

Within a year the report on the brains of Mommsen, Bunsen and Menzel has been published by Hansemann[29] who has also given an account of the anatomical findings in the brain of Helmholtz. Hansemann also concludes his study with a healthy scepticism, for he says that within physiological limitations we cannot tell the brain of a distinguished person from that of an ordinary one. He then falls back on the analogy that muscular men are not necessarily athletic, but under proper conditions could easily become so. Furthermore, he predicts that individuals with unusual qualities in one direction, but who are otherwise quite inferior, like mathematical prodigies or remarkable chess players, may possess brains with portions unusually well developed. The recent study by Stieda[30] of the brain of a man

[27]Wagner. Vorstudien zu einer Wissenschaft. Morphol. d. Menschl. Gehirns, etc., 1862.

[28]Retzius. Biol. Unt., VIII, 1898, IX, 1900.

[29]Hansemann. Ueber die Gehirne von Mommsen, Bunsen und Menzel, Stuttgart, 1907.

[30]Stieda. Zeit. f. Morph. u. Anthropol., XI, 1907.

who spoke fifty languages gave a negative result, for nothing peculiar was found in it. However, Hansemann states that we should expect to find a morphological basis to account for geniuses of the first rank, for they possess qualities peculiar to themselves. In fact the configurations of the brains of Helmholtz and Menzel showed some peculiarities which may support this theory.

The one ray of hope in the study of the peculiarities of the configuration of the gyri and sulci comes from the comparison of brains of members of the same family which often show many similarities. This important discovery was made by Spitzka,[31] who observed that there were hereditary resemblances in the brains of three brothers. This was fully confirmed by Karplus[32] in studying the brains of 21 groups of relations in each of which he found a marked similarity of the gyri and sulci. The configuration of the right side has a tendency to repeat itself on the right side, and the left on the left, but peculiarities on the right side are not found on the left in near relatives. There is an hereditary tendency in the fissuration of the brain as there is in the other features.

Nevertheless, even if we should find that the brains of two eminent men of the same family were much alike we have by no means shown that the genius has an anatomical basis. Furthermore, it seems to have been established that anatomical variations often show different percentage in different communities. Schwalbe and Pfitzer[33] have shown, for instance, that the absence of the psoas minor is as follows.

	Men			Women		
	No. of Cases.	No. of times absent.	Per Cent.	No. of Cases.	No. of times absent.	Per Cent.
St. Petersburg.....	900	405	45.	600	326	54.3
Strassburg.....	386	219	56.7	175	99	56.6
Boston	400	223	55.8	208	145	69.7
England.....	210	125	59.5	130	93	71.5

[31]Spitzka. American Anthropologist, VI, 1904.
[32]Karplus. Obersteiner's Arbeiten aus d. Neurol. Inst., XII. Wien, 1905.
[33]Schwalbe and Pfitzer, Morph. Arbeiten, Bd. 3.

In each group the percentage had reached a constant value, that is with an increase of the number of cases the percentage in a given locality did not change. The same condition may exist in brain configuration, and Merkel[34] states that the brains from cadavers used for dissection in Göttingen, and which come from Brunswick, of which Gauss was a native, were often very rich in gyri and sulci. On the other hand, in Mecklenburg, where Merkel also had had a large experience, brains of the Gauss type were never seen in the dissecting room, but instead a very simple type prevailed.

It certainly would be important if it could be shown that the complexity of the gyri and sulci of the brain varied with the intelligence of the individual, that of genius being the most complex, but the facts do not bear this out, and such statements are only misleading. I may be permitted to add that brains rich in gyri and sulci, of the Gauss type, are by no means rare in the American negro.[35]

While there seems to be no evidence to show that the configuration of the brain of genius is different from that of other brains, there is some evidence in favor of the statement that there are slight differences due to sex. It is often said that the brains of women are of a simple type, but if their weight is not considered it is questionable whether a collection of brains could be assorted according to sex with any degree of certainty. Furthermore, even the more pronounced differences of eurygyrencephaly and stenogyrencephaly are not easily recognizable because they are not easily measured. Of course, when gyri of the simple type are twice as broad as those of the complex type, as pictured on Plate 54 in Retzius' *Menschenhirn,* it is not difficult, but there are many intermediate stages and the observer can only express an opinion, for there is nothing that can be weighed or measured. Waldeyer states that to determine whether a brain

[34]Merkel. Top. Anat., I, Braunschweig, 1885-1890.

[35]Spitzka, Amer. Phil. Soc., Vol. 21, has arranged a number of figures in plates showing the evolution of the complexity of the gyri. For example, in his Fig. 8 the gorilla with a simple brain is below, the brain of a Bushwoman is in the middle and that of Gauss, the most complex, is above. In another plate, Fig. 10, the brain of Gambetta holds the lower position, Altmann the middle and Skobeleff the upper. Comparing Figs. 8 and 10 it appears that Gambetta's brain resembles the gorilla's more than it does that of Gauss.

came from a man or woman is much like identifying the sex of the individual from which a given skull came. I am not so optimistic and would rather take my chances with the skull.

In the article by Schwalbe and Pfitzer mentioned above many anatomical variations are tabulated and there do not seem to be more variations in the male than in the female, but the percentage of variations is by no means always alike in the two sexes. If there is a percentage difference according to sex in a special variation it tends to remain constant in various sets of statistics and does not become the same as the records are increased in number. Moreover, "bei den weiblichen Fällen werden in der Regel die Werthe viel rascher constant als bei den männlichen." In other words, a smaller number of records are required in the female than in the male to obtain the true percentage of variations. How much this indicates is by no means clear, but this conclusion should be that there is not a simpler type, but less variations in the female, which appears to be the opinion of Retzius regarding the female brain.

We have tested this difference by grouping the illustrations of brains in the great *Atlas* of Retzius under simple and more complex types, without knowing whether the picture of a brain in question was from a man or from a woman and obtained the result given on page 19. In the first line in the table my estimates are found with the percentages below them. In the second line another estimation by Dr. Sabin is given, and in the third line one by Dr. Mellus. In general the opinion expressed in these estimations does not bear out the notion that the configuration of the brains of women is of a simpler type than in those of men.

This, however, is only our opinion regarding the complexity of the gyri and sulci of pictures of brains. But Retzius has tabulated in an excellent way a number of concrete data of 100 brains which can easily be tested in other specimens. These include a number of variations, such as the central sulcus communicating with the fissure of Sylvius, regarding which there can be little difference of opinion. There are in all 73 such records, 19 being of the norm and 56 of variations. Each of these records can be entered a second time by subtracting its frequency in percentage from 100. Thus, if the central

sulcus communicates with the fissure of Sylvius in 3 per cent of the cases it is called a variation in 3 per cent of the cases, while in the remaining 97 per cent it is normal. In this way I obtained a column of 73 records, representing the norm as well as the variations for each hemisphere both of the male and the female. The average of these figures is as follows.

Men.				Women.			
Right Side.		Left Side.		Right Side.		Left Side.	
Norm.	Variations.	Norm.	Variations.	Norm.	Variations.	Norm.	Variations.
78%	22%	75%	25%	81%	19%	81%	19%

This table indicates that the brain of woman is not nearer the norm but varies less than does that of man. Could all the variations found be grouped together in single brains, leaving the rest as perfectly normal, then 76 brains of men and 81 of women out of our 100 would be exactly normal in the arrangement of the gyri and sulci.

Retzius has done us a great service in pointing out the way by which this problem can be attacked by the statistical methods. A few remarks regarding his conclusion may be made, but before they can be criticised properly it will be necessary to tabulate many other brains, as he has done, of both men and women.

In the first column of figures in Retzius' table regarding the fissure of Sylvius both the norm and the variation is given, but the missing figures can easily be obtained by subtracting the given percentage from 100. In case the average of a given record is more than 50 in both male and female, it is called normal, while when it is less it is called a variation. Thus the central sulcus anastomoses with the sulcus precentralis superior in 18 per cent of the cases and therefore these do not anastomose in 72 per cent. It may be remarked that the number of brains of men studied by Retzius is somewhat small, while that of women is decidedly too small, for in the latter each single record equals 8 per cent when reduced to the scale of 100.

The data given by Retzius regarding differences in the gyri and sulci due to sex may be criticized from two standpoints. Those in

which there is a marked difference between the brains of men and women may be tested by other records. For instance, according to Retzius the anterior branch of the fissure of Sylvius is divided and forms an operculum frontale intermedium in 82 per cent of the brains of men and in 100 per cent in those of women. At this point woman's brain forms a perfect norm, being richer in all cases in gyri and sulci. However, only four specimens of brains of women without an intermediary operculum would have made the results for the two sexes exactly alike. No doubt a larger number of records would have shown, even in Stockholm, that the operculum frontale intermedium is not always present in the female brain. I notice that Karplus, in the article mentioned above, figures four brains of women without the operculum frontale intermedium, and states expressly that it is missing in those four specimens which were found in a relatively small number of brains. His record will bring the chief difference, given by Retzius, pretty close to the male average of 82 per cent. The second criticism can only be made by collecting many more statistics along the lines laid down by Retzius in his great monograph.

At any rate what has been written by Karplus is to the point: "Auf die von den Autoren angegebenen einzelnen Geschlechtsmerkmale der Gehirne, die ja von vielen bestritten werden, will ich hier nicht näher eingehen. Auch hier muss zunächst viel mehr Material gesammelt werden, bisher bin ich nicht davon überzeugt, dass sich aus dem Furchenbild eine Inferiorität des weiblichen Gehirns ableiten liesse."

The question of the type of the female brain, a subject which has been discussed so much, is therefore still far from being solved in a satisfactory manner.

Furthermore, it is by no means established that there are male and female types of the brain due to the form and arrangement of the gyri and sulci, as has been so frequently asserted. Each claim for specific differences fails when carefully tested, and the general claim that the brain of woman type is fœtal or of simian type is largely an opinion without any scientific foundation. Until anatomists can point out specific differences which can be weighed or measured, or until they can assort a mixed collection of brains, their assertions

TABLE OF BRAINS STUDIED.

No.	Length of body.	Color.	Sex.	Weight of Brain.	Weight of Parts of the Brain. (Hardened)				Cerebellum.	Per cent to be added to or subtracted from hardened brain to make it equal fresh.	Area of Section of Corpus Callosum.		
					Right side.		Left side.				Whole.	Genu.	Splenium.
					Anterior.	Posterior.	Anterior.	Posterior.					
	cm.			gm.	gm.	gm.	gm.	gm.	gm.		sq. cm.	sq. cm.	sq. cm.
1405	170	W.	M.	....	290	340	...	...	180		...	...	...
1451	153	B.	M.	....	265	335	265	345	170		6.3	3.0	1.6
1452	153	B.	F.	....	250	330	240	320	160		5.5	2.4	1.4
1453	162	B.	M.	....	212	319	201	304	166		5.9	2.1	1.3
1454	160	B.	M.	....	205	265	200	270	160		5.9	2.3	1.6
1457	173	W.	M.	....	230	300	220[1]	295[1]	160		...	...	...
1458	173	W.	M.	....	270	345	270	330	170		...	...	...
1478	178	B.	M.	....	220	295	215[2]	280[2]	170		5.8	2.3	1.8
1485	162	W.	F.	....	170	230	160	220	130		2.7	.6	1.3
1489	191	W.	M.	....	240	305	245	295	145		6.9	2.8	1.4
1493	158	B.	F.	....	205	250	205	250	160		5.4	2.3	1.2
1510	170	W.	F.	....	205	290	210	285	155		...	...	...
1519[3]	130	B.	M.	....	190	250	194	245	150		4.3	1.7	1.0
1521	158	B.	F.	....	225	295	235[4]	280[4]	135		6.6	2.5	1.6
1527	158	W.	F.	....	185	255	200	225	120		...	...	...
1591	165	W.	M.	....	220	290	220	300	180		6.4	2.1	1.7
1682	165	W.	M.	....	245	305	240	300	165		6.9	2.7	1.5
1683	173	W.	M.	1410	210	295	205[5]	275[5]	160	+23	6.6	2.5	1.5
1686	165	B.	F	....	185	252	195	242	128	..	6.4	2.3	1.7
1690	168	W.	M.	....	262	335	270	332	182	..	7.0	2.4	2.0
1696	178	W.	M.	1440	250	350	250	330	180	+ 6	...	...	...
1697	158	W.	F.	1000	175	215	175	215	110	+12½	4.9	2.2	1.0
1699	163	B.	M.	1230	235	315	240	305	155	— 2	6.1	2.5	1.5
1707	160	W.	M.	1300	190[6]	230[6]	200	260	150	+26	4.2	1.4	1.0
1716	168	W.	M	1320	235	310	230	320	150	+ 6	5.6	2.5	1.4
1720	186	W.	M.	1430	225	280[7]	220	305[7]	170	+20	7.1	2.3	2.0
1728	163	B.	M.	1393	230	315	230	310	150	+13	5.9	2.5	1.5
1787	...	B.	M.	1200	205	290	200	280	210	+ 1	6.1	2.0	1.4
1788	175	B.	M.	1470	...	...	...	...	190	..	...	...	...
1789	170	B.	M.	1210	205	275	210	260	175	+ 9	5.3	1.9	1.4

1790	163	B.	M.	1280	235[8]	300	250	285[8]	155	+ 4½	5.9	2.3	1.3
1811	168	B.	F.	1195	210	280	210	275	170	+ 4	6.2	2.1	1.5
1835	175	W.	M.	1420	260	330	260	330	170	+ 5	5.8	2.5	1.4
1836	165	B.	M.	1190	215	265	210	260	160	+ 7	6.3	2.2	1.2
1837	186	W.	M.	....	280	375	272	382	196		5.4	2.3	1.8
1840	158	W.	M.	1370	253	315	255	315	175	+ 4	5.7	2.4	1.4
1847	163	B.	F.	1195	180	240	180	250	130	+22	4.3	1.8	1.0
1867	180	B.	M.	1250	235	285	235	280	160	+ 5	5.5	2.0	1.4
1868	175	W.	M.	1620	280[9]	395[9]	295	410	195	+ 3	8.8	3.4	1.9
1869	163	B.	M.	1150	230	280	240	270	120	0	6.7	2.3	1.8
1874	178	W.	M.	1200	205	265	205	265	140	+11	5.0	2.0	1.5
1877	165	B.	M.	1340	250	300	255	300	180	+ 4	...	...	...
1895	175	W.	M.	1480	247	295	247	300	165	+18	..–	...	...
1896	186	B.	M.	1310	215	300	225	295	185	+ 7	5.9	2.2	1.5
1906	186	B.	M.	1450	260	320	255	335	180	+ 7	5.8	2.2	1.6
1907	158	B.	M.	1205	205	270	205	270	170	+ 8	6.0	2.1	1.6
1908	163	B.	M.	1240	213	285	218	298	155	+ 6	5.7	2.2	1.3
1909	158	B.	F.	1120	210	242	200	250	150	+ 7½	...	...	...
1950	178	B.	M.	1350	230	320	235	315	140	+ 9	7.1	2.7	1.5
1962	147	B.	F.	1220	235	280	230	280	140	+ 4	5.6	2.2	1.8
1963	165	B.	M.	1250	225	275	220	280	150	+ 9	4.0	1.5	1.3
1971	175	B.	M.	1330	240	330	240	330	160	+ 2	...	...	...
1972	158	W.	M.	1360	250	280[10]	245	320[10]	152	+ 9	6.4	2.7	1.6
1976	158	B.	F.	1050	173	250	177	245	140	+ 7	5.6	2.1	1.7
2004	168	W.	M.	1390	215	300	210	302	170	+16	...	...	...
2005	158	B.	F.	1110	200	250[11]	220[11]	240	160	+ 3	6.3	2.8	1.5
2021	163	B.	F.	1400	265	325	255	330	170	+ 4	7.0	2.7	1.8
2022	165	W.	M.	1450	260	340	270	350	165	+ 5	...	...	...
2027	165	B.	F.	1300	235	305	230	300	175	+ 4	5.7	2.3	1.4
2028	175	B.	M.	1200	230	290	230	285	165	0	5.3	2.2	1.2
2469	...	B.	M.	....	205	295	215[12]	280[12]	155		5.1	1.9	1.6
2536	170	B.	M.	....	190	265	200	255	170		5.9	2.3	1.5
2612	169	B.	M.	1350	250	290	240	300	150	+10	5.1	2.2	1.5
2614[13]	173	W.	M.	1500	230	290	230	290	160	+25	7.3	2.8	1.7
2620	175	B.	M.	1450	220	280	220	285	165	+24	5.7	2.0	1.6
2621	160	B.	M.	1350	225	280	230	280	170	+10	5.7	2.4	1.5

No.	Length of body.	Color.	Sex.	Weight of Brain.	Weight of Parts of the Brain. (Hardened.)				Cerebellum.		Area of Section of Corpus Callosum.		
					Right side.		Left side.				Whole.	Genu.	Splenium.
					Anterior.	Posterior.	Anterior.	Posterior.					
	cm.			gm.	gm.	gm.	gm.	gm.	gm.		sq. cm.	sq. cm.	sq. cm.
2622	172	B.	M.	1450	200	245	205	235	155	+40	6.6	2.9	1.6
2629	172	B.	M.	1400	210	295	210	295	160	+20	7.5	2.6	1.5
2632	176	B.	M.	1450	215	275	215	275	150	+28	4.4	1.7	1.2
2639	165	B.	M.	1320	200	295	200	295	155	+15	...	...	...
2647	157	B.	M.	1350	205	275	210	270	170	+20	5.3	2.1	1.4
2649	174	B.	M.	1300	250	260	264	280	160	+10	4.8	1.7	1.2
2660	163	B.	M.	1270	205	275	200	280	120	+18	5.4	2.5	.9
2665	163	B.	F.	1270	165	245	175	245	150	+30	5.4	2.6	1.1
2666	170	W.	M.	1050	190	270	195	260	135	0	...	...	...
2667	166	B.	M.	1170	190	250	200	255	140	+13	5.3	2.0	1.4
2672	170	W.	M.	1450	245	330	250	325	160	+10	7.1	3.0	1.5
2683	170	B.	M.	1450	250	315	260	310	155	+ 4	5.9	2.2	1.4
2689[14]	167	B.	F.	1320	220	285	222	295	155	+ 3	5.4	2.1	1.2
2697	155	B.	F.	1150	225	310	230	309	160	— 7	4.3	1.8	1.0
2703	167	B.	M.	1350	240	335	245	337	170	+ 2	7.4	2.9	1.6
2719	...	B.	M.	....	230	355	235	353	182		6.1	2.2	1.6
2722		B.	F.		207	286	198[15]	302[15]	159		5.0	2.2	1.1
2731		W.	M.		225	297	217	305	157		7.2	2.7	1.7
3732	...	B.	M.	....	225	298	227	302	165		4.5	2.0	1.3
2743	...	W.	F.	....	227	360	242	340	152		...	...	...
2746	...	W.	M.	....	240	295[16]	237	315	170		4.9	1.9	1.2
2748	...	W.	M.	....	280	345	275	340	290 (?)		5.1	2.0	1.3
2751	...	B.	F.	....	230	250	225	250	155		5.0	2.2	1.2
2752	...	W.	M.	....	210	277	210	285	130		4.6	2.0	1.0
2753	...	B.	F.	....	202	275	202	270	135		...	...	...
2759	...	B.	M.	....	262	345	262	352	165		5.4	2.3	1.2
2762	...	B.	M.	....	230	335	235	320	165		5.8	2.4	1.4
2789	...	B.	M.	....	255	320	250	330	185		5.4	2.0	1.6
2796	...	W.	M.	1500	295	368	285	380	170		5.3	2.7	1.2
2801	164	B.	F.	1500	245	317	250	300	195		5.2	2.1	1.1

2807	167	B.	M.	1250	235	300	235	300	170		4.6	1.9	1.0
2810	150	B.	F.	1200	245	305	240	315	160		4.6	1.8	1.2
2826	162	B.	M.	1450	280	370	280	375	170	— 2	6.2	2.4	1.3
2828	155	B.	M.	1500	310	375	290	365	180	— 1	7.3	3.0	1.8
2829	180	W.	M.	1650	335	420	320	430	195	— 3	7.5	3.1	1.4
2833	161	B.	M.	1400	275	360	280	355	170	— 3	6.6	2.8	1.5
2834	170	B.	M.	1500	290	410	300	400	195		5.9	2.2	1.8
2837	180	B.	M.	1370	270	385	280	375	170		5.5	2.2	1.4
2842	167	B.	M.	1320	265	335	260	325	160		6.7	2.5	1.8
2861	...	B.	M.	1250	270	345	265	340	205		5.6	2.4	1.6
2864	185	W.	M.	1440	270	340	265	340	160		6.6	2.4	1.7
2865	167	W.	M.	1440	330	400	330	400	220		6.9	2.5	1.7
2867	167	B.	F.	970	185	250	190	250	155		5.3	2.0	1.4
2878	169	W.	M.	1330	250	350	265	340	195		5.5	2.5	1.4
105	Infant	B.		....	170	206	157[16]	219[16]	137		3.8	1.9	.8
108	Infant	B.	F.	....	90	120	90	120	55		1.3	.2	.4
169		W	M.(?)	1110	195	250	210	270	150		3.4	1.8	.6
193	175	B.	M.	950	160	205	170	220	130		4.2	1.7	1.1
196	...	W.	M.	....	250	325	250	325	170		6.4	2.9	1.4
3x	...	B.	M.	....	230	300	230	290	195		...	...	...
4x	...	B.	M.	....	190	280[17]	200	260[17]	...		4.6	2.0	1.0
	..	B.	F.	....	210	273	209	276	155		...	...	...
	163	W.	M.	1200	230	305	230	305	160		...	...	...
	...	W.	M.	1130	235	295	225	305	165		...	...	..
	...	W.	M.	1200	240	325	240	315	180		...	...	..
	...[18]	W.	M.	1200	240	315	240	315	169		...	...	...
	175	B.	M.	1520	260	320	265	315	170		...	...	...
	...	W.	F.		230	310	230	305	180		...	...	...
	168	W.	M.	....	240	320	230	320	140		...	...	...
....	...	W.	M.	....	...	...	...	...	...		5.0	1.9	1.3
....	...	B.	F.	....	...	...	...	...	...		4.8	2.0	1.4
1538	...	W.	M.	....	...	...	...	...	...		5.8	2.2	1.5
1839	...	B.	M.	....	...	...	...	...	...		7.0	2.6	1 4
1969	...	W.	M.	....	...	...	...	...	...		4.7	1.8	1.1
2668	...	W.	F.	....	...	...	...	...	...		4.2	2.0	1.1
2670	...	W.	M.	....	...	..	...	...	...		5.8	1.9	1.8

regarding male and female types are of no scientific value. It may turn out, however, that variations in the gyri and sulci will not be of the same percentage in both men and women and that the constant value in the latter will be found more readily, as is the case with other anatomical variations (Schwalbe).

In this study of several anatomical characters said to vary according to race and sex, the evidence advanced has been tested and found wanting. It is found, however, that portions of the brain vary greatly in different brains and that a very large number of records must be obtained before the norm will be found. For the present the crudeness of our method will not permit us to determine anatomical characters due to race, sex or genius and which if they exist are completely masked by the large number of marked individual variations. The study has been still further complicated by the personal equation of the investigator. Arguments for difference due to race, sex and genius will henceforward need to be based upon new data, really scientifically treated and not on the older statements.

Note to the Preceding Table.

The data given in the preceding table have been aranged in a great variety of ways, but only three of these bear upon the subject under discussion. They are given in Figs. 1 to 3. The individiual records are appended to enable those who are interested in the subject to make further comparisons with those given by Bean and by Spitzka, as well as for further use to those who may collect new data.

The genu and splenium were outlined by Bean's method, given on page 8.

FOOTNOTE TO THE TABLE.

[1]Pia on left side. [2]Pia off on left side. [3]Boy. [4]Break not even on left side. [5]Pia off on left side. [6]Ventricle on right side greatly dilated. [7]Break unsatisfactory. [8]Sulci on both sides very irregular. [9]Pia off on right side. [10]The posterior left is decidedly larger than the posterior right. [11]Left operculum is very large and right parietal convolutions are very atrophic. [12]Curious interlacing of fiber bundles below central fissure on the left side. [13]Boy. [14]Central fissure seems to be double on both sides. [15]Break unsatisfactory. [16]Large cavity in right brain; break also unsatisfactory; break on left side is not accurate. [17]Breaks unsatisfactory. [18]Insane murderer.

ON THE CERVICAL VEINS AND LYMPHATICS IN FOUR HUMAN EMBRYOS,

With an Interpretation of Anomalies of the Subclavian and Jugular Veins in the Adult.

BY

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In order to explain an anomaly of the subclavian vein occurring in a man 68 years old, reconstructions of the cervical veins and lymphatics in four human embryos were made, with the following results. In an embryo having a maximum measurement of 10 mm., the right arm is drained by a primitive ulnar vein which unites with a thoraco-epigastric vein to form the dorsal subclavian vein. These vessels are shown in Fig. 1. The primitive ulnar vein is seen to receive a branch near the elbow, and distally it has many smaller tributaries which have been omitted from the drawing. There are no veins larger than capillaries along the radial border of the arm.

The thoraco-epigastric vein, which is found in the lateral body wall, is represented in fishes and in all the higher classes of vertebrates. Unfortunately it has been given a great variety of names. In the rabbit it is called the external mammary vein (Krause,[1] Lewis[2]) and this name has been applied to it in man (Poirier[3]). In man it has been called, in part at least, the long, lateral, or inferior thoracic vein, but the term *thoraco-epigastric* is preferable to any of these.

The primitive ulnar and thoraco-epigastric veins unite to form a subclavian vein which passes dorsal to the brachial plexus to enter the

[1]Krause, W. Die Anatomie des Kaninchens. Leipzig, 1868.

[2]Lewis, F. T. The development of the lymphatic system in rabbits. Amer. Journ. of Anat., 1905, Vol. 5, pp. 95-111.

[3]Poirier, P. Traité d'anatomie humaine. Paris, 1896. Vol. 2, p. 911.

anterior cardinal vein. Small branches of the anterior cardinal vein are found ventral to the brachial plexus. In a slightly older embryo (Fig. 2) these ventral branches are continuous with the thoraco-epigastric vein so that the brachial plexus is enclosed in a venous ring. This arrangement of the subclavian veins has been described in the rabbit and man by Hochstetter,[4] and the corresponding stage in the rabbit has been figured.[5]

In addition to the subclavian vein, the anterior cardinal has another important branch. This begins as a median vessel in the lingual region (Fig. 1). It turns sharply to the right, receives tributaries from the lateral superficial tissues and descends in front of the vagus nerve. In the lower part of its course it is close to the dorsal portion of the pericardial cavity. It passes on the lateral side of the vagus nerve to enter the anterior cardinal vein. In the older embryo this linguo-facial branch (*V. ling-fac.*) is easily recognized. The median vessel in the sublingual region is present, but it has not been drawn, since in this embryo it is drained by the linguo-facial branch of the *left* cardinal vein. As seen in the reconstruction, the linguo-facial vein has acquired a new outlet, which is anterior to the place where the hypoglossal nerve crosses the cardinal vein. The hypoglossal nerve, which before was lateral to the cardinal vein, is now surrounded by it,[6] and the venous loop through which it passes is shown near the top of Fig. 2. That part of the linguo-facial vein which descended in front of the vagus nerve has apparently disappeared, and the vein is no longer in relation with the pericardial cavity.

It is perhaps worth while to call attention to the scattered data regarding this linguo-facial vein. It was discovered by Grosser in embryo bats and described as follows:[7]

"The veins of the branchial arches consist in young embryos of Rhinolophus of a median longitudinal vein near the ventral surface

[4]Hochstetter, F. Ueber die Entwicklung der Extremitätsvenen bei den Amnioten. Morph. Jahrb., 1891, Vol. 17, pp. 26-27 and 32-33.

[5]Lewis, F. T. Amer. Journ. of Anat., 1905, Vol. 5, p. 98.

[6]This accords with Tandler's observation that the hypoglossal nerve is lateral to the internal jugular vein in human embryos of 8 and 9 mm. and medial at 12.5 mm. Anat. Anz., 1907, Vol. 31, pp. 473-480.

[7]Grosser, O. Zur Anatomie und Entwickelungsgeschichte des Gefässsystems der Chiropteren. Anat. Hefte, 1901, Heft 55, p. 131.

of the mandibular and hyoid arches, which at its caudal end divides into two symmetrical parts. Each of these turns abruptly to one side, receives small tributaries from the lateral parts of the branchial arches, and empties, lateral to the aortic arches, into the anterior cardinal vein. Later the vessel cannot be demonstrated. A part of it may perhaps be incorporated in the external jugular vein."

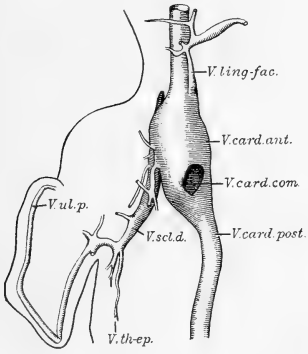

FIG. 1

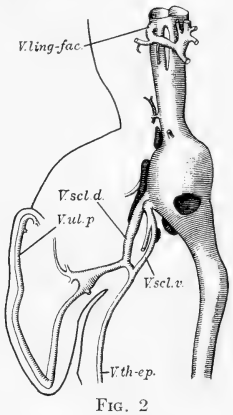

FIG. 2

FIG. 1. Reconstruction, as seen from the ventral side, of the right arm and adjacent part of the body of a human embryo of 16 mm. (Harvard Embryological Collection 1,000), to show the veins and lymphatic vessels. The latter, in this and the three following figures, have been heavily shaded. × 20 diam.

FIG. 2. Similar reconstruction from a human embryo of 11.5 mm. (H. E. C. 189). × 20 diam. *V. card. ant.*, Vena cardinalis anterior; *V. card. com.*, V. cardinalis communis (duct of Cuvier); *V. card. post.*, V. cardinalis posterior; *V. ling-fac.*, V. linguo-facialis; *V. scl. d.*, V. subclavia dorsalis; *V. scl. v.*, V. subclavia ventralis; *V. th-ep.*, V. thoraco-epigastrica; *V. ul. p.*, V. ulnaris prima.

This description is illustrated by an excellent reconstruction. Two years later these veins were recorded in pig embryos of 6, 12, 14 and 20 mm.[8] Because of their course across the throat they were called "transverse veins." It was stated that the median vessel, instead of bifurcating, sometimes passes wholly to the right and sometimes to the left. The early appearance of corresponding veins in the rabbit

[8]Lewis, F. T. The gross anatomy of a 12 mm. pig. Amer. Journ. of Anat., 1903, Vol. 2, p. 221.

was reported subsequently.[9] They are present in an embryo of 9½ days (3 mm.). Usually the linguo-facial vein arises from the anterior cardinal near its outlet, but sometimes it connects with the common cardinal vein (duct of Cuvier) and in one exceptional case—the left side only of an embryo of 12 days (5 mm.)—it emptied into the posterior cardinal vein. In a 7 mm. rabbit the median lingual portion of the vein has been seen to bifurcate symmetrically, agreeing with Grosser's description for the bat. In rabbits from 9.5 to 29 mm. the main trunk of this vein has been shown in a series of six reconstructions.[10] Its terminal branches are the anterior and posterior facial veins, the latter receiving a posterior auricular branch; thus it corresponds with the external jugular vein of the adult rabbit as described by Krause, and it has been so labeled. This vein, however, seems to be homologous with the common facial vein in man, the external jugular vein of human anatomy arising independently as will be seen presently.

In a recent publication Grosser has shown that the linguo-facial vein is homologous with the *inferior jugular vein* of fishes, amphibia, and reptiles,—a paired ventral vessel draining the floor of the branchial region.[11] In the same paper he states that this vein is well developed in cat and guinea-pig embryos and in a human embryo of 6.5 mm. Grosser's comparative studies demonstrate the fundamental importance of this vein. The name *inferior jugular* has, however, not been adopted in this paper since it is an unfortunate designation for a ventral vessel in fishes, which has nothing to do with the anterior (*i. e.,* ventral) jugular vein of man, but gives rise to veins which empty anterior or superior to the other jugulars. The term *linguo-facial* is justified by the embryonic distribution of the vessel,—in part to the lingual region (hyoid and mandibular arches) and in part to the superficial tissues of the mandibular region. Although there is a complex rearrangement and new formation of branches, a single large vein drains this territory from early embry-

[9]Lewis, F. T. The intraembryonic vessels of rabbits from 8½ to 13 days. Proc. Amer. Assoc. of Anat., 1903, pp. 12-13.

[10]Lewis, F. T. The development of the lymphatic system in rabbits. Amer. Journ. of Anat., 1905, Vol. 5, pp. 95-111.

[11]Grosser, O. Die Elemente des Kopfvenensystems der Wirbeltiere. Verh. d. anat. Gesellschaft, 1907, pp. 179-192. F. R. Lillie refers to this vein in the chick as the external jugular. Development of the Chick, Chicago, 1908.

onic stages to the adult. Thus in man, according to Sebileau and Demoulin,[12] "Faraboeuf has well shown that the facial vein, lingual vein, and superior thyreoid vein unite to form a common canal very happily termed the thyreo-linguo-facial venous trunk. This arrangement is in fact very frequent." Except for the superior thyreoid vein, which has not yet developed, this description may be applied to the embryonic vessels shown in Fig. 2.

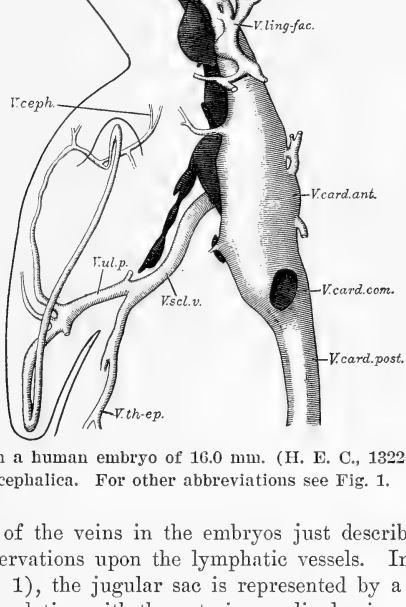

FIG. 3. From a human embryo of 16.0 mm. (H. E. C., 1322). × 20 diam. *V. ceph.*, Vena cephalica. For other abbreviations see Fig. 1.

The study of the veins in the embryos just described led to the following observations upon the lymphatic vessels. In the 10 mm. embryo (Fig. 1), the jugular sac is represented by a single lymph space in close relation with the anterior cardinal vein. It appears to be lined with endothelium, which in some sections has shrunken away from the surrounding mesenchyma. It contains a few blood corpus-

[12]Sebileau, P., and Demoulin, A. Comment il faut comprendre le système des veines jugulaires antérieures. Bull. de la soc. anat. de Paris, 1892, année 67, pp. 120-132.

cles, and appears to communicate with the vein by a slender oblique passage which is completely filled by a single file of blood corpuscles. This lymphatic space is larger and more irregular in outline than the neighboring small tributaries of the vein. No

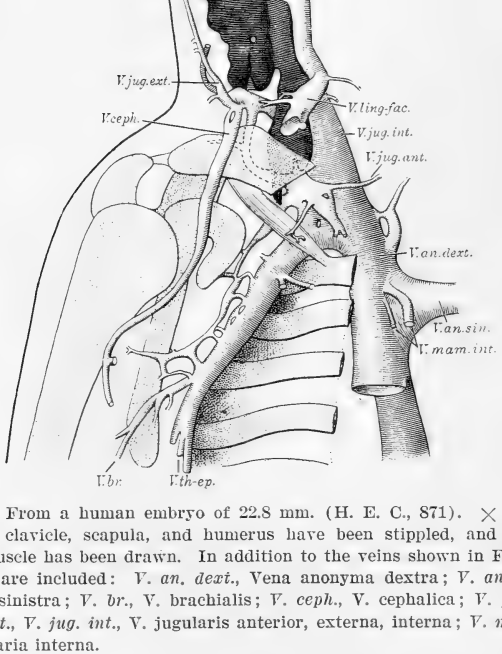

FIG. 4. From a human embryo of 22.8 mm. (H. E. C., 871). × 20 diam. The ribs, clavicle, scapula, and humerus have been stippled, and the subclavius muscle has been drawn. In addition to the veins shown in Fig. 1, the following are included: *V. an. dext.*, Vena anonyma dextra; *V. an. sin.*, V. anonyma sinistra; *V. br.*, V. brachialis; *V. ceph.*, V. cephalica; *V. jug. ant.*, *V. jug. ext.*, *V. jug. int.*, V. jugularis anterior, externa, interna; *V. mam. int.*, V. mammaria interna.

lymphatics could be found in a 9.2 mm. embryo, so that the jugular lymphatics probably arise in human embryos of about 10 mm. This accords with the observation that they first appear in rabbits of 9.5-10.0 mm., but does not agree with Ingalls' opinion that in a 4.9 mm. human embryo certain vessels represent "the first anlage, or

earliest forerunners, perhaps, of the lymphatic system in man."[13] The vessels in question are clearly veins.

In the 11.5 mm. embryo (Fig. 2) there are four or perhaps five lymphatic spaces, apparently separate from each other. They contain some blood corpuscles and in two cases they seem to connect with the vein, but the apertures are very small. At 16 mm. (Fig. 3) the lymphatics have increased and extend along a considerable portion of the anterior cardinal vein. There is a separate space in relation with the linguo-facial vein. A lymphatic vessel extends from the jugular sac dorsal to the brachial plexus, but the dorsal subclavian vein which it accompanied in the earlier stage has disappeared. Similarly in the rabbit the dorsal subclavian vein is accompanied by a lymphatic vessel and later both vein and lymphatic disappear. No outlet from the jugular sac into the cardinal vein was found in the transverse sections studied, but, as Professor Sabin has recently demonstrated, frontal sections are more favorable for detecting the valvular orifice. In an embryo of 22.8 mm. (Fig. 4) there is a very large jugular sac which has grown around certain nerves. The upper small aperture transmits a branch of the third cervical nerve, and the lower one is for branches of the third and fourth. In a rabbit of 14.5 mm. the jugular sac showed similar openings for the third and fourth cervical nerves.

Returning to the veins in Fig. 3, it will be seen that the branch of the primitive ulnar vein near the elbow now joins the radial extremity of the ulnar vein, and from their junction a vessel can be followed along the radial border of the limb toward the shoulder. It is very probable that at this stage there is a capillary union between this cephalic vein and the lateral branch of the anterior cardinal which is shown in the figure. An obvious connection between them is seen in Fig. 4. Here the lateral branch of the anterior cardinal may be identified as the external jugular vein and it can be traced upward behind the ear as the posterior auricular vein. It does not yet connect with the linguo-facial vein.

Fig. 4 shows the brachial vein (derived from the primitive ulnar) joining the larger thoraco-epigastric vein to make the (ventral) subclavian vein. Along the latter, several branches anastomose to make

[13]Ingalls, N. W. A contribution to the embryology of the liver and vascular system in man. Anat. Record, 1908, Vol. 2, p. 343.

a small vena comitans for the subclavian artery. Higher up both the subclavian vein and the vena comitans are separated from the artery by the scalenus anterior muscle, and both are dorsal to the sub-

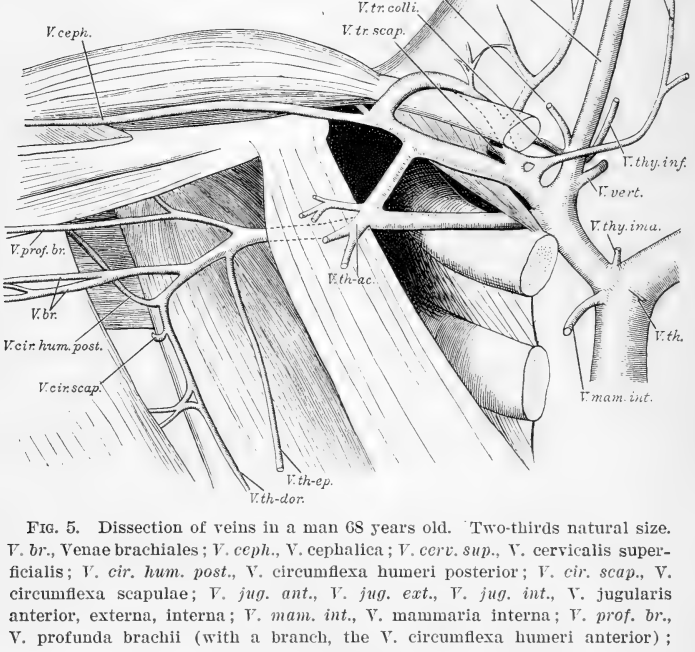

FIG. 5. Dissection of veins in a man 68 years old. Two-thirds natural size. *V. br.*, Venae brachiales; *V. ceph.*, V. cephalica; *V. cerv. sup.*, V. cervicalis superficialis; *V. cir. hum. post.*, V. circumflexa humeri posterior; *V. cir. scap.*, V. circumflexa scapulae; *V. jug. ant.*, *V. jug. ext.*, *V. jug. int.*, V. jugularis anterior, externa, interna; *V. mam. int.*, V. mammaria interna; *V. prof. br.*, V. profunda brachii (with a branch, the V. circumflexa humeri anterior); *V. th.*, V. thymica; *V. th-ac.*, V. thoraco-acromialis; *V. th-dor.*, V. thoracodorsalis; *V. th-ep.*, V. thoraco-epigastrica; *V. thy. ima*, *V. thy. inf.*, V. thyreoidea ima, inferior; *V. tr. colli*, V. transversa colli (from vertebral border of scapula); *V. tr. scap.*, V. transversa scapulae (from scapular notch); *V. vert.*, V. vertebralis.

clavius muscle, which has been drawn in the figure. Ventral to the subclavius muscle there is a small branch distributed beneath the pectoral muscles; this branch probably is the principal factor in the

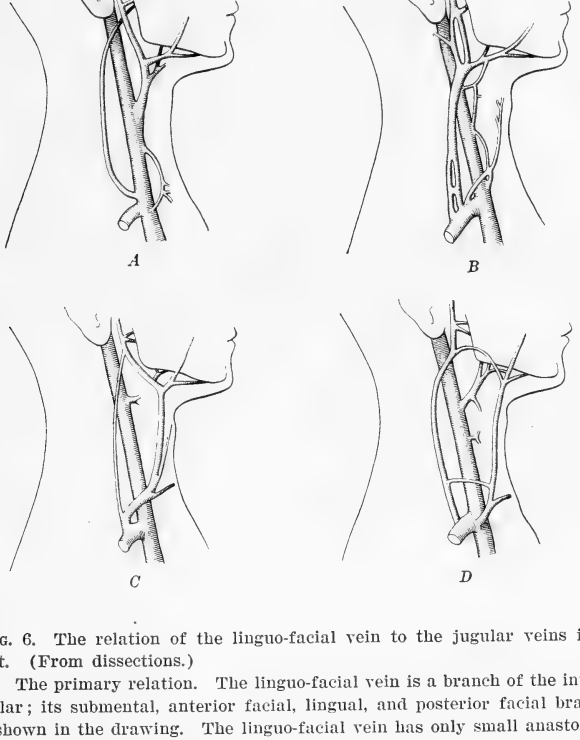

Fig. 6. The relation of the linguo-facial vein to the jugular veins in the adult. (From dissections.)

A. The primary relation. The linguo-facial vein is a branch of the internal jugular; its submental, anterior facial, lingual, and posterior facial branches are shown in the drawing. The linguo-facial vein has only small anastomoses with the external jugular vein, and none with the anterior jugular, the latter vessel being scarcely represented in this case. The similarity to the embryonic relations shown in Fig. 4 is apparent.

B. The linguo-facial vein has been tapped by the external jugular so that its branches appear to belong to the latter.

C. The linguo-facial vein is drained chiefly by the anterior jugular, to which its branches appear to belong.

D. The linguo-facial vein is sub-divided, so that its posterior facial branch empties into the external jugular, its anterior facial branch empties into the anterior jugular, and the lingual branch remains as a tributary of the internal jugular.

anomaly to be described. At the outlet of the subclavian vein there are several branches, and among them the anterior jugular vein can be identified.

The anomaly which led to the examination of the embryos is pictured in Fig. 5. If the small vein ventral to the subclavius muscle in Fig. 4 is considered to have enlarged and anastomosed both with the cephalic vein and the subclavian vein, the conditions found in the anomaly will be strikingly reproduced. Both the clavicle and the subclavius muscle will then be surrounded by a venous ring. In the anomaly the "jugulo-cephalic" vein, which is above the clavicle, is as large as the subclavian vein. The "accessory subclavian vein," which is between the clavicle and the subclavius muscle, is somewhat larger. The subclavian vein occupies its normal position between the subclavius muscle above, the scalenus anterior behind, and the first rib below.

Finally it may be noted that the formation of a venous ring around the scalenus anterior muscle, occasionally recorded in the adult, is suggested in the 22.8 mm. embryo. Where the subclavian vein empties into the jugular, these vessels are molded about the muscle, and since a vertebral branch joins the jugular at this point, nearly two-thirds of the circumference of the scalenus anterior are in relation with the veins. The completion of this ring by venous outgrowths would account for the anomaly. The conclusion may be drawn that although the jugulo-cephalic vein in man is a persistence of an important and normal embryonic vein, the accessory subclavian vein, whether situated behind the scalenus or above the subclavian, is an abnormal vessel.

Of wider interest is the conclusion that the linguo-facial vein is a morphological constant. In mammals it appears at an early stage, and although it often becomes resolved into a group of branches, it is present in the adult. Some of its transformations in man are shown in Fig. 6, on the preceding page. It may have large anastomoses with the external jugular vein or the anterior jugular vein or with both, but these can be readily described and understood on the basis of a primary linguo-facial vein.

THE LYMPHATIC SYSTEM IN HUMAN EMBRYOS, WITH A CONSIDERATION OF THE MORPHOLOGY OF THE SYSTEM AS A WHOLE.

BY

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When we consider the history of our knowledge of the lymphatic system, it is clear that there have been two wholly different lines of thought with regards to our general conceptions. To establish its general morphology is the fundamental task for each of the systems of the body, and upon such a general conception is based all future elaboration of the system. I need only to refer to the neurone theory as establishing such a foundation for our knowledge of the nervous system. In connection with the lymphatic system, the idea that it arises from mesenchyme spaces dominates anatomical and zoölogical literature as is evidenced by examining most of the text books. This conception is based on the work of Budge, Sala, Gulland and many others. It allies the lymphatic system with tissue spaces and serous cavities. The other theory, which seems in a fair way to displace the earlier conception, is that the lymphatics are derived from the veins, that they are vascular rather than mesenchymal in origin. This theory, only recently crystallized, has had an interesting evolution; beginning with Langer and Ranvier, it has been formulated and developed by a group of American anatomists. In this paper I hope to add evidence for this theory and give a general picture of the primitive lymphatic system as a whole. The great usefulness of this theory, aside from the fact that we believe it to be true, is that it gives a key by which to work out the entire development of the lymphatic system down to its ultimate capillaries, and it will be readily conceded that the old theory of the relation of the lymphatics to the tissue spaces gave us no such point of attack.

The first theory, that the lymphatics arise from tissue spaces, received its strongest support from Budge.[1]

In his first paper Budge described injections of Berlin blue into the false amnion of three-day chick embryos. He found that the injection mass ran out into the area vasculosa in a series of irregular canals forming an abundant network immediately under the epiblast and hence dorsal to the vascular layer. This network of canals extended out to a marginal canal around the area vasculosa similar to the marginal vein. Budge interpreted this system of canals as a primitive lymphatic system which in his injections arose in connection with the cœlom and its extra embryonal expansion, the false amnion. This primative lymphatic system he said never had any connection with the veins, so that the interchange of fluid must have been through the walls. Dr. Mall[2] has studied Budge's specimens and is convinced that they are injections showing simply the extent of the extra embryonal cœlom.

I repeated Budge's experiments, using India ink instead of Prussian blue, as it flows more readily, and found that I could duplicate Budge's figures exactly.[3] The fluid ran out in blunt processes simulating canals, but readily distinguished from the lymphatic injections. The fluid runs exactly as it would, if forced between two glass plates held closely together, that is, blunt processes push out which form an advancing network, but this network soon fills into a solid mass. With a careful injection of true lymphatics on the other hand the individual vessels often remain absolutely distinct from the very point of the needle as is shown in Fig. 4, of the article in Volume I, of the American Journal of Anatomy, where the needle was introduced into two places, one just over the shoulder and the other over the crest of the ilium. The injections in the area vasculosa of the chick are like the pictures obtained by injecting into a

[1]Budge. Ueber ein Canalsystem im Mesoderm von Hühnerembryonen. Arch. f. Anat. u. Phys., Anat. Abth., 1880, s. 320.

Untersuchungen über die Entwickelung des Lymphsystems beim Hühnerembryo. Arch. f. Anat. u. Phys., Anat. Abth., 1887, s. 59.

[2]Buck's Handbook of Medical Sciences. The Cœlom.

[3]Sabin. The Development of the Lymphatic System. American Journal of Anatomy, Vol. I, 1901-1902.

mass of embryonic connective tissue which has no lymphatics. The fluid runs out in the lines of least resistance, simulating performed canals but easily distinguished from true lymphatic capillaries, both in form and from the fact that as the injection proceeds the network fills into a solid mass. Serial sections of the area vasculosa showed no preformed channels, but rather that the space between the germ layers is bridged by delicate fibrils, the processes of mesenchyme cells. It seems certain then that Budge's primitive lymphatic system is simply a study of the extent of the early cœlom and morphologically has no relation to the lymphatic system.

In the understanding of the lymphatic system this point is of great importance, as will be shown later. None of the serous cavities, hollowed out of the mesenchyme, that is, the pleural and peritoneal cavities, the joints, the various bursæ, and the chambers for the vitreous and aqueous humors in the eyes, though they contain serous fluid ever form a part of the true lymphatic system. In Budge's second paper, which is unfortunately just a fragment of his work published from the notes after his death, are pictured beautiful figures of true lymphatic injections made at a much later stage, namely in embryo chicks, 18 days old. These, the true lymphatics, Budge thought belonged to a second, the permanent system, distinguished from the first by the presence of the thoracic duct which emptied into the veins. Budge thought that the thoracic duct arose from spaces derived from the cœlom. He also discovered the posterior lymph hearts in chick embryos between 10 and 20 days old.

The theory of the origin of the lymphatic system from tissue spaces was further illustrated by Gulland.[4]

He found spaces hollowed out in the mesenchyme along the course of the blood vessels of the limbs and thought that these flowed together to form ducts.

The next exponent of the theory that the lymphatics arise from the tissue spaces in Sala.[5]

Sala has studied the origin and the development of the lymphatic

[4]Gulland. Journal of Pathology and Bacteriology, Vol. II, 1894, p. 466.
[5]Sala. Ricerche Lab. di Anat. Norm. d. r. Univ. di Roma, Vol. VII, 1899-1900.

system in chick embryos. Basing his work on Budge's, he worked out with care the origin of the posterior lymph hearts which Budge had discovered. He found that the posterior lymph hearts begin at the middle of the seventh day in connection with the lateral branches of the first five coccygeal veins. He says that corresponding to these veins there are excavations in the mesenchyme which soon enter into communication with the lateral branches, and in fact one would say that these fissures are simply dilatations of the veins themselves. These two statements of course exclude one another, for the spaces can not be both fissures in the mesenchyme and dilatations of the veins. ("Esaminando in serie le sezioni caudali di un emb. di g. 6 + ore 18, si scorge che nel mesenchima che sta lateralmente ai miotomi ed in corrispondenza dei rami laterali delle prime cinque vene coccygei, si vanno scavando dei piccoli spazi o fessure che ben presto entrano in comunicazione cogli stessi rami laterali venosi: si direbbe anzi che esse non sono che semplici dilatazioni, ramificazioni delle stesse vene.")

Then he describes these fissures as becoming more abundant and confluent. By opening up communications with each other they form a sac or lymph heart in the mesenchmye. This sac he says is lined with flattened mesenchyme cells, which, if it were so, would, according to our standpoint, exclude it from being a vein. He found muscle in the wall of the hearts on the ninth day and was able to inject the heart directly by the second half of the tenth day. Sala's description of the origin of the posterior lymph hearts in the chick is so clear and graphic that it is perfectly evident to those who are familiar with the method of origin of the lymph sacs in mammals, that the two processes are the same, that the sacs arise from the veins in both cases. The fact that Sala had the old conception of the lymphatic system as coming from the tissue spaces too firmly fixed in mind to really accept the evidence of his own material does not need to confuse the picture.

The lymphatic ducts he thought began as fissures in the mesenchyme along the hypogastric veins on the ninth day. By the eleventh day these spaces communicated and formed a plexus of lymphatic ducts which connected with the lymph hearts and the thoracic duct.

The thoracic duct, which he found extended only from the beginning of the cœliac artery to the outlet of the superior vena cava, began on the eighth day in the following manner. First a series of mesenchyme spaces around which occur clumps of mesenchyme cells which develop into a solid cord. These solid cords become excavated and form the thoracic duct. There is nothing to correspond with this in connection with the lymphatic system in mammals.

To trace the development of the idea that the lymphatic system is derived from the venous system it is necessary to begin with the work of Langer,[6] published in 1868.

In this important paper, Langer makes clear a number of fundamental points. He distinguished the lymphatics in the tadpole's tail from the arteries and the veins by injecting them. He found the two longitudinal lymphatic vessels of the tail, and the branches forming a plexus leading from them. He distinguished the lymphatic vessels clearly from the surrounding connective tissue, and determined that the lymphatics were closed tubes. He was studying a border zone of developing lymphatics and saw that the lymphatics here were really terminal blind ends. He noted the endothelial sprouts from the sides and ends of the vessels and interpreted these sprouts to mean that the lymphatic vessels grow by the same method as do blood capillaries.

Thus he says: "Ich zweifle nicht, dass Lymph und Blutcapillaren nach dem einen und demselben Bildungsmodus sich vermehren, die Elemente sind dieselben." This in reality is his great contribution and upon this idea as a foundation rests the new conception of the lymphatic system as derived from the veins. Another of his observations must not be omitted, namely that in the course of a lymphatic capillary, a portion of the vessel may be greatly narrowed, that is to say, even completely collapsed. "Ich traf aber auch Röhrchen, welche sich ziemlich rash verengten und in der Mitte ihres Verlaufes einen dünnen, anscheinend ganz soliden Faden darstellten." The meaning of this phenomenon and its relation to the general theory will be made clear later.

[6]Langer. Ueber das Lymphgefässsystem des Frosches. Sitzb. d. k. Akad. d. Wissensch., LVIII Bd., I Abth., 1868.

Between the years 1895 and 1897, Ranvier published a series of articles on the development of the lymphatic system.[7] He also studied the development of the lymphatic system in the frog and added an extensive study of the growth of the lymphatics in pig embryos from 9 to 18 cm. long. He observed endothelial sprouts in growing lymphatics and interpreted them as Langer had done 27 years before to mean that the growth of the lymphatic capillaries is by the process of sprouting. Some of the very large lymphatic vessels which he found in the mesentery he interpreted to mean degeneration or retrogression of the system. Ranvier suggested the theory that the lymphatic system comes from the veins, on the basis that the growth is from centre to periphery rather than from the connective tissue spaces to the veins—but he did not prove his theory, for he did not find lymphatics in embryos below 9 cm. in length, at which time the lymphatic capillaries have already covered the surface of the body.

W. J. MacCallum was the next one to call attention to this method of growth by sprouting and he has given graphic descriptions of the process. He studied developing lymphatics in the skin of embryo pigs, 5 to 15 cm. long, watching the injection under the microscope in order to determine the relation of the lymphatic capillaries to the connective tissue cells and spaces.[8]

In studying the growth of the lymphatic capillaries in the skin of the embryo pig, I found that the early lymphatics started from certain centres and gradually spread over the surface of the body.[9] The first of these areas is in the neck, from which vessels grow over the head, shoulder and back. The second is over the crest of the ilium for the vessels over the back and hip, while subsequent centres form the axilla and inguinal region for vessels to the ventral aspects of the body wall and limbs. By studying the figures in Volume III,

[7]Ranvier. Comptes Rendus de l'Acad. d. Sciences, 1894 to 1896, and Archives d'Anatomie microscopique. Paris, 1897.

[8]MacCallum. Die Beziehung der Lymphgefässe zum Bindegewebe. Arch. f. Anat. u. Phys., Anat. Abth., 1902.

[9]Sabin. American Journal of Anatomy, Vol. I, 1901-1902, Vol. III, 1904, and Vol. IV, 1905.

of the American Journal of Anatomy, which show complete injections of the skin for each stage, it will be seen that the lymphatics invade non-lymphatic areas, even in the last figure of the series where all of the systems have anastomosed over the body there is a marked non-lymphatic area over the top of the head as well as over the feet. In pigs longer than 5.5 cm., it is difficult to obtain such extensive injections because valves begin to develop and tend to make the lymph flow from periphery to centre. During this early period of the spread of the lymphatics over the body there are no valves whatever, which accounts for the wide extent of the injection shown for a pig 5.5 cm. long.

To trace these vessels back to their source was fundamental to an understanding of the lymphatic system, and I began with the group in the neck as it was the primary group. The vessels in the neck converge to a sac which is readily demonstrated by injection as is shown in Fig. 1, Vol. IV, American Journal of Anatomy. This sac, which lies against the internal jugular vein, is the beginning or anlage of the lymphatic system. In embryo pigs from 14.5 to 16 cm. long there are symmetrical jugular sacs opening into the vein. Saxer made mention of these sacs as a part of the lymphatic system, but did not realize their fundamental significance.[10] These sacs are either empty or contain a few blood corpuscles. F. T. Lewis worked on the stages before this lymphatic sac is formed and carried our knowledge a step farther by showing that they are preceded by a plexus of veins opening into the jugular vein.[11] This plexus of veins gradually becomes cut off from the main vein and by the coalescence of the small veins a sac is formed which is entirely free from the jugular vein for a time. Subsequently the symmetrical sacs rejoin the veins. The endothelial lining of these sacs is thus derived from the endothelium of the veins. In studying the lymphatics Dr. Lewis used the method of graphic reconstruction. The fact that the jugular sacs are transformed venous capillaries, I was able to entirely confirm by the method of injection in pig embryos.[12] In pig embryos

[10]Anat. Hefte, Vol. VI, 1896.

[11]Lewis. The Development of the Lymphatic System in Rabbits. Amer. Jour. Anat., Vol. V, 1906.

[12]Sabin. Anat. Record, Vol. II, 1908.

13 and 14 mm. long there is an abundant plexus of capillaries anterior to the junction of the primitive ulnar vein with the internal jugular vein, readily injected from the veins. In embryos slightly older this plexus of capillaries is being transformed into a sac, and these sacs are less readily injected from the veins. For example in an embryo 15 mm. long, the sac was injected on the side from the vein and not in the other. About this time then the primary connections with the vein become severed. In my specimens the sacs are filled with blood. When the secondary opening into the veins is formed the sacs become empty and this is true in pig embryos 16 mm. long. In connection with human embryos I shall show how to determine the presence of these secondary openings or valves.

This method of formation of the jugular sacs was also confirmed by Huntington and McClure in studying cat embryos.[13] They have followed all the details of the transformation of the simple veins to the abundant venous plexus and the sac formation by Born's method of reconstruction. Thus the origin of the jugular sac has been worked out in the pig, the rabbit and the cat by the methods of injection and of reconstruction both in two and in three dimensions. The formation of this jugular sac will also be illustrated in the human embryos in this paper.

Besides the jugular sacs two other paired sacs and two unpaired have been described. Lewis described symmetrical subclavian sacs in the rabbit, which in human embryos are, however, an extension of the jugular sacs; the other paired sac is the posterior or sciatic one, noted in the pig and more fully marked out in this paper for human embryos. The unpaired sacs are the cisterna chyli and the mesenteric or better retroperitoneal sac.

This sac was discovered by Lewis its origin and development have been worked out by Baetjer; its significance is brought out in Heuer's work in connection with the lymphatics of the intestine. Mr. Baetjer[14] has shown conclusively that the retroperitoneal sac begins as a series of small veins which bud off from the renal veins.

In his figures are shown the small veins in the root of the mesen-

[13]Huntington and McClure. The Anatomical Record, Vol. II, 1908.
[14]Baetjer. Amer. Jour. Anat., Vol. VIII.

tery of a pig embryo 17 to 19 mm. long. It is readily noted that these small veins are injected from the main veins as the drawings show the injected ink of the specimens. As the embryo develops, these small veins enlarge and coalesce to form a sac, which shows a few connections with the veins, as proved by injection until the embryos are 23 mm. long. The sac is completely formed at 30 mm., when it is cut off from the veins entirely and clearly connected with the cisterna chyli. Baetjer's series of nine drawings show every stage in process of the transformation of the veins into the sac and its subsequent connection with the lymphatic system.

Thus to sum up, it will be seen that the lymphatic system begins as a series of sacs of which eight have been described; three paired, the jugular sacs, the subclavian and the posterior lymph sacs; and two unpaired, the retroperitoneal and the cisterna chyli. In the human embryo there are only six, for the subclavian sacs are extensions of the jugular sacs. All of the sacs are shown in Fig. 12, in a human embryo 30 mm. long. The method of origin of two of them, namely the jugular sacs and the retroperitoneal sacs, has been worked out with care showing that they are clearly derived from the veins. The jugular sacs form a secondary connection with the jugular veins, the other sacs forming in regions where there is great shifting of veins do not form secondary communications with their own veins but join the other lymph sacs to make a primitive system.

The question now arises whether these sacs can be considered as analogous with the amphibian lymph hearts. None of the mammalian sacs studied develop any muscle in their walls; throughout their history they have a lining simply of endothelium, but they all are in regions from which ducts radiate out to drain wide areas, so that as the system begins to function the lymphatic stream converges to these sacs and in this sense they represent the lymph hearts. In the chick the posterior lymph sacs are true lymph hearts, for they develop a muscular wall, and from Sala's description it is easy to see that these hearts really arise by exactly the same process as the mammalian sacs. The fate of the lymph sacs has some bearing on the subject.[15] This has been followed for the jugular sac in the pig and

[15]Sabin. Amer. Jour. Anat., Vol. IV, 1905.

for all the sacs in the human. They all become completely transformed into a group of lymph nodes except the cisterna chyli, which is partially though to a varying degree transformed. The lymph sacs make the great primary groups of nodes for each region through which lymph must pass before entering the veins. Thus for example in the intestines the preaortic nodes are the primary group and they come from the retroperitoneal sac, while the nodes of the mesentery are secondary, tertiary, etc. Thus we may define primary lymph nodes as those that are derived from the lymph sacs, and they are also primary in the sense of being the first to develop for a given region. It therefore seems to me that it is fair to conclude that the lymph sacs of the mammals, which represent the lymph nodes, take the place of the lymph hearts of the amphibia. They do not of course represent the same function, for they never have any muscle, so they never pulsate, and from the beginning they must cause a slowing of the lymph flow rather than a hastening of it and this slowing must become much more marked as they are transformed into lymph nodes. Thus they seem to me analogous to amphibian lymph hearts.

From the preceding analysis of the literature, it is clear that there is a general agreement among recent workers that the mammalian lymph sacs precede the lymph vessels, and hence form a primary lymphatic system and that these sacs are derived from the veins. This position has been very greatly strengthened by the work of Favaro[16] and Allen,[17] on the lymphatic system in fishes, and by Knower[18] and Hoyer,[19] in the amphibia.

Favaro discovered that in fishes the lymphatics come from the veins, and that here the relation of the lymphatics to the veins is much more primitive than in mammals. Lymph hearts and vein hearts may be present, moreover one and the same vessel may carry either blood or lymph either at the same time or at different times. Thus

[16]Favaro. Atti R. 1st Veneto di sc. lett. ed arti, 1905-06, T, 65, Parte seconda. Appendice alla Dispensa 10. Octobre 1906. S. 279. Venezia 1906.

[17]Allen. Proceedings of the Washington Academy of Sciences, Vol. IX, 1907.

[18]Knower. Anat. Record, Vol. II, 1908.

[19]Hoyer. Bulletin de l'Acad. d. Sciences d. Cracovie, 1908.

he speaks of venæ lymphaticæ and vasa lymphaticæ. The system varies much in the different forms; in Urodeles he finds that the lymph hearts begin as a swelling of one of the primitive lateral longitudinal veins. This abstract is taken from Schwalbe's Jahresberichte.

Knower and Hoyer have shown independently that the fundamental points maintained for mammals are true also for the amphibia. They have found that the anterior lymph hearts are the first structures of the lymphatic system to appear in the embryo and have described their origin from the veins. They have found that the first lymph vessels are derived from the lymph hearts, this being stated by Hoyer on page 463 of his article as follows:

"Berücksichtigen wir weiterhin die Art und Weise, in welcher sich die Lymphgefässe entwickeln, namentlich das Auftreten der vorderen Lymphherzen an der vorderen Vertebralvene sowie der Lymphgefässe, welche aus dem Lymphherzen hervorgehen, so kann man sich dem Gedanken nicht verschliesen, dass das Lymphgefässsystem eben an diesen Stellen symmetrisch auf beiden Körperseiten seinen Anfang nimmt und sich von dort aus über den ganzen Körper verbreitet. Als wichtige, diese Ansicht stützende Tatsachen hebe ich aus der vorliegenden Arbeit hervor: Die weite Kommunikation des sich entwickelnden Lymphherzens mit der Vene, die anfänglich mit einer kegelförmigen Zelle endigende freie Spitze des spindelförmigen Lymphherzens, welche sich später zu einem Zellstrange verlängert und sich schliesslich zu einem Lymphgefässe umbildet, ferner die rege Zellvermehrung im Gebiete des sich entwickelnden Lymphherzens und schliesslich die Entwicklung der zwei auf den Kanten der Myomeren einander parallel verlaufenden Lymphgefässe." Both of them state that they will give further evidence of the central origin of the lymph vessels and their growth toward the periphery in their final papers.

We come now to the relation of the peripheral lymphatics to the sacs and to the origin of the thoracic duct. Here we have a diversity of opinion and certain unsettled points which for the sake of the development of the subject it is fundamental to have perfectly clear. In the first place, Sala, who, in connection with the origin of the posterior lymph hearts, really describes them as coming from the

veins, though he confuses the picture by considering them as coming from tissue spaces at the same time, describes the thoracic duct as coming from solid cords of mesenchyme, and the peripheral vessels as derived from spaces in the mesenchyme. We find nothing to correspond to the solid cords of mesenchyme as an anlage of the thoracic duct in mammals, and believe that the lymph vessels grow out from the primitive sacs. That is, we believe that the conditions found by H. Hoyer for amphibia, that the vessels come from the hearts, is true also in mammals. This being the disputed point however, it will be necessary to review the literature in this connection with care.

In 1901 I showed that the jugular lymph sacs are the primary lymphatics in mammals, that they are derived from the veins, that from these sacs, and others, vessels grow out to invade the body and that therefore there are non-lymphatic areas and one can study the invasion of these areas by lymphatic vessels. In the study of the skin this general law was found to hold, that there are areas which at first cannot be injected either directly or through the sacs. This I believe to be because there are no lymphatics to inject. That gradually lymphatics invade these areas and at first a primary subcutaneous plexus can be injected, later a secondary more superficial plexus, and finally terminal capillaries in the papillæ. The same law holds for the lymphatics of the intestine as shown by Dr. G. Heuer in the same number of this journal. In the intestine the lymphatics first form a plexus in the submucosa; secondarily a mucosal capillary plexus forms and from this mucosal plexus the lacteals grow out. In connection with the intestine the fundamental point that the lymphatics grow out from the sacs is also shown. In all the early work the injections of the intestine were made through the thoracic duct, but later it proved that by far the best place to inject is the retroperitoneal sac. This sac gives the key for working out the development of the lymphatics of the viscera. For years I have been trying to get injections of the lymphatics of the lungs and diaphragm and have never succeeded until I introduced the needle directly into the retroperitoneal sac. In connection with the intestine, injections into the retroperitoneal sac at

first show no vessels in the mesentery, next vessels inject from the sac into the mesentery and these vessels gradually extend to the bowel wall which they reach in embryos between 4 and 4.5 cm. long. Thus injections of the retroperitoneal sac make it possible to trace the development of the lymphatics to the viscera, and this is an important point in the proof of the general theory.

In 1904 F. T. Lewis published an important contribution to our knowledge of the lymphatic system. He studied perfect serial sections of rabbit embryos, worked out the early history of the jugular sacs, and discovered the retroperitoneal sac as has been mentioned. In studying the peripheral lymphatics, Lewis pictured a series of small isolated vessels extending along the external mammary and umbilical veins. These isolated vessels are distinguished in sections by being slightly larger in caliber than the neighboring vein. In sections they are clearly isolated. I have had the privilege of examining Dr. Lewis' specimens and can confirm his observations entirely. In one or two places there was evidently great difficulty in determining whether some of these vessels were isolated or were connected with the vein. Moreover, I can find some of these isolated vessels in pig and human embryos. These numerous lymphatic anlagen of Lewis are now the crucial point in connection with the lymphatic system. They exist undoubtedly in perfect sections, they are always lined by a perfectly formed endothelium and never show any transitions toward tissue spaces. The question is simply, Are they lymphatic vessels which have grown from the sacs and are only apparently isolated or do they arise in situ? I believe them to be true lymphatics derived from the sacs and will give my reasons shortly.

In connection with Lewis' observations it is important to make clear the work of Huntington and McClure.[20] They have strengthened the theory that the lymphatics come from the veins the more because they began with a vigorous attack upon the theory.

In 1906 they described elaborate models of the developing lymphatics in cat embryos which showed lymphatic vessels along the veins previous to the formation of the lymph sacs. These early lymphatics

[20]Huntington and McClure. Anat. Record, Vol. I, 1906-07, and Vol. II, 1908.

which they termed subintimal, proved to be only tissue spaces and they withdrew this work in 1907.

At this time they presented the development of the jugular lymph sacs in the cat, agreeing entirely with the work of Lewis and myself; but in connection with the rest of the system they at that time agreed entirely with Sala, believing that the peripheral vessels were dilated tissue spaces. In the Anatomischer Anzeiger of 1908, McClure gives up entirely the theory of the origin of the lymphatics from tissue spaces and comes to agree with Lewis that sections show multiple anlagen. As I have already said, the multiple anlagen of Lewis are undoubtedly in sections and to interpret them is the crucial point. They cannot be interpreted through sections alone; and merely repeating the observation of them in sections does not add to our knowledge of their interpretation. They must be subjected to some kind of an experiment. The inadequacy of simple observation to interpret them was clear to Lewis for he refrained from making an interpretation. The experience of Huntington and McClure serves to emphasize strongly the inadequacy of sections alone, and the large part of that personal equation plays in interpretation, for from practically the same type of material they have taken successively three different standpoints.

In this laboratory under the direction of Professor Mall a group of people have been subjecting these numerous anlagen of Lewis to some sort of experiment. Dr. Eliot R. Clark[21] has been studying the blood vessels and lymphatics in the living tadpole's tail. His specimens prove Langer's suggestion that lymphatics grow by the sprouting of their endothelial lining cells by making it possible to watch them grow. His observations and descriptions of these lymphatic capillaries, sending out long sprouts that now move forward, now bend out of their course to pick up some stray blood corpuscle and now retreat, make one realize how little sections show us. Certain of his observations are exceedingly fundamental, first in the non-lymphatic zone in the living specimen there are no isolated anlagen. This you can never say with certainty in sections because as will be shortly proven lymphatics can be demonstrated by injection where they cannot be

[21]Clark, Anat. Record, Vol. III, 1909.

seen in sections. But in these specimens of the entire living tail, endothelium can be distinguished from mesenchyme, and the lymphatics grow out from their own endothelium and do not add any peripheral anlagen.

A second point which Dr. Clark observed, but did not publish, is the sudden collapsing of a part of a lymphatic vessel. Once or twice while a red blood cell was pushing its way into the vessel, its central end collapsed suddenly to an endothelial thread, while the peripheral end remained dilated. These collapsed lymphatics right in the middle of a vessel were noted and figured by Langer; they have been noted many times in blood capillaries, for example, see Fig. 49 of Stricker's Handbuch der Histologie, but they have been interpreted as evidences of growth simply, while it may be that these collapsed vessels are a part of the functional activity of the lymphatic capillaries. The reverse of this process of collapsing of the vessels, namely the sudden opening up of tiny vessels during an injection, I have often observed and used as an argument in favor of continuous lymphatics rather than isolated anlagen. (See Symposium on the Lymphatic System. Anat. Record, Vol. II, 1908.) It can be readily seen that in cross section these entirely collapsed vessels might be wholly lost and only the dilated portions shown, and thus the suggestion is that Lewis' anlagen represent a transitory phase of the functional activity of the lymphatic capillaries.

The question of the multiple anlagen has resolved itself wholly into a question of method, with the study of the living lymphatics and injected lymphatics on the one hand and the method of serial sections on the other. Ludwig's famous phrase, "die Methode ist alles," was never more apt than in this connection, for it sums up the whole situation. Having long worked with injections we are convinced that uninjected serial sections are wholly inadequate to show all the blood capillaries or lymph capillaries, moreover, we are convinced that Dr. Lewis has carried the observations as far as they can be carried with sections and that sections will always show these apparently isolated anlagen. To put the contention that serial sections are inadequate to the test, Dr. Clark has made the following

experiment. He made a careful camera lucida drawing of the lymphatics in the living tadpole's tail, then killed the tadpole and cut it in serial sections and tried to reconstruct the lymphatics. The failure in reconstructing these amphibian lymphatics confirms similar attempts of my own on mammalian lymphatics and makes me feel sure that uninjected capillaries cannot be completely reconstructed.

The same point in connection with the blood vascular system, namely that the blood capillaries cannot be reconstructed from uninjected specimens no matter how perfect, will be conceded, but has been brought out much more strikingly by the work of Dr. Evans, soon to be published from this laboratory, for he has shown that a blood capillary plexus can be demonstrated by injection where it was not known to exist before.

Thus the question of the relation of the peripheral vessels to the sacs is becoming more and more clear. There is a primary lymphatic system which consists of sacs that are formed directly from the veins. These primary lymphatic sacs are transformed from a series of isolated sacs into a system by means of the thoracic duct and the right lymphatic duct. These two structures form a part of the primary system. The secondary system consists of the peripheral vessels which, it is becoming more and more sure, are an outgrowth from the sacs. Thus we can say that the primary system, as far as it is made up of sacs, comes from transformed veins, and that the secondary system, characterized by being formed of lymphatic ducts and capillaries, develops by endothelial sprouting from the sacs. It remains now to be determined whether the thoracic duct develops after the manner of the primary sacs as transformed branches of the azygos veins or whether it develops as the other lymphatic ducts of the body do, from endothelial sprouts from the sacs. No theorizing can decide between these two ideas. We must wait some decisive method of getting at the facts. The presumption seems to us to lie on the side that the thoracic duct develops in the same manner as all the other ducts, since wherever the isolated anlages of ducts can be tested, as, for example, in the living tadpole's tail, they prove not to exist. Moreover, Dr. McClure, who is at present the advocate of the idea that the thoracic duct arises as a series of

independent spindle spaces along the aygos vein, shows himself the weakness of his own position. He says, referring to serial sections,[22] "These outgrowths, in the writer's estimation, constitute the veno-lymphatic anlages of the thoracic and right lymphatic ducts." That is to say, the entire argument rests on the interpretation of appearances in sections, and it is becoming more clear each year that interpretation of sections is not proof.

The Lymphatic System in Human Embryos.

Based upon these studies of the lymphatic system in the pig, rabbit and cat embryos, I have studied through the Mall collection of human embryos. I do not believe that the subject could have been worked out with human embryos alone, for the real advances in the study of vascular problems have always come from the method of injection. The method cannot be well applied to human material on account of its scarcity, but the points determined in other mammalian embryos can be verified in serial sections of human embryos. Moreover, the Mall collection is sufficiently ample to illustrate all the essential points of the origin of the lymphatic system. In several points I think it adds new evidence to that already gained from other mammals; for example, in connection with the history of the thoracic duct, in gaining a conception of all of the primitive sacs as forming a primitive system, in tracing the posterior sacs which had thus far been seen only in pig embryos among mammals, and in following the transformation of all the sacs into lymph nodes. It is a very great pleasure to thank Professor Mall for the privilege of studying his valuable collection and for many helpful suggestions during the progress of the work.

In the Mall collection no trace of a lymphatic system can be made out in embryos of the first four weeks, from 2 to 8 mm. long. In these there are certain spaces which might be confused with a lymphatic system, first certain areas where the meshes of the connective tissue are especially large, as, for example, around the developing cœlom, and secondly spaces which follow the course of the nerves.

[22]McClure. Anat. Anz., XXXII Bd., 1908, p. 536.

These spaces along the nerves, which may be termed perineural spaces, are especially important to note both on account of their physiological significance and because they have been confused with lymphatics. They may be injected from the space around the spinal cord and they are especially large around the growing tips of the nerves. Their constancy, their presence in perfectly prepared specimens, and especially their size at the growing tips of the nerves, leads one to think that they are physiologically of great importance to the nerves, but they never form a part of the lymphatic system. No injections of these spaces ever run over into the lymphatic system. An injection into the developing arachnoid spaces around the spinal cord will often pass into the veins, entering them around the fourth ventricle, but I have never succeeded in injecting any lymphatic vessels from the arachnoid nor in tracing any lymphatic vessels to the arachnoid, so that I believe the older anatomists, for example Breschet, were right in believing that the lymphatic system does not drain the great arachnoid lymph space which rather retains its primitive relation to the veins while other parts of the body become drained by a new system of capillaries, namely the lymphatics, derived from the veins.

In studying Professor Mall's collection it seems that there are two stages to be made out in the development of the system as a whole. This has been illustrated in the table on the next page. The first includes a study of the origin of all of the primitive sacs and their fusion into a primitive lymphatic system through two factors, namely the formation of the valves of the jugular sacs which make the permanent openings into the veins, and secondly the connection of the various sacs by means of the cisterna chyli and thoracic duct. This period includes embryos up to 30 mm. in length, of which there are seventeen in the series. This first stage may be divided into two periods, one of which there are fourteen specimens, measuring up to 20 mm., which have the jugular sacs alone; the other shown in three specimens, which mark the time of origin of the other sacs and of the thoracic duct.

The second stage involves the transformation of the sacs into the primary lymph nodes and the spread of the peripheral lymphatics.

The first point is well illustrated in the Mall collection; the second, namely the spread of the peripheral lymphatics, needs ample material for injection. It has been worked out only for the lymphatics of the intestine and skin in the pig.

To return to the first stage, namely that of the origin of the sacs, one can outline the course of development as follows. The jugular lymph sacs begin in an embryo 8 mm. long, the valves are first seen at 10.5 mm. The sac reaches its maximum development at 30 mm. when it attains a size of 5 x 3.6 mm. The beginning of the process of the bridging of the sac, which is the process by which the sac is ultimately turned into a chain of lymph nodes comes early, namely in an embryo 14 mm. long. The process of the transformation of the jugular sac into nodes is about complete in an embryo of 80 mm. At 20 mm. there begin to be signs of the formation of the other sacs in the presence of a plexus of veins in the region of the mesenteric sac and the posterior lymph sacs, and at 23 mm. there is a definite retroperitoneal sac and a cisterna chyli. By 24 mm. all three of the sacs are well formed, namely the mesenteric, the cisterna chyli and the posterior lymph sacs. All the sacs together with the thoracic duct are illustrated in Fig. 12, for an embryo of 30 mm., which marks the stage of the completion of the primitive system. The posterior lymph sac which is second in size to the jugular apparently reaches its maximum in an embryo 80 mm. where it measures 2.8 x 2 x 3.5 mm. I have no higher stages but judge from its appearance that it will soon be entirely cut into lymph nodes. The retroperitoneal sac, so large in the pig embryos, is always small in the human, and the cisterna chyli is the smallest of all. The thoracic duct is complete in an embryo 30 mm. long. These facts are summed up in the accompanying table. In the table, where one measurement is given, it represents the length of the sac or its antero-posterior diameter; where two measurements are given the first is the length, the second the width or lateral diameter.

I shall now describe in detail the lymphatics in each of the 22 embryos listed in the table. The earliest specimen in the Mall collection to show any traces of the beginning lymphatic system is an embryo (No. 397) 8 mm. long. In this embryo, as shown in

Length of Embryo in mm.	No. of Embryo in Mall Collection.	Direction of Section.	Thickness in Microns	Jugular Lymph Sac.		Other Lymph Sacs.
				Size in mm.		
8	397	Transverse	10	.3 x .19	Prelymphatic plexus of veins.	
9	163	Transverse	20	.36 x .14	Same.	
10.5	109	Transverse	20	.7 x .28	Symmetrical sacs, empty, with valves.	
11	353	Coronal	10	1.2 (Ant. post.)	Sac full of blood Extensive plexus of veins along jugular vein. Valve formed but apparently not open. Extension of jugular sac along primitive ulnar vein.	
12.5	317	Coronal	20	1.5	Definite long sac out of preceding plexus of veins. Valve.	
14	144	Sagittal	40	1.5	Sac empty, beginning of bridging.	
15	350	Coronal	10		Very abortive sac.	
15	423	Transverse	50	.9	Very small sac.	
17	106	Transverse	50		Very abortive sac.	
17	424				Region damag'd.	
17	296	Coronal	20	1.5	Sacs large, valve undoubtedly open. Small extension along primitive ulnar vein.	

Length of Embryo in mm.	No. of Embryo in Mall Collection.	Direction of Section.	Thickness in Microns.	Jugular Lymph Sac.		Other Lymph Sacs.		
				Size in mm.				
16	74	Transverse	50	1.8	Symmetrical jugular sacs. No extension along primitive ulnar vein.			
20	22	Transverse	50	1.6	Sacs wider, cut by developing nerve. First vessels from the sac to the skin.	Small groups of vessels along renal anastomosis.		
20	128	Coronal	50	.75	Abortive.	Large median anastomosis of sciatic veins. Groups of veins along sciatic vein, anlage of posterior lymph sacs. Median anastomosis of renal veins.		
						Cisterna Chyli.	Retroperitoneal Sac.	Posterior Lymph Sac.
23	382	Sagittal	50	2 x 1	Sacs and valves	Present	Present	
24	6	Transverse	20		Large.	Present	Present	Present
30	86	Coronal	50	5 x 3.6	Maximum size, begin'g lymph-nodes along jugular sac and subclavian vein.	Present, thoracic duct complete.	Present	4.6 x 1 with begin-ni'g lymph-hnode.
46	95	Sagittal	10	3.75 x 1.5	Sac with few lymph nodes.	Not found.	Present, no nodes.	Present 2.5 x .75
50	96	Sagittal	100	4 x 1.5	Sac turning into lymph nodes.	Not found	Present, no nodes.	3 x 1.75
50	84	Transverse	50	3 x 1.5	Many follicles.	1.2	Large 1.6 x 1	1.7 x 2.5
50	224	Sagittal	50 and 100	4 x 1.75	Fine bridges all thin.	Dam	aged	2 x 1
80	172	Transverse	100	1.75 x 1	Chains of lymph nodes.	Present, surrounded by nodes	Mass of lymph-nodes.	Sac with nodes measuring 2.8 x 2 x 3.5

diagrammatic form in Fig. 1, there is a group of vessels lying external to the internal jugular vein near its junction with the primitive ulnar vein. The figure can be interpreted by reference to Fig. 4. These vessels are completely filled with blood and yet I cannot find any openings from them into the veins, or into each other, and thus interpret them after a study of corresponding stages in other mammalian embryos as a plexus of veins which have separated from the jugular vein preparatory to the formation of the anterior or jugular lymph sac. These vessels are small, the largest measuring

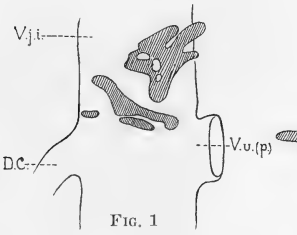

Fig. 1

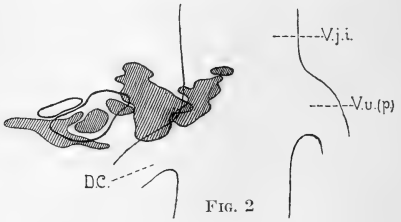

Fig. 2

Fig. 1. Reconstruction of the plexus of small veins lateral to the V. jugularis interna in a human embryo, 8 mm. long, (crown rump). Mall collection, No. 397. × about 50. The plexus of veins is shaded. D. C., ductus Cuvier; V. j. i., vena jugularis interna; V. u. p., vena ulnaris primitiva.

Fig. 2. Reconstruction of the small veins lateral to the right V. jugularis interna in a human embryo 9 mm. long. Mall collection, No. 163. × about 50. Of the veins, six are shaded, indicating that they are full of blood, while the two with heavy outlines are nearly empty. Lettering same as Fig. 10.

approximately .3 mm. in the antero-posterior diameter, by .19 mm. laterally.

The next specimen in the series is an embryo (No. 163) measuring 9 mm. This specimen shows a similar condition but with certain differences. In the first place the plexus of isolated vessels occupies a slightly different place, as seen in Fig. 2. They lie farther ventralward, extending over the body wall external to the heart. Most of these vessels are well filled with blood, while two are nearly empty. This embryo differs also in having an asymmetrical development, the

vessels representing the forerunners of the lymphatics being much larger on the right side than on the left. The three largest sacs of this series measure .27 x .19 mm., .36 x .14 mm. and .27 x .14 mm. respectively.

The next specimen is an embryo (No. 109) measuring 10.5 mm. In this embryo there are symmetrical jugular sacs as seen in Fig. 3, just external to the internal jugular vein. The relation of the sac to the venous system as a whole is shown in Fig. 4, which is a reconstruction from serial sections. This embryo has been figured

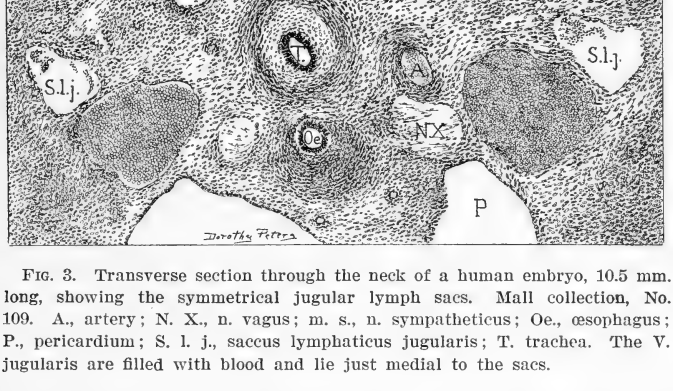

Fig. 3. Transverse section through the neck of a human embryo, 10.5 mm. long, showing the symmetrical jugular lymph sacs. Mall collection, No. 109. A., artery; N. X., n. vagus; m. s., n. sympatheticus; Oe., œsophagus; P., pericardium; S. l. j., saccus lymphaticus jugularis; T. trachea. The V. jugularis are filled with blood and lie just medial to the sacs.

by Bardeen and Lewis, American Jour. of Anat., Vol. I, 1901-1902, and by Dr. Mall, American Jour. of Anat., Vol. IV, 1905; the outline and some of the details of the figure are taken from their reconstructions. As will be seen in Fig. 4, the sac lies external to the jugular vein and anterior to the primitive ulnar. In this embryo the question of a valve is an interesting one.

In studying through Dr. Mall's collection it has proved that the finding of the valves depends wholly on the plane of the section. There is only one plane which is at all adequate for determining the

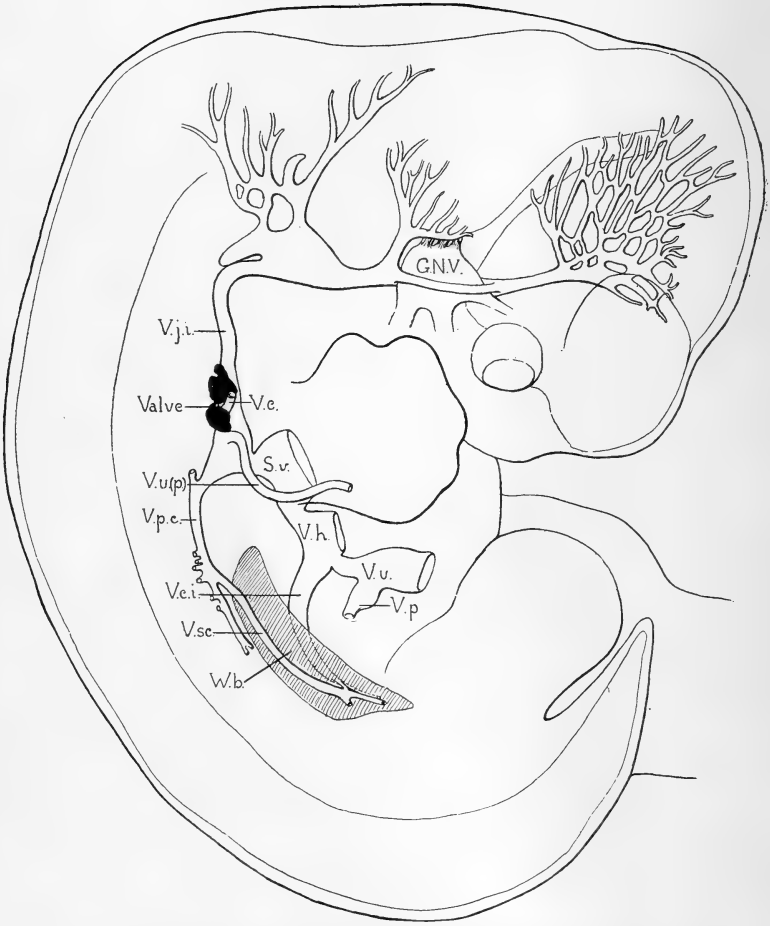

FIG. 4. Reconstruction of the right jugular lymph sacs, shown in solid black, in a human 10.5 mm. long. Mall collection, No. 109. × about 14. G. N., gasserian ganglion; S. v., sinus venosus; V. c., V. cephalica: V. c. i., vena cava inferior; V. h., vena hepatica; V. j. i., vena jugularis interna; V. p., vena porta; V. p. c., vena cardinalis posterior; V. s. c., vena subcardinalis; V. u. (p.), Vena ulnaris (primitiva); V. u., vena umbilicalis; W. b, Wolffian body.

valves, namely the coronal. This will be readily seen in Fig. 10, which shows that the valve is made by a long projection of the lymphatic duct into the angle of the internal jugular vein with the cephalic vein. Imaginary cross sections through this figure will show that the place of the valve would be represented by a small duct in the angle between two veins and this is exactly what is seen in Fig. 5 for this embryo. A study of Fig. 10 will also show that there could be nothing distinctive of the actual opening of the lymphatic to the vein in cross sections, for they would consist simply of a double layer of endothelium between the veins. In like manner sagittal sections are still more difficult than transverse ones for locating the valves and indeed only in an occasional, fortunate section can it be accurately done.

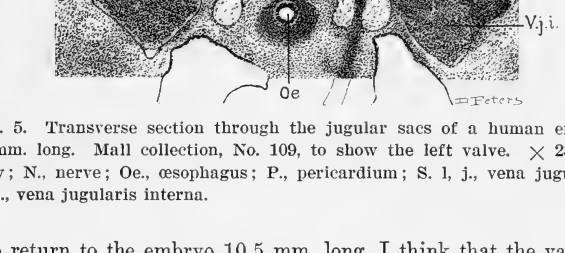

Fig. 5. Transverse section through the jugular sacs of a human embryo, 10.5 mm. long. Mall collection, No. 109, to show the left valve. × 28. A., artery; N., nerve; Oe., œsophagus; P., pericardium; S. l, j., vena jugularis; V. j. i., vena jugularis interna.

To return to the embryo 10.5 mm. long, I think that the valve is present, for, as is seen in Fig. 5, there is a small duct in the angle between two veins and the duct connects with the sac as traced in serial sections. Secondly, the lateral vein in this section is the cephalic, see Fig. 4, and is therefore in the exact position of the undoubted valves seen later in Figs. 10 and 13. Whether this valve is open or not it is impossible to say. The sacs are both empty, and in the earlier stages where there are no valves they are often, though not always, full of blood. They measure .7 x .28 mm., showing a considerable increase over the two preceding specimens. This embryo

shows one further point of interest, namely a possible beginning of the thoracic duct in the shape of a duct running over toward the aorta as shown in Fig. 6. The entire question of a thoracic duct will be discussed later, on page 77.

The next embryo of the series (No. 353) is 11 mm. in length. This embryo is cut in coronal sections which proves to be the best plain not only for seeing the valves but for understanding all of the cervical lymphatics. This embryo is represented in a series of three figures, 7, 8 and 9, two of them sections and the third a diagram

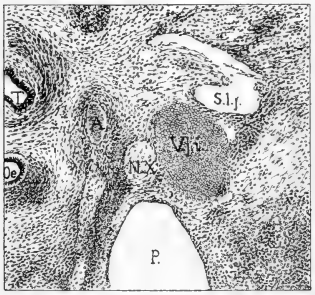

Fig. 6. Transverse section through the left jugular sac to show the possible beginning of a vessel growing down to form the upper part of the thoracic duct. × 40. A., aorta; N. X., N. vagus; Oe., œsophagus; S. l. j., saccus lymphaticus jugularis; V. j. i., vena jugularis interna.

from the same series. In Figs. 7 and 8 will be seen the extension of the lymphatic plexus along the external border of the jugular vein. These two figures show a number of important things, first in connection with the veins, they show the relations of the primitive ulnar and the cephalic to the jugular vein; for the lymphatics they show the relation of the lymphatics to the cephalic vein and in general to the arm bud. These relations are all brought together in the diagram of Fig. 9. Here it will be seen that this plexus which appears isolated in Fig. 7 is really continuous. The plexus is actually much more complex than is shown in Fig. 9. Measuring

from the valve, which is in the angle between the cephalic and internal jugular veins, it extends 1.2 mm. along the jugular vein. By a comparison with the reconstruction of the preceding stage, Fig. 4, I think that the sections shown in Fig. 7 and 8 suggest that the sac is extending along the jugular vein by means of a plexus of veins.

The next point of interest is the location of the valve. In Figs. 7 and 8, it will be seen that the beginning cephalic vein is easily recognized by its position opposite the upper curve of the arm bud. The lymphatic sac runs deep into the angle between the cephalic and the internal jugular veins, Fig. 7, but in no section is there any break in the endothelium of the sac, which leads one to think that the valve may not yet be open and that this fact may account for the complete filling of the lymphatic sacs with blood. The internal jugular vein is only partially filled with blood. The blood of the vein itself was omitted in the drawing.

In this embryo there is an extension of the jugular sac along the primitive ulnar and lateral thoracic veins. This extension forms the subclavian sac which gives rise to the lymphatics of the arm, Fig. 9. This is especially interesting in connection with F. T. Lewis's observations on the subclavian sac in rabbits where it begins as an isolated sac. I was able to confirm Lewis' observations on his specimens of rabbit embryos, but feel sure that in human embryos the sac in the arm bud is an extension of the jugular sac. The sac along the ulnar veins measures .8 mm. beginning from the valve. This makes 2 mm. the total extent of the lymphatics in this embryo.

By putting together Figs. 7 and 8, relating them by the position of the cephalic veins, it will be noted that following along the external border of the internal jugular vein there are a series of branches which we might call segmental, some of them, as for example above the lymphatics in Fig. 7 or between the lymphatics and the primitive ulnar vein in Fig. 8, are obviously small veins, others like the cephalic and primitive ulnar are large veins, while still others are being transformed into lymphatics. This suggests the process of transformation of the various branches of the internal jugular vein from the original simple segmental type into the adult system. In these transformations some of the branches become

enlarged, others reduced and dropped out, while still others are changed into lymphatic sacs.

The next embryo of the series (No. 317), measuring 12½ cm., has not been illustrated because it is exactly like the preceding except that the lymphatics have much less blood, and the plexus along the

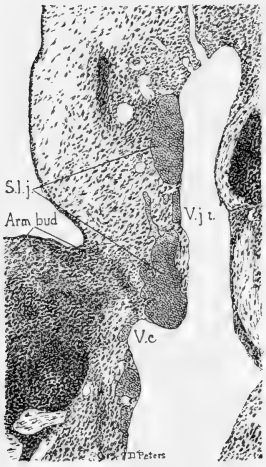

Fig. 7

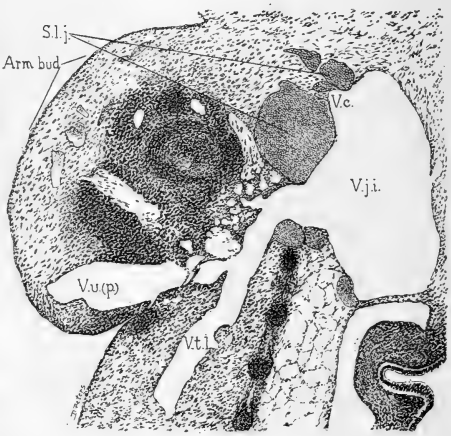

Fig. 8

Fig. 7. Coronal section through the arm bud of a human embryo, 11 mm. long. Mall collection, No. 353, to show the plexus of veins or lymphatic sacs along the internal vein. This section is to be related to Fig. 8 by means of the composite section, Fig. 9. × about 36. S. l. j., saccus lymphaticus jugularis; V. c., vena cephalica; V. j. i., vena jugularis interna.

Fig. 8. Coronal section through the arm bud of the same embryo as Fig. 7, to show the relation of the lymphatic sac to the primitive ulnar vein. The larger lymphatic sac is the upper part of the extension along the primitive ulnar vein, shown in Fig. 18. × about 36. S. l. j., saccus lymphaticus jugularis; V. c., vena cephalica; V. j. i., vena jugularis interna; V. t. l., vena thoracicus lateralis; V. u. (p.), vena ulnaris (primitiva).

jugular vein has been definitely transformed into a single sac. The extent of the lymphatics along the jugular vein is practically the same. The valve is definite, showing the same type as seen in Fig.

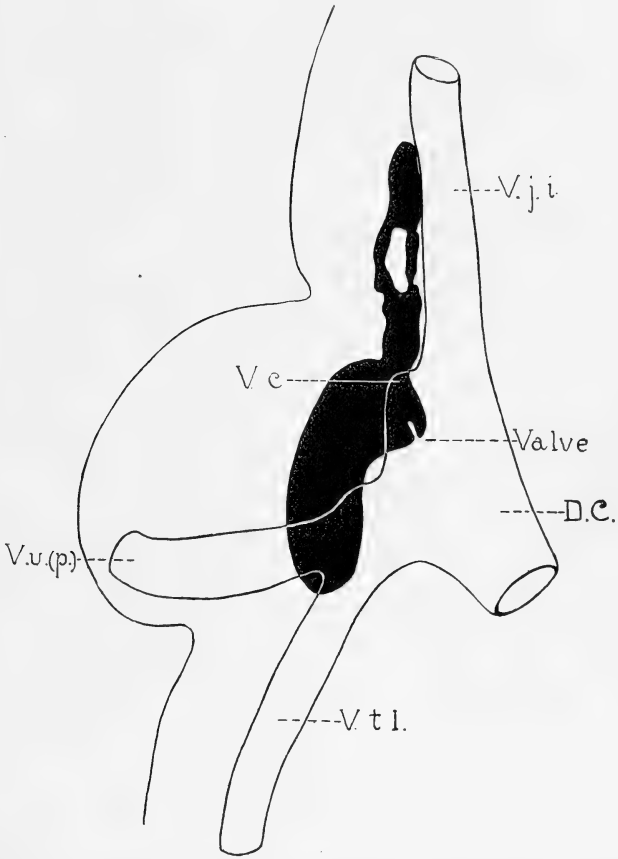

Fig. 9. A composite diagram made by superimposing the sections showing the jugular sacs, as shown in Figs. 16 and 17, of human embryo, 11 mm. long, Mall collection. × about 36. D. C., ductus Cuvier; V., position of valve; V. j. i., vena jugularis interna; V. t. l., vena thoracicus lateralis; V. u. (p.) vena ulnaris (primitiva).

10, except that an opening cannot be made out. It is impossible to say whether the opening is not present or whether the shrinkage of preservative is sufficient to conceal it.

The sixth embryo of the series (No. 144), 14 mm. long, shows only one new point in the formation of the jugular sac, namely the beginning of the process of bridging of the sac which is illustrated for a later stage in Fig. 14. This cutting of the sacs by slender connective tissue bridges, which has already been described in the development of the jugular lymph sacs in the pig, is, I believe, the beginning of the transformation of the sac into a lymph node.[21] This will be considered later.

Two of the embryos of the series, one (No. 350) measuring 15 mm. and the other (No. 106) measuring 17 mm., have very abortive sacs near the junction of the primitive ulnar with the jugular vein. In both instances the preservative is not good enough to show the endothelium, so there is no way of telling these small sacs, which measure less than half a millimeter in their longest diameter, from mesenchyme spaces except by their position in comparison with other embryos. They certainly are an evidence that there are marked irregularities in the development of the lymphatic system.

Another embryo of 15 mm. (No. 423) has also only a small sac, this one measuring .9 mm. In the collection there are some embryos in which the preservative is too poor to admit determining the lymphatics at all, but out of the series of 22 which have been studied there are four cases of abortive jugular sacs, or 18 per cent. These embryos measure 15, 17 and 20 mm.

The next specimen (No. 424), measuring 17 mm., is valuable, for it has a double vascular injection. An extravasation along the jugular region interferes with a study of the jugular lymph sacs, but the vascular injection of the posterior part of the embryo gives conclusive evidence that the other sacs, namely the retroperitoneal, the posterior and the receptaculum chyli have not begun.

Embryo (No. 296) measuring 17 mm. is the earliest specimen in which I have found a valve undoubtedly open. This is shown in

[21]Sabin. Amer. Jour. Anat., Vol. IV, 1905.

Fig. 10. It is in exactly the position found in the embryo 11 mm. long (Fig. 7) and in No. 317 which is 12.5 mm., which are cut in coronal sections, but in the two earlier embryos I could not make out the break in the endothelium. The extent of the lymph sac in the section is 1.5 mm. and there is a slight extension along the primitive ulnar vein.

The next embryo of the series (No. 74) measures 16 mm. In Dr. Mall's catalogue it is placed after those measuring 17 mm., for

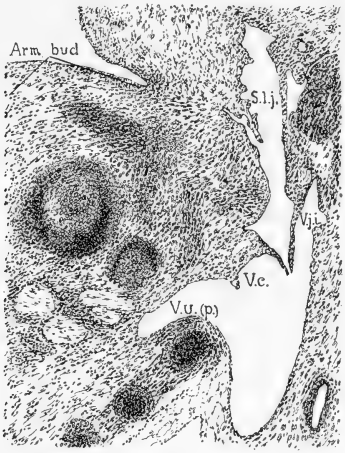

Fig. 10. Coronal section through the arm bud of a human embryo, 17 mm. long, Mall collection, No. 296, to show the open valve of the jugular lymph sac in relation to the veins. × about 26. S. l. j., saccus lymphaticus jugularis; V. c., vena cephalica; V. j. i., vena jugularis interna; V. u. (p.), vena ulnaris (primitiva).

it is undoubtedly further developed. This embryo, in which the sections are 50 microns thick, is a very satisfactory one for determining the sacs, for the veins are unusually distended with blood and the lymph sacs are filled with a serum which takes a definite stain. The sacs extend a distance of 1.8 mm. along the internal jugular vein. There is no sac on the primitive ulnar vein and there are

no traces of the other sacs in the posterior part of the body. The veins are especially large in the posterior part of the body. The sections are too thick to show the valves well. The sacs appear as in Fig. 3 except that they are larger.

The next embryo of the series (No. 22) measures 20 mm. and shows several interesting points. The series is cut transversely and the sacs also appear much as they are shown in Fig. 3 for an embryo 10.5 long except that they are much wider. The sacs measure 1.6 x 7. The new point of interest is, that in this series the third nerve cuts through the sac; in a later stage, in an embryo measuring 30 mm., Fig. 12, three nerves cut through the sac, namely the third, fourth and fifth. For the first time, in this stage, there are vessels extending from the sac toward the skin. It will be remembered that this is the point in which the recent American workers on the lymphatic system differ.

A further point of interest in this series is a group of small vessels along the renal anastomosing vein. These vessels, I think, are forerunners of the mesenteric sacs. The indications of lymphatics for the posterior part of the body appear at this stage.

In another embryo (No. 128) measuring 20 mm. the jugular sacs are again abortive, measuring only .75 mm. The specimen is, however, very interesting in connection with the lymphatics for the posterior part of the body. In the neck, as we have seen, the early lymphatics are the two jugular sacs, with either an extension or a supplementary sac along the primitive ulnar vein, in the arm bud. In the posterior part of the body three sacs have been found, two of them median, the mesenteric sac and the cisterna chyli; and one paired, namely the posterior lymph sac. In this embryo, in the place of the future cisterna chyli, there is an extensive median vein connecting the two sciatic veins. Just ventral to this, compare with Fig. 12, is the renal anastomosis running through the great mass of the sympathetic system in the hilum of the two adrenal bodies. Around these two large median anastomosing veins there is as yet no evidence of the future median lymphatic sac. However, to the side of the two sciatic veins, just posterior to the median anastomosis, is an abundant plexus of veins on the one side and a possible beginning pos-

terior lymph sac on the other, making a definite indication of the posterior lymphatic sacs. This stage is, I believe, just preliminary to the formation of the three abdominal sacs. In the next specimen these sacs become definite.

Thus in the first fourteen specimens of the series, measuring from 8 to 20 mm., simply the jugular sacs are present. From now on, that is in embryos above 20 mm., we shall have to follow not only the jugular sacs but the mesenteric sac, the cisterna chyli and the posterior lymph sacs as well.

The first embryo above 20 mm. in the series, is one (No. 382) measuring 23 mm. This series is cut in sagittal sections. It shows the jugular sac beautifully, which has now reached a size of 2 x 1 mm., and lies opposite the third, fourth and fifth cervical vertebræ. The series, however, is much more important in connection with the other lymphatic sacs. I cannot find the posterior lymph sac, but both the mesenteric sac and the cisterna chyli are present. For these two median sacs the sagittal plane proves to be by far the best. In Fig. 11, which was made by graphic reconstruction, is shown the retroperitoneal sac in its relation to the renal vein and the suprarenal body. It is designed to relate the mesenteric sac and cisterna chyli to the surrounding structures. The point at which the vena cava turns ventralward, opposite the second lumbar vertebra, marks the position both of the renal veins and also the suprarenal branch which is a large vein running anteriorly along the ventral surface of the suprarenal body. The retroperitoneal sac extends along the renal and suprarenal veins, the latter being hidden in the diagram by the vena cava. In following the suprarenal veins the lymphatic vessels approach the superior mesenteric artery, along which they subsequently grow out into the mesentery, as has been shown by Heuer.[22] The line of mesentery is shown in the diagram. The diagram shows the mass of sympathetic ganglia closely related to the suprarenal body. In this early stage the lymphatic ducts are not likely to be confused with the sympathetic ganglia, but later when nodes begin to develop care must be exercised to distinguish them. The diagram also shows

[22]Heuer. Amer. Jour. Anat., Vol. IX, No. 1.

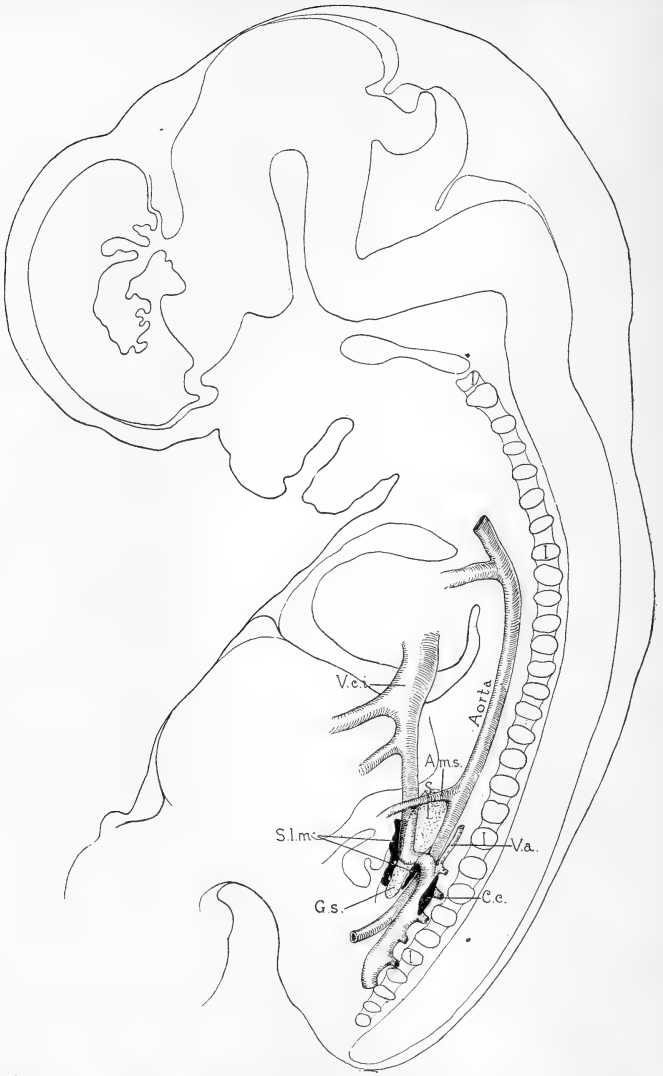

FIG. 11. A composite diagram made by superimposing the sections showing the relations of the mesenteric sac and cisterna chyli to the veins, in a human embryo measuring 23 mm., Mall collection, No. 382. X about 8. A. m. s., A. mesenterica superior; C. c., cisterna chyli; G. s., gangli sympathetica; S. l. m., saccus lymphaticus mesentericus; S., suprarenal body; V. a., v. azygos; v. c. i., vena cava inferior.

interesting relations of the cisterna chyli. It arises opposite the second, third and fourth lumbar vertebræ, closely adjacent to the inferior vena cava where it anastomoses with the azygos veins. In studying through Dr. Mall's collection I have become convinced that the cisterna chyli forms one of the primitive sacs and that the thoracic duct may grow forward, *i. e.*, anteriorly from it. Baetjer has shown that the mesenteric sac soon becomes connected with the cisterna chyli. In this series I cannot find any evidence of a thoracic duct. The cisterna chyli differs from the other sacs simply in not being completely transformed into lymph nodes, though its lower border develops into a large group of them, as will be shown in the last series.

The next embryo of the series (No. 6), measuring 24 mm., has a large jugular sac. The series is incomplete so that I cannot get the length of the sac, but its width is the same as the preceding, namely 1 mm. The valves are present and the sac shows much bridging. There is a well defined subclavian sac. This series is also more interesting in connection with the other sacs. It shows three things, the retroperitoneal sac, the cisterna chyli, the beginning thoracic duct and the anlage of the posterior lymph sacs. A point of especial interest in this series is in connection with the cisterna chyli. This sac is present as a few vessels dorsal to the aorta; and from the sac ducts extend anteriorly immediately adjacent to the azygos veins. On the left side, this duct extends into the thoracic cavity almost to the neck. I cannot trace it to the jugular sac nor is the series perfect enough to enable one to say whether it is present in every section or not, but there is sufficient evidence to indicate that the thoracic duct may be an outgrowth of the cisterna chyli.

The thoracic duct has proved to be the most difficult part of the lymphatic system to work out for this reason, we have not yet found a way to inject it in early stages and uninjected sections are not adequate. The evidence of sections is as follows, the jugular sac and the cisterna chyli, which the duct subsequently connects, develop before the duct. The question is, does the duct develop from multiple anlagen from the azygos veins for which there is no proof except that lymphatic vessels can be seen in sections adjacent to

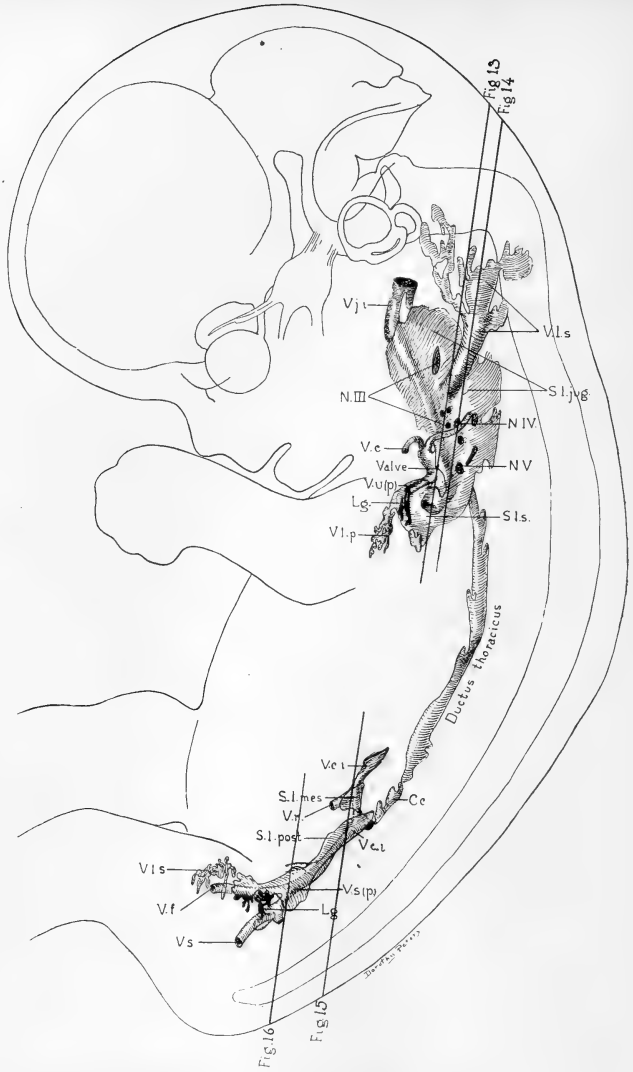

FIG. 12. Flat reconstruction of the primitive lymphatic system in a human embryo, 30 mm. long, Mall collection, No. 86. X about 5.4. C. c., cisterna chyli; L. g., lymphoglandula; N. III., N. IV., and N. V., Nn. cervicales; S. l. jug., saccus lymphaticus jugularis; S. l. mes., saccus lymphaticus mesentericus; S. l. post., saccus lymphaticus posterior; S. l. s., saccus lymphaticus subclavius; V. c., vena cephalica; V. c. i., vena cava inferior; V. f., vena femoralis; V. j. i. vena jugularis inferior; V. l. p., vasa lymphatica profunda; V. l. s., vasa lymphatica superficialis; V. r., vena renalis; v. s., vena sciatica; V. u. (p.), vena ulnaris (primitiva).

these veins, or does the duct grow from the two sacs, the cisterna chyli and the jugular one. For this second view the evidence is also weak, it consists in this, that other lymph ducts wherever we can study them grow from the sacs; and secondly in pig embryos and in human embryos one can trace a duct forward from the cisterna chyli and caudalward from the jugular sac, and in later stages these two ducts have joined. The weakness of this evidence lies in the fact that in the earlier stages the picture is always liable to be confused by Lewis' multiple anlagen. In both pig and human embryos the stages to be studied for the thoracic duct lie between 20 and 30 mm. In an embryo pig the complete thoracic duct can be injected at 27 mm. It should be quite clearly noted that whichever method of formation of the thoracic duct proves ultimately to be correct, that is whether it arises from the azygos veins in situ or from an outgrowth of the lymphatic sacs, the most fundamental point remains the same, that its endothelium is vascular.

However, it should be stated here that wherever growing lymphatic capillaries have been absolutely tested, they grow by the sprouting of their own endothelium rather than by additions of new anlagen from the veins. This has already been noted for the living lymphatics; it is also shown by the work of Dr. H. M. Evans[23] on the growth of new lymphatic capillaries into a sarcoma of the intestine. His injections show that the new lymphatic capillaries are derived from the mucosal plexus and that these new vessels are analogous with the central lacteals of the villi. In the tumor, however, they are developing beyond the normal limits of the terminal lacteals into a spreading plexus. This plexus shows all gradations as seen in Evans' figure to the normal lacteal.

From the next specimen (No. 86), measuring 30 mm., a graphic reconstruction has been made of the entire primitive lymphatic system, Fig. 12. It does not show the extent of the peripheral lymphatics, but does show the relations of the primitive system. At this stage, as will be seen in the reconstruction, the lymphatic system

[23]Evans. On the occurrence of newly-formed lymphatic vessels in malignant growths. Johns Hopkins Hospital Bulletin, 1908.

consists of the large jugular sac, measuring 5 x 3.6 mm., with its large extension along the ulnar vein to the arm bud. Emptying into the jugular sac on one side is the thoracic duct which connects with a small cisterna chyli. Ventral to the cisterna chyli is the sec-

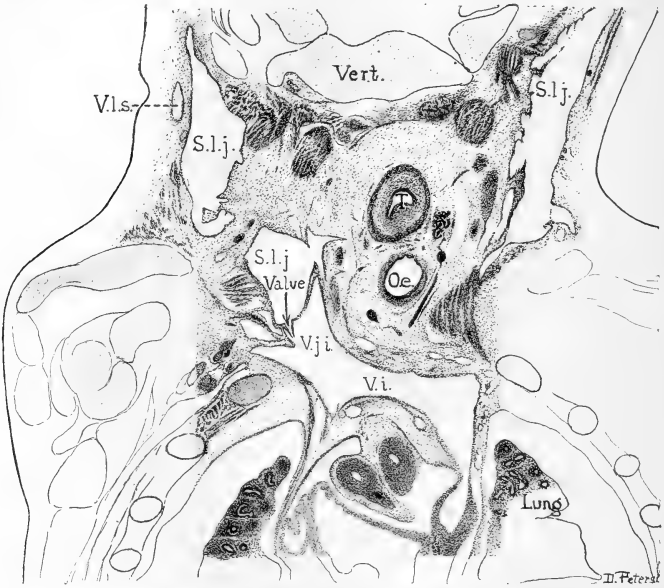

FIG. 13. Coronal section through the jugular lymph sacs in a human embryo of 30 mm., Mall collection, No. 86. × about 11. The level of the section is shown on the reconstruction of Fig. 21. The section shows the complete lymph sac on the right side and is cut to show the valve on the left. S. l. j., saccus lymphaticus jugularis; V. i., V. innominata; V. j. i., V. jugularis interna; V. l. s., vasa lymphatica superficialis.

ond median sac, the retroperitoneal, which is adjacent to the renal veins. At this stage a connection between the cisterna chyli and the mesenteric sac, which has been so well shown by Baetjer for the pig of the same size, could not be made out. The posterior sac has now become a long narrow sac, along the course of the primi-

tive sciatic veins and inferior vena cava. It measures 4.6 x .5 x .96 (dorso-ventral), and runs almost to the cisterna chyli, with which a connection cannot be made out. The plane of the section, coronal, is not especially advantageous for determining whether the connection has been made or not.

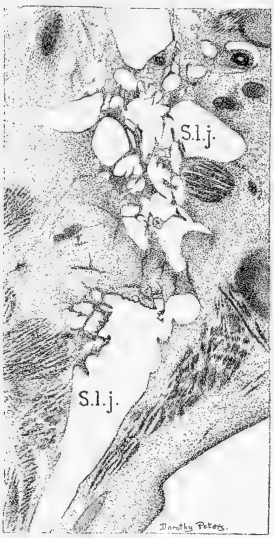

Fig. 14. Coronal section through the jugular lymph sac of the same embryo, at the level shown in Fig. 21, to show the simple bridging of the sac which is the anlage of the first lymph node. × about 19. S. l. j., saccus lymphaticus jugularis.

The jugular sacs show a number of points of interest. First their increase in size, this being the stage of the maximum size. The valve is very beautifully shown, as is seen in Fig. 13. The level of this section is shown on the diagram. A second point of interest is the extensive bridging of the sac. This occurs especially near the dorsal border, as is shown in Fig. 14. At this stage the bridges of connective tissue, which cut the sac, show more connective

tissue cells than the surrounding mesenchyme. This thickening of the mesenchyme around a plexus of lymph ducts makes the anlage of a lymph node.

A third point of interest is the spreading of the ducts from the jugular sac to the skin. I want to call especial attention to the great size of these ducts, one especially which leaves the lateral surface of the sac. These ducts are the first lymphatics to reach the skin; as has been said, they first reach the skin in a human embryo of about 20 mm., and by this stage they have grown over the head and down over the shoulders. These peripheral vessels have not been reconstructed.

Fig. 12 shows that the sac has now been cut through by three of the cervical nerves, the third, fourth and fifth. This is interesting in connection with the shifting of the structures in the neck and in the placing of the sacs. Just at the edge of the subclavian sac is a second small beginning lymph node. This lymph node is like the jugular one, consisting of bridges of thickened connective tissue between a rich plexus of lymphatic capillaries. The beginning of the deep lymphatics for the arm is also shown. I could not trace them farther in the sections.

The thoracic duct shows beautifully in the sections. It begins at the cisterna chyli as a double duct, but the right one soon crosses obliquely in the plane of the coronal section to the left side and joins its fellow. The duct lies adjacent to the azygos veins and has many irregularities. At this stage, the duct reaches the jugular sac, an advance from embryo No. 6, of 24 mm., in which it only extended into the thoracic cavity.

In the angle of the bifurcation of the trachea in this embryo is a clump of lymphatic vessels which possibly connect with the thoracic duct, though the connection could not be made out in the sections. These vessels extend a short distance along the bronchi and are the first visceral lymphatics I have found in the series.

The retroperitoneal sac is shown in Fig. 15, which corresponds with the line on Fig. 12. The section shows the relation of the sac to the renal vein and brings out the especially large masses of the sympathetic ganglia in this region.

The posterior lymph sac shows on one side in Fig. 15, but much better in Fig. 16. The posterior lymph sac is a double sac extending

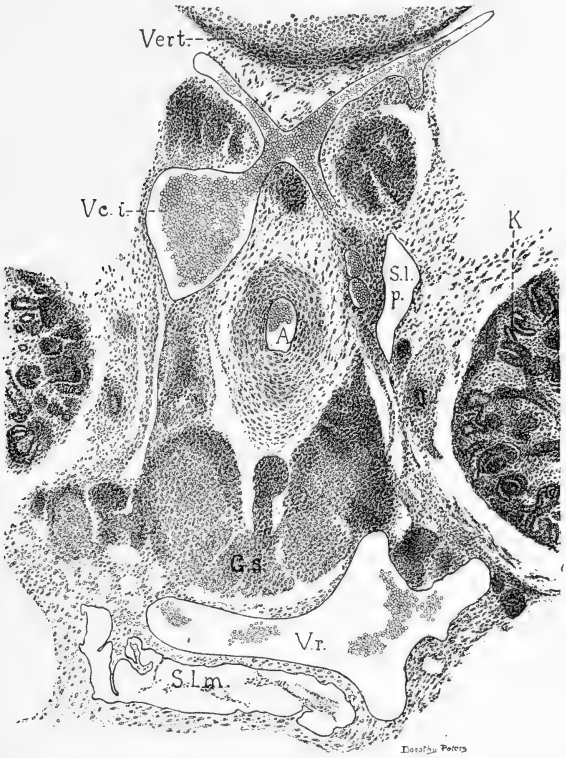

Fig. 15. Coronal section through the retroperitoneal sac of the human embryo, at a level indicated on Fig. 21. × about 39. A., aorta; K., kidney; S. l. m., saccus lymphaticus mesenterica; S. l. p., saccus lymphatica posterior; V. c. i., vena cava inferior; V. r., vena renalis.

along the primitive sciatic veins. The reconstruction is made of the left side, but shows where the left primitive sciatic vein joins the

right to form the inferior vena cava, and shows that the sacs now extend forward almost to the cisterna chyli. The cisterna chyli being median and the posterior sacs being lateral, the plane of the section made it impossible to trace whether the connection has been made or not. The two sacs, however, run to the same level and probably do connect. The posterior sac measures 4.6 x .2 (lateral x .9 (dorso-ventral). In the angle where the femoral vein branches

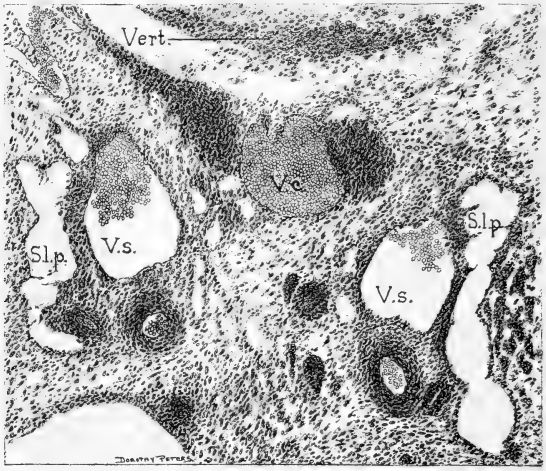

Fig. 16. Coronal section through the posterior lymph sacs as they lie along the primitive sciatic veins, of the same embryo, at a level shown in Fig. 21. × about 49. S. l. p., saccus lymphaticus posterior; V. c., vena candalis; V. s., vena sciatica primitiva.

off from the primitive sciatic is a lymph node and it will be seen that deep lymphatic vessels follow both the sciatic and femoral veins. There is also a group of superficial lymphatics covering the skin of the hip in the groove between the body wall and the leg. These superficial lymphatics could be traced to a connection with the sac on the opposite side, but not on the side reconstructed. This gap is probably due to the accidental plane of the section; it comes where

the vessels turn directly outward and are so cut in cross section. These gaps to be found in serial sections have already been discussed, they occur in thin sections, but much more often in thick ones like these, this embryo being cut at 50 microns, where the slender lymphatics must often be missed. The extent of these superficial lymphatics has not been shown in the reconstruction, they are readily made out in the skin over the back and hip. There is no difficulty in telling them, they are so sharply lined by endothelium, are empty and about three times the size of the blood capillary. This specimen then shows all the primitive sacs and their relations to the thoracic duct. It marks also the beginning of the peripheral lymphatic system, both visceral, to the lungs, and superficial to the skin.

The next specimens consist of a group of four embryos of about the same stage, No. 95 measuring 46 mm. and three others (No. 96, No. 84 and No. 224) all measuring 50 mm. They all prove to be especially interesting in connection with the development of the posterior lymph sac. In connection with the jugular sac the measurements are given in the table. These sacs show certain differences. In No. 95 the transformation into lymph nodes is not extensive and is chiefly at the upper end. No. 224, on the other hand, shows a fine bridging throughout the sac. The other two specimens show an important stage in the evolution of lymph nodes. By referring back to Fig. 14 it will be seen that when the nodes first begin in an embryo, 30 mm. long, they consist simply of a thickened connective tissue between a plexus of ducts. But at this stage, 50 mm., there appear round clumps of lymphocytes in the connective tissue bridges. These clumps of lymphocytes are the primary lymph follicles and they occur around the blood vessels of the connective tissue bridges. These primary follicles are well illustrated in Fig. 17, in the femoral lymph node, or in Fig. 18. The evolution of the lymph node depends on the balance between the two elements; the lymph ducts which multiply until they are sinuses and the vascular part with its attendant lymphocytes which make the follicles and cords. It will be seen in the figures of these embryos, that in early stages the lymphatic element by far predominates.

In embryo No. 84, the size of the lymph ducts coming from the

jugular sac is particularly striking. In one section, one of these ducts measures 2.75 x .5 mm. When it is considered that these vessels are really capillaries, being lined by a single layer of endothelium, one sees that they are really enormous in size, almost as

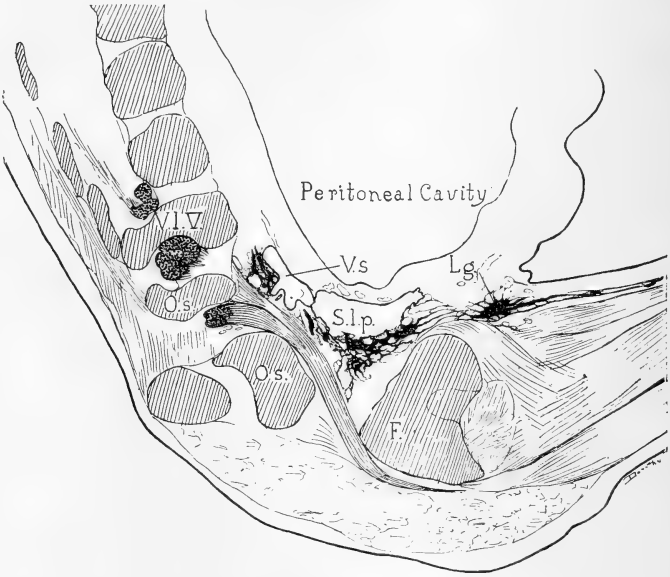

Fig. 17. Sagittal section of a human embryo measuring 50 mm., Mall collection, No. 96, showing the posterior lymph sac within the pelvis and its extension along the femoral vein. × about 8. F., femur; Lg., lymphoglandula (femoralis); O. s., os sacrum; S. l. p., saccus lymphaticus posterior with lymph node in the border; V. s., vena sciatica; V. l. v., vertebra lumbalis v.

big as the inferior vena cava itself. In general, the lymphatic vessels are considerably larger than the blood capillaries.

The cisterna chyli could not be found in No. 95, but there is a small lymph node near its usual location and there is a thoracic duct. The second embryo (No. 96) was damaged at the area; the

other two show the cisterna chyli well with large connections with the mesenteric sac. This is especially true in No. 84, when the series is transverse, the sections looking like Fig. 9 of Mr. Baetjer's series. Both the cisterna chyli and the retroperitoneal sac are easily located from Fig. 11. They are bridged from the very beginning.

These four series, however, are much more interesting in connection with the posterior lymph sacs. As we have seen, these sacs begin in an embryo about 24 mm. long as sacs along the primitive sciatic veins. In an embryo of 30 mm. they are long, narrow sacs. In Fig. 17 it will be seen that in an embryo 50 mm. long they have become large sacs lying in the side of the pelvis opposite the first three sacral vertebræ. The entire dorsal wall of the sac is occupied by a lymph node. In one of the other series it is plain that the sac is opposite the bifurcation of the vein into the sciatic and femoral veins, and that there is a large lymph node in the angle of these two veins. From the sacs extend vessels, both along the femoral vein, as shown in the figure and along the sciatic; both of these groups of vessels have developing lymph nodes. These are secondary nodes in contrast with the primary nodes which come from the sacs. The primary groups of nodes are the jugular, subclavian, retroperitoneal and posterior. The early secondary nodes are near the sacs, a point also in support of the outgrowth of lymphatics from centre to periphery.

The last embryo of the series (No. 172), measuring 80 mm., is especially valuable in connection with the fate of the jugular lymph sacs, the development of lymph nodes and the spread of the peripheral lymphatics. The jugular sac is fast becoming transformed into a large group of lymph nodes. In a few sections there are remnants of the sac measuring 1.75 x .5 or even 1.75 x 1 mm., but most of the sac has disappeared. There are also secondary lymph nodes along the other veins of the neck, for example along the external jugular vein next the parotid gland, and along the facial vein at the angle of the jaw.

In connection with the arm there is an extensive group of nodes over the shoulder. In the axilla there are four groups—one posterior to the vessels and nerves, one along the subclavian vein, and two groups anterior to the pectoralis minor muscle.

Along the trachea is a group of nodes, of which the mass at the bifurcation is especially large. Nodes are also seen along the bronchi within the hilum of the lung, and large lymph vessels extend into the pleura while smaller ones are to be seen in the septa of the lung itself. No nodes are to be made out within the lung.

The thoracic duct is easy to follow as a plexus of vessels along the aorta. Along the vertebral column there are three chains of lymph nodes—one on either side of the bodies of the vertebræ not far from the mid-line and closely associated with the thoracic duct. The other two sets are farther to the side, against the body of the vertebræ near the base of the transverse processes. These drain the body walls. So abundant are these vertebral lymph nodes that scarcely a section lacks them, the sections being 100 microns thick.

In passing into the abdominal cavity the cisterna chyli is readily located. Along its lateral borders is a complete chain of nodes, and at the lower end is a large clump of similar nodes.

The retroperitoneal sac has been transformed into a group of nodes except at the upper end, just below the superior mesenteric artery where the sac still persists. Fig. 18 is taken just below the more open part of the sac and shows the bridging and some extension of the sac to the right. The retroperitoneal sac then becomes the group of nodes ventral to the aorta. It will be remembered that at the beginning, the sac extended along the veins of the adrenal bodies. At this stage there is an extensive mass of lymphatic tissue continuous with the mesenteric sac, extending along the hilum of the suprarenal bodies. The same mass of lymphatic tissue lies at the base of the mesentery at the portal of the liver. In no section, however, are there any nodes within the hilum of the liver.

The most extraordinary development has taken place in the mesentery. A group of nodes follows the pancreas and there is a small node at the hilum of the spleen. A similar node lies against the stomach. In the center of the mesentery is an exceedingly large node, measuring 2 mm. on a side, see Fig. 18. This large central lymphatic mass in the mesentery is connected with the mesenteric sac by a chain of nodes running along the superior mesenteric artery. From this central mass vessels run out in the mesentery toward the intestine.

The only structures with which the developing lymph nodes could be confused are the sympathetic ganglia. On this account care must be exercised, especially around the retroperitoneal sac, where both structures are very abundant. The sections of these stages are thick (50 to 100 microns) and the low powers of the

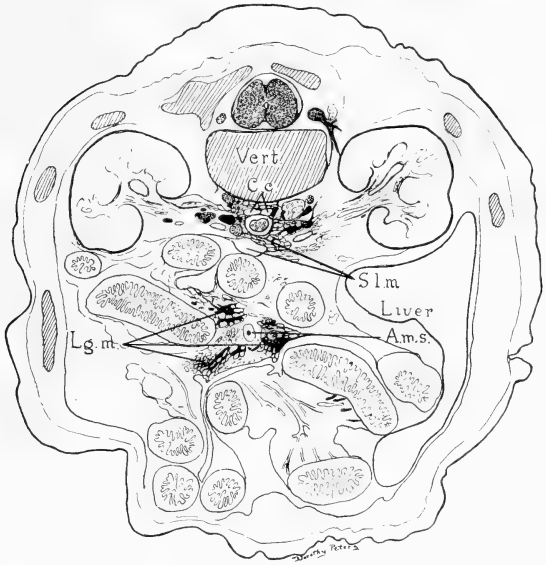

Fig. 18. Transverse section through the abdominal cavity of a human embryo, 80 mm. long, Mall collection, No. 172. It shows the kidneys, a little of the liver, and many loops of the intestine. × about 8. A. m. s., arteria mesenterica superior; C. c., cisterna chyli at its lower border; Lg. m., lymphoglandulae mesentericae; S. l. m., saccus lymphaticus mesenterica.

microscope are inadequate to distinguish them, especially when the connective tissue around the ganglia is broken. With care and serial sections the lymph nodes can be absolutely determined.

The significance of this retroperitoneal sac is brought out in the injected specimens in Dr. Heuer's paper. It will be noted that in

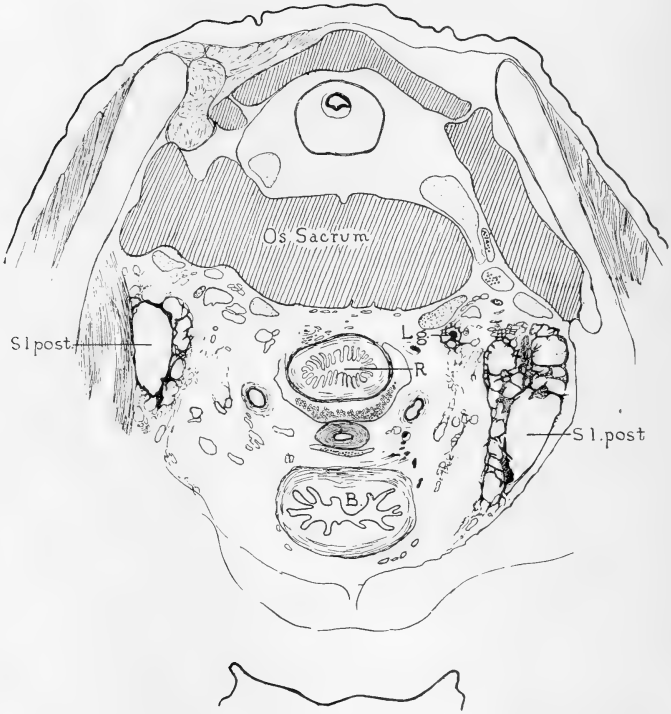

Fig. 19. Transverse section through the pelvis of a human embryo, 80 mm. long. Mall collection, No. 172, to show the posterior lymph sacs. × about 9. B., bladder; Lg., lymphoglandula; R., rectum; S. l. post., saccus lymphaticus posterior.

the injected pig embryos the sac seems much larger than in the sections of human embryos. Its importance is that it is the anlage of the visceral lymphatics; it is transformed into the preaortic nodes of which the most anterior group is around the cœliac axis.

In Fig. 18 is shown the lower part of the cisterna chyli; here the sac is being transformed into lymph nodes while farther an-

terior the sac itself persists. In tracing the series caudalward, the central mass of lymph nodes corresponding with the cisterna chyli, can be traced to the pelvis, where the mass turns a little to the side and joins the posterior lymph sacs. The posterior lymph sacs are really enormous in size, measuring 2.8 x 2 x 3.5 mm. (dorso-ventral). These measurements include the glandular masses in the edge of the sac.

The sacs are well shown in Fig. 19, which illustrates that the posterior sacs are being transformed into lymph nodes. In some sections of the pelvis these masses of lymphatic tissue seem to take up almost two thirds of the area of the cross section. From the posterior sac two sets of vessels extend, one along the sciatic vein and the other along the femoral. There is one lymph node along the sciatic vessels and a chain of nodes along the femoral. In Fig. 19 is a tiny lymph node, labeled Lg., which illustrates well the simplest form of a lymph node, a central mass of lymphocytes with a plexus of lymph ducts around. This plexus of ducts is so close that it may already be termed a sinus, so the node consists of a single follicle with its peripheral sinus. It is the structural unit of the lymph node.

From the description of this specimen it will be seen that the foundations of the lymphatic system as it is found in the adult have been laid down in an embryo of 80 mm.

The primitive system is complete, and the sacs are forming the primary nodes. The peripheral vessels have extended to the skin and to the viscera, and secondary nodes are forming along these vessels. I think that we have the key for working out the peripheral spread of the lymphatics and carrying them to their capillary bed. Injections of the retroperitoneal sac give us the material for tracing this development.

THE DEVELOPMENT OF THE LYMPHATICS IN THE SMALL INTESTINE OF THE PIG.

BY

GEORGE HEUER.

From the Anatomical Laboratory of the Johns Hopkins University.

WITH 17 FIGURES.

The recent American work on the lymphatic system has given us a new conception of the morphology of the system as a whole. The primitive lymphatic system consists of a number of sacs which are derived from the veins and which become united into a system for the most part by the thoracic duct. A further essential of this primitive system is that all of the sacs give up their connection with the veins, and only the two in the neck rejoin the vein to form the permanent opening. Thus far three sets of paired sacs and two unpaired ones have been described: the jugular sacs, the subclavian which in human embryos are an extension of the jugular, and the sciatic are the paired, the retroperitoneal sac and the cisterna chyli are the unpaired. It is the retroperitoneal sac which especially concerns us in this paper, for it is the source of the lymphatics of the intestine. This primitive system is complete and can be injected in pig embryos 2.7 cm. long.

The idea that the sacs form a primitive lymphatic system is not the most fundamental conception of this new theory, but rather that these sacs arise from the veins and in turn give rise to the lymphatic vessels, so that we may say that the lymphatic system as a whole is derived from the blood vascular system, that lymphatics are modified veins, and that the growth of lymphatics is always from center to periphery. There is now a general agreement in regard to the origin of the primitive sacs, but in regard to the second

exceedingly important point, namely, that the lymphatic vessels grow from the sacs, there are still differences. It was to test this point that the present study was undertaken at the suggestion of Dr. Sabin. I believe that this work, showing that the vessels of the intestine grow from the retroperitoneal sac, strengthens her position that the lymphatic system is derived from the sacs. Certainly the theory is a fruitful one, for injections of the retroperitoneal sac give the key for tracing the growth of the lymphatic vessels to the viscera.

The literature and general relations of the problem are given by Dr. Sabin in the article on the development of the lymphatics in human embryos in this same journal and therefore only the work on the retroperitoneal sac will be mentioned here. This sac was discovered by F. T. Lewis[1] who described it as a part of the lymphatic system in 1906.

It has been thoroughly worked out by Mr. W. Bætjer,[2] in this laboratory.

Mr. Bætjer has shown that in pig embryos 17-19 mm. long there are small branches of the large renal anastomosing vein in the root of the mesentery. These small veins are ventral to the renal vein and run in an antero-posterior direction. In embryos 19 and 20 mm. long these veins increase markedly in size and number, and by the time the embryo is 21 mm. long show sac-like dilatations which are still readily injected from the renal vein. From this time on the sac formation goes on rapidly; in embryos 22-23 mm. long, these small sacs have been completely transformed into a large median sac entirely cut off from the veins and likewise independent of the cisterna chyli. By the time the pig is 2.7 cm. long, this sac is abundantly connected with the cisterna chyli, which forms from the veins dorsal to the aorta, and an injection into the thoracic duct will flow through the cisterna chyli into the retroperitoneal sac.

Methods and Material. This paper begins with the stage at which Mr. Bætjer left off, namely, where the retroperitoneal sac

[1]Lewis, Amer. Jour. of Anat., Vol. V., 1906.
[2]Bætjer, Amer. Jour. of Anat., Vol. VIII.

is connected with the thoracic duct through the cisterna chyli. Such an injection is shown in Fig. 1 from an embryo 3 cm. long. The earliest stage in which the thoracic duct and sac have been injected is 2.7 cm., where the appearances do not differ from the specimen shown in Fig. 1, except that the sac is a little smaller.

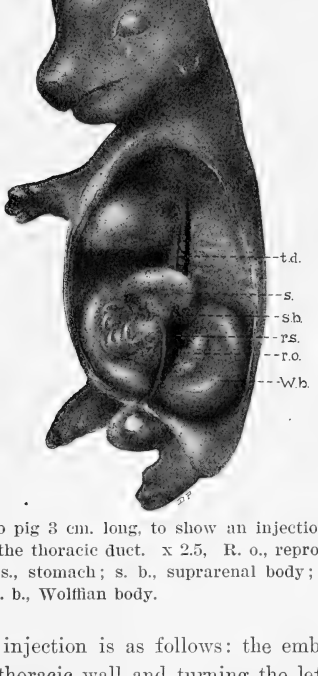

Fig. 1.—An embryo pig 3 cm. long, to show an injection of the lymphatics made by puncturing the thoracic duct. x 2.5, R. o., reproductive organ; r. s., retroperitoneal sac; s., stomach; s. b., suprarenal body; t. d., thoracic duct around the aorta; W. b., Wolffian body.

The method of injection is as follows: the embryo is opened by cutting away the thoracic wall and turning the left lung far to the right or removing it entirely. For injections of the retroperitoneal sac it is better to remove the liver. The aorta and azygos vein should be thoroughly exposed, and the hypodermic needle plunged down behind the aorta, inserting the needle just at the arch where

it is easy to distinguish the vein and thus avoid it. In Fig. 2 is shown a section of an embryo 20 mm. long, taken at the level of the apex of the lung, to show the thoracic duct in its relation to the aorta and to the azygos veins. It shows why it is necessary to insert the needle behind the aorta. It is best to use the finest possible needles; my injections are made with No. 28. For an injection mass either saturated aqueous Prussian blue or India ink are used; the Prussian blue gives beautiful total specimens which

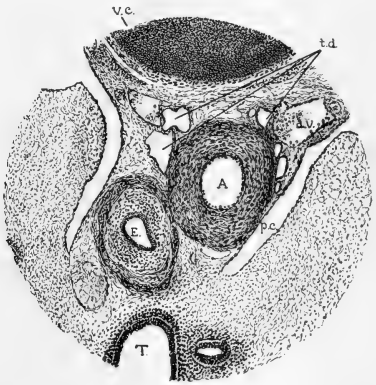

Fig. 2.—Transverse section of an embryo pig, 2 cm. long at the level of the apex of the lung to show the relation of the thoracic duct to the azygos veins and to the aorta. x 50. A., aorta; a. v., azygos vein; E., esophagus; p. c., pleural cavity; t. d., thoracic duct; v. c., vertebral cartilage.

can be kept either in formalin or hardened in bichloride acetic and kept in alcohol; the India ink runs farther, and the total specimens can be cleared by the Schultze method. The ink is more useful for determining complete injections.

For studying the development of the lymphatics to the intestine it was found best at first to inject by means of the thoracic duct in embryos from 3.0 to 12.5 cm. Subsequently after the exact position of the retroperitoneal sac was determined, it proved that it was far better to introduce the needle directly into the sac. This

is done as follows: The embryo is thoroughly opened and the two Wolffian bodies spread apart as far as possible and the entire intestine turned to the right. This exposes the sac, as can be seen in Fig. 3. By looking closely one can see three or four small arteries

FIG. 3.—Embryo pig 3.5 cm. long to show an injection of the retroperitoneal sac made through the thoracic duct. x 2.5. r. s., retroperitoneal sac; r. o., reproductive organ; s, stomach; W. b., Wolffian body.

to the Wolffian body running perpendicular to the rectum; the needle must enter just dorsal to these arteries. The retroperitoneal sac is the best point for injecting the viscera. To determine the zones in the intestine, the sac has been filled until it ruptured. Good injections are only to be obtained in embryos in which the heart is

still beating. For any stage it is easier to obtain complete injections through the sac than through the thoracic duct, for in the later case the injection mass must fill the sac before it runs out into the vessels, and the size of the sac decreases the pressure.

Figs. 1 to 4 and 7 show that the thoracic duct is fairly symmetrical below the heart, that there are two vessels, one on either side connected by many cross channels making a plexus around the aorta. Dorsal to the heart, the right duct crosses over and joins the left. Dorsal to the kidneys, the two ducts unite in a median cisterna chyli.

In embryos above 12 cm. it was found impossible to obtain injections of the intestinal wall through the thoracic duct. This is due to the developing lymph nodes, which at first do not check the flow of the injection mass, but later retard it very much. It has been shown that in human embryos the retroperitoneal sac is changed into the group of preaortic lymph nodes extending from the cœliac axis to the bifurcation of the aorta, and this change is being made in an embryo 8 cm. long. A secondary, larger group of lymph nodes forms in the center of the mesentery, along the superior mesenteric artery, and this group is also being formed in the same embryo. The early lymph nodes, however, consist of a great plexus of wide lymphatic ducts with very few follicles and hence injection through them is easy. By the time the pig is 12 cm., however, the injection mass passes to the nodes, and increased pressure results in an extravasation at the node. For these stages, therefore, it was found necessary to inject into the wall of the intestine itself. To get good injections by this method it was found best to pierce both muscle coats, and thus have the needle just enter the sub-mucosa and inject slowly under low pressure. In embryos from 12 to 13 cm. this was difficult, in the older stages, from 16 cm. up, it could be done readily.

The areas injected by these two methods, in the younger embryos through the thoracic duct or retroperitoneal sac, in the older pigs directly into the wall of the intestine, were of course very different. By injecting into the sac, a general lymphatic injection was obtained, the lymphatics reaching in embryos 5 or 6 cm. long all the thoracic

and abdominal viscera. By injecting into the wall of the intestine only the lymphatics of the intestine and the mesenteric lymph nodes were filled with the injection mass.

The lymphatics were studied both macroscopically and microscopically. In the younger stages, 2.7 to 12 cm., in which a general lymphatic injection was made, a single embryo could be used for both purposes. The best view of the lymphatics is obtained while injecting; there is absolutely no difficulty in distinguishing the lymphatics nor in telling any extravasations whatever. After noting the extent of the injection, small pieces were removed for microscopic sections, while the rest of the embryo was placed in a large amount of strong alcohol, 95 to 96 per cent, until shriveled, then cleared in caustic potash, 1 to 2 per cent, and mounted in glycerine, according to the Schultze method.

Specimens cleared in this way show the course of the lymphatics beautifully and with a dissecting microscope they can be followed to all the organs. The walls of the lymphatic vessels are so delicate, however, that the specimens are not permanent.

For the study of the lymphatics of the intestine, cleared pieces of the intestine were mounted in glycerine in hanging drop slides and studied with the microscope in conjunction with serial sections. The portion of the intestine which was most readily identified and which was in all cases particularly examined is a loop of the duodenum which coils around the root of the mesentery. This loop is seen in Figs. 1, 3 and 4. It is the loop most apt to be injected if the needle is introduced into the thoracic duct, probably for mechanical reasons, for when the needle enters the retroperitoneal sac itself, the mesentery becomes uniformly injected.

General Description. The lymphatics of the intestine arise from the retroperitoneal sac. This sac is shown in Fig. 1 in an embryo 3 cm. long. The specimen was made by injecting into the thoracic duct. It will be seen that the sac is triangular in shape and lies opposite the hilum of the Wolffian bodies. In an embryo 2.7 cm. long the sac measures about 2 mm. in length, at this stage 3 cm., it is about 2.7 mm. long. That it is connected with the cisterna chyli is proved by the injection and is shown in Bætjer's Fig. 9 for an embryo of the same stage.

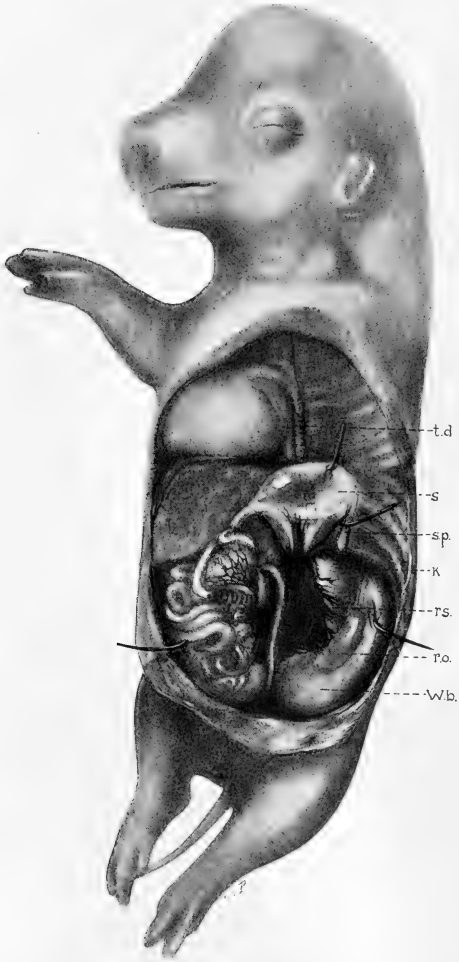

Fig. 4.—Embryo pig 4.5 cm. long to show an injection of the retroperitoneal sac and mesentery made through the thoracic duct. x 2.5. K., kidney; r. o., reproductive organ; r. s., retroperitoneal sac; sp., spleen; s., stomach; W. b., Wolffian body.

In Fig. 3 is shown a lymphatic injection of a pig 3.5 cm. long. At this stage the sac is readily injected either through the thoracic duct or directly. It is diamond shape with a slight indentation opposite the suprarenal bodies. This indentation indicates a division of the sac into two portions, an anterior portion which, as is seen in Fig. 4, sends vessels to the stomach, spleen and duodenum while the ducts for the intestine come from the posterior portion. At this stage, namely, at 3.5 cm., there are a few vessels extending on to the suprarenal bodies, as well as numerous blunt processes to the Wolffian body. It is especially to be noted that the sac is a solid mass, in the injected specimen, that is, it has a single cavity in contrast to the later stage of Fig. 7, where the sac is broken up into a mass of vessels making the anlage of a lymph node or a group of nodes.

In Fig. 4 is shown an injection of an embryo 4.5 cm. long. This stage is particularly interesting to us, for it shows the lymphatics extending into the mesentery. The thoracic duct is plain, and there is a vessel running to the heart, as well as a back flow from the point of injection to the jugular lymph sac. Below the diaphragm, the stomach has been pulled up and the spleen turned over to the left to show the lymphatics passing to its dorsal border. The mesenteric sac is now considerably larger, measuring 5 by 4 mm. From its anterior border three groups of vessels are seen, one to the spleen, a large group which reaches the stomach wall, and the third group which pases on to the duodenum. In the mesentery of the coil of the lower end of the duodenum, the vessels form a beautiful plexus and have reached the mesenteric border of the gut. The sac itself, which still retains its character as a large sac, is connected with the thoracic duct in three places, one, the principal group of several ducts opposite the hilum of the kidney which is the primary connection seen in sections at 3 cm., as shown in Bætjer's Fig. 9; secondly, a small duct which connects the anterior end of the sac with the thoracic duct; this duct runs just anterior to the suprarenal body; and thirdly, an anastomosis between the posterior end of the retroperitoneal sac and the posterior lymph sac. These relations are all shown in Fig. 5,

which is taken from a pig of the same litter as Fig. 4, and is drawn with the Wolffian body and kidney removed in order to show the cisterna chyli which lies dorsal to the aorta. In watching an injection from the thoracic duct, the fluid usually runs down the duct to a point just anterior to the suprarenal body, here the stream divides and part runs into the anterior end of the mesenteric sac, while the rest runs on into the cisterna chyli. From here the central part of the retroperitoneal sac fills up. As soon as this central part is filled, the fluid runs from it into the anterior part of the sac. An incomplete injection might lead one to think that the

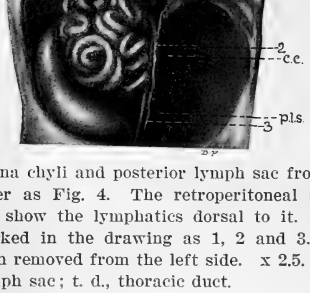

Fig. 5.—The cisterna chyli and posterior lymph sac from a pig 4.5 cm. long, from the same litter as Fig. 4. The retroperitoneal sac has been almost entirely removed to show the lymphatics dorsal to it. The three points of anatomosis are marked in the drawing as 1, 2 and 3. The Wolffian body and kidney have been removed from the left side. x 2.5. c. c., cisterna chyli; p. l. s., posterior lymph sac; t. d., thoracic duct.

anterior end of the sac was a separate sac, while as a matter of fact there is one continuous sac, corresponding with the position of the entire group of preaortic nodes of the adult, a chain of nodes, which extend from the level of the cœliac axis to the bifurcation of the aorta.

It does not correspond in position with the so-called mesenteric lymph nodes which are within the folds of the mesentery. Therefore the sac is termed here the retroperitoneal lymph sac, rather than retaining the name of mesenteric sac used by Dr. F. T. Lewis and Mr. Bætjer.

The anatomosis of the posterior lymph sac with the retroperitoneal sac is shown in Fig. 5. This posterior sac receives three sets of

vessels, a sciatic group, a femoral and an umbilical. It is clear then that in the embryo, lymph from the legs has the direct course through the posterior sac to the cisterna chyli, or the indirect path through the posterior sac and retroperitoneal sac. This, in connection with the fact that the abdominal viscera, the diaphragm and the lymphatics of the lungs are most readily injected from the retroperitoneal sac, is of importance in emphasizing the significance of the retroperitoneal sac and the preaortic group of nodes into which it develops. In an embryo 5.5 cm. long a single puncture into the retroperitoneal sac injected the lymphatics of the abdominal viscera, the skin of both hips and legs, the diaphragm, lungs, esophagus and lymphatics of the skin of the head. Such very extensive anastomosis of all the lymphatic vessels of the embryo is of significance as a basis for variations in the adult.

To sum up the lymphatics at this stage, namely at 4.5 cm., the primary system is complete, that is the primary lymph sacs are formed and connected into a system by the thoracic duct. Above the diaphragm the vessels have reached the heart, and the esophagus, probably the lungs also. Below the diaphragm vessels from the anterior part of the sac have reached the spleen, the stomach, the intestinal wall, the kidneys, suprarenal bodies and Wolffian bodies. There is an anastomosis with the posterior lymph sac. From the posterior lymph sac vessels follow the sciatic, femoral and umbilical veins.

For the small intestine the lymphatics extend along the superior mesenteric artery. In human embryos it has been shown that the mesenteric sac spreads along the suprarenal veins to the root of the superior mesenteric artery. In the study of the growth of lymphatic capillaries, it proves that these delicate walled vessels grow along some thicker walled vessel; the earliest lymphatics grow along the veins, but in the case of some of the viscera other vessels or ducts may be followed, as for example the mesenteric arteries or the bronchi in the lungs. The border zone of the injected lymphatic capillaries is marked by the rounded blunt ends which are characteristic of injections of terminal lymphatics. In studying the border zones of growing lymphatics it has been shown that the growing tips are either smooth and rounded or have long slender endothelial sprouts

which Dr. Clark has shown are collapsed vessels capable of rapidly expanding according to their state of functional activity. Hence we may say that the border zone consists of terminal vessels either collapsed or expanded. An injection mass may either open up these ends or may rupture them. A considerable increase of pressure will always rupture the terminal vessels of a border zone. The best evidence that we have at present of these border zones of growing lymphatics is in Dr. Clark's work in the living tadpole's tail where one can see a row of growing tips beyond which there are no lymphatic vessels whatever, only blood capillaries. The next best evidence

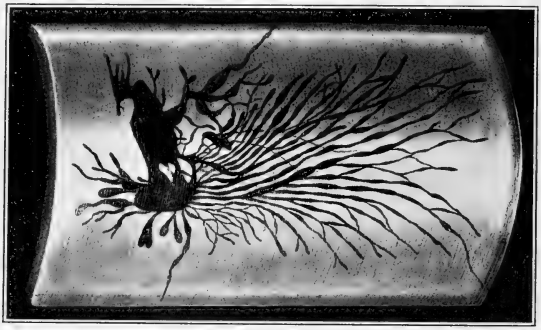

Fig. 6.—Injected lymphatics in the stomach wall of an embryo pig 6 cm. long. The large mass is at the lesser curvature.

comes in making numerous injections of succeeding stages and noting that as the embryos increase in size the zone of injected lymphatics spreads.

Injections at 5.5 and 6 cm. show an increase in size of the retroperitoneal sac; for example, at 5.5 cm. the sac measures 7 mm. in length, and there is a great increase in the number of ducts from it and an extension of their zone. At 6 cm. lymphatic vessels have extended far over the surface of the stomach and intestine (Fig. 6). The retroperitoneal sac is being cut up into a mass of vessels so that it has not the solid appearance in injections as at 3.5 and 4.5 cm. The breaking up of the sac into a plexus of

vessels is marked at 8 cm., as is shown in Fig. 7. During the stages from 4 to 10 cm. long, a number of changes take place, in the first place the plexus in the mesentery becomes exceedingly complex. For example, at 8.5 cm., an injection directly into the retroperitoneal sac will fill the entire mesentery with an abundant plexus of ducts; moreover, at the root of the mesentary, along its entire length, the injection mass fills in so as to appear solid to the naked eye. This long line of exceedingly abundant injection corresponds with the long line of single mesenteric nodes which are characteristic of the pig. Thus both the primary, that is retroperitoneal, and the secondary or mesenteric lymph nodes are forming at this stage.

To return to the wall of the intestine, it is shown in Fig. 4 that lymphatic vessels have reached the stomach and intestine in an embryo 4.5 cm. long. Injections made directly into the sac show that the vessels reach the intestine in embryos about 4 cm. long. The injections shown in Figs. 8, 11 and 12 were all made into the thoracic duct before it was found that puncturing the sac gives better results; and some of them are incomplete, but they serve to illustrate the progression of the lymphatics. In Fig. 4, it is seen that the lymphatics form a plexus in the mesentery and from this plexus a series of lymph vessels grows into the intestine along the arteries. These vessels enter the submucosa and form there a primary plexus. This primary submucosal plexus, at a stage when there is no secondary plexus, is shown for the stomach for a pig 6 cm. long. The drawing is not made so that it can be oriented readily, but the heavy mass is at the lesser curvature, and the plexus shown is in the submucosa.

In Fig. 8, is shown a section of the duodenum of a pig 8.7 cm. long. By this time there is not only a submucosal plexus but a secondary mucosal plexus as well. As has been said, the lymphatics which grow into the intestine at the mesenteric border penetrate the longitudinal and circular muscle coats, and enter the embryonic submucosa. In following through a large number of series, no deviation from this course has been observed. The point at which the lymph ducts penetrate the intestine, however, is subject to variation within certain

limits, and the lymphatic ducts follow one of two courses. In the one type, the lymphatic trunk penetrates the wall of the intestine immediately on reaching it, that is that portion along the mesenteric

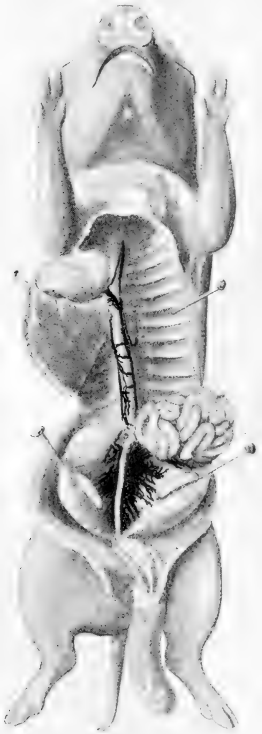

Fig. 7.—Injected lymphatics in a pig embryo 8 cm. long to show the thoracic duct and that the retroperitoneal sac is being transformed into a plexus of ducts the anlage of a group of lymph nodes. x 1.3.

attachment included between the two folds of the peritoneum, as shown in Fig. 8. In the other type the lymph vessel may run in the serosa a variable distance, usually not more than one quarter or

one half around the intestine, before penetrating the muscle coats, as shown in Fig. 8. In the latter case, by the branching of this vessel, a subperitoneal plexus may develop, a consideration of which will be taken up later.

The variations in the course of the lymphatic vessels above described may be seen in any part of the intestine and in all stages of embryos where lymphatics have entered the intestine as well as

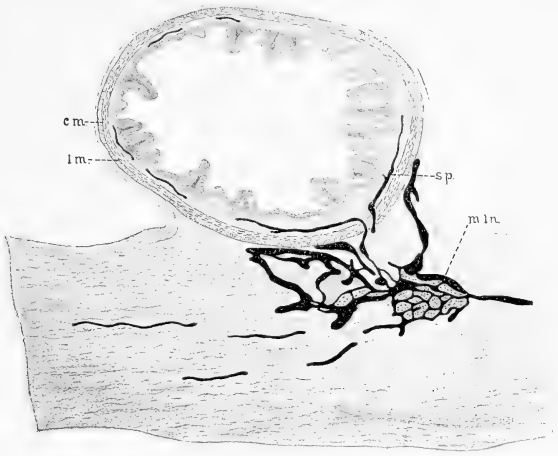

Fig. 8.—Injected lymphatics in the duodenum of a pig embryo 8.7 cm. long to show the entrance of the lymphatics into the submucosa, the submucosal plexus, and the beginning of the mucosal vessels. C. m., circular muscle; l. m., longitudinal muscle; m. l. n., mesenteric lymph node.

in the new-born and adult pig. The same relation is seen in an older embryo, 16 cm. long, in Fig. 14. In injecting the lymphatics of the intestine of the new-born or adult pig, the variations in the course of the large collecting trunks through the intestinal wall to reach the mesentery is frequently observed.

This variation in the course of the lymph vessels through the bowel wall is dependent upon the distribution of the blood vessels. It was shown in tracing the growth of the lymphatics through the

root of the mesentery that these vessels followed the artery in their development. It is equally true that the lymphatic ducts follow the arteries through the wall of the intestine. This can be seen in serial sections and in whole specimens which have been cleared by the method of Schultze. In serial sections of injected material, one can readily find a section showing an artery passing through the wall of the intestine; and in such a section often one and sometimes two lymphatic vessels are seen closely accompanying the artery through the bowel wall.

The arteries in their course to the intestine show the variations which we have described for the lymphatics; that is, the artery either penetrates the wall of the intestine immediately on reaching it, or passes some distance under the serosa before doing so. Since the lymphatic vessels follow the arteries to the intestine, the variation in this course is accounted for by the distribution of the blood vessels.

To return to Fig. 8, from an embryo 8.7 cm., beside showing the beginning of the mesenteric lymph nodes and the method of the entrance of the lymphatics into the wall of the gut, the section also shows that the submucosal plexus has spread entirely around the wall of the intestine.

On entering the submucosa, the lymphatic trunk divides into two branches, one of which extends around either side of the wall of the intestine. In its course, each branch lies entirely in the submucosa. In Fig. 9 is shown an important point in connection with these branches of the intestinal wall. It is from a pig 9 cm. long, and shows that these primary branches form a series of more or less complete lymphatic loops lying in the submucosa, near the circular muscle coat. This is, probably, an incomplete injection at this stage, but it serves to illustrate the point, for in making the injections the primary vessels of an area the vessels that develop first, fill first, and those that develop later inject later. Thus a vascular unit can be shown by a partial injection as seen in Fig. 17 for the new born pig. This can be observed even in the adult.

The growth and the arrangement of these vessels indicate a segmental development, and each with its future branches may be thought

of as a unit of structure. These primary lymph ducts can be followed in the ascending series of embryos and appear as the large collecting trunks, such as are seen for example in Fig. 14.

The formation of simple lymphatic loops in the submucosa as the primary event in the development of the lymphatics of the intestine has been demonstrated by repeated injections in embryos from 6 to 10 cm. long. It has been found constant that lymphatic vessels first appear in the submucosa and that the vessels branch to form loop-like structures. By means of serial sections, these loops are found to be continuous with the lymphatics of the mesentery.

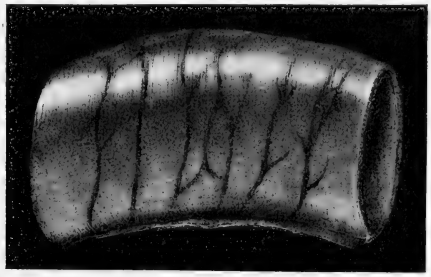

FIG. 9.—Loop of small intestine of an embryo pig 9 cm. long to show the primary lymphatic loops in the submucosa.

By the branching of these primary loops and the fusion of these branches with those of adjacent loops, a primary lymphatic plexus is formed in the submucosa. This is shown in Fig. 10, from a pig 10 cm. long.

In cleared whole preparations and in serial sections, branches from the primary lymph ducts are seen extending out into the submucosa toward one another. In different preparations it is found that some of the branches from neighboring trunks have fused, while others have approached one another but have not fused. Cleared specimens from embryos 8.5 to 11 cm. long show this plexus formation very well.

It seems clear, therefore, that a primary lymphatic plexus is formed in the submucosa by the branching of the primary loops and

the fusion of these branches. One can readily demonstrate outgrowing branches in injected material, and from the appearance of the peripheral ends of these branches and by the section one feels reasonably sure that the injection has been complete. Moreover, if one takes a series of embryos from 8.5 to 11 cm. long, injected from the same place along the thoracic duct and under about the same pressure, he finds in each succeeding larger embryo a more complete plexus formation.

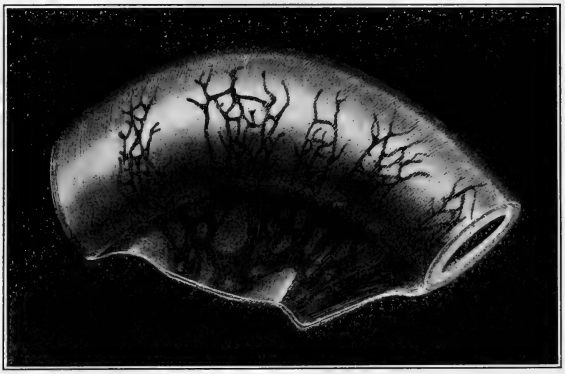

Fig. 10.—Loop of small intestine of an embryo 10 cm. long to show the development of the submucosal plexus from the primary loops shown in Fig. 9.

The plexus thus formed in the submucosa never becomes complex and close-meshed. In younger embryos it appears most complex, for here the primary lymph ducts are close together. With the increase in the size of the embryo and the elongation of the intestine, it becomes a wider meshed and less complex appearing structure and is recognized as the plexus of large vessels in embryos 16 cm. long, as in Fig. 14, and in new-born and adult pigs.

From the primary submucosal plexus above described, there is developed a second lymphatic plexus in the mucous membrane of the intestine, namely, a plexus in the mucosa at the base of the villi. From a study of the material at hand,it is believed that this plexus,

like the preceding, is the result of the peripheral spread of the lymphatics through the intestine, a plexus being formed by branching and the fusion of neighboring branches. The beginning formation of this second plexus is seen in stages in which the primary plexus is but incompletely formed and, therefore, we must think of the two plexuses as developing more or less simultaneously. In embryos 8.5 to 9.0 cm. long, in which primary lymph ducts and an incomplete submucosal plexus are found, branches are seen coming off from the primary plexus, these branches extending toward the villi. These branches mark the beginning development of the mucosal

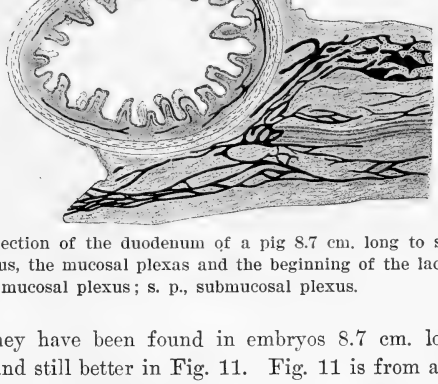

Fig. 11.—Section of the duodenum of a pig 8.7 cm. long to show the submucosal plexus, the mucosal plexus and the beginning of the lacteals. l., lacteals; m. p., mucosal plexus; s. p., submucosal plexus.

plexus. They have been found in embryos 8.7 cm. long, as seen in Fig. 8, and still better in Fig. 11. Fig. 11 is from a section 100 microns thick and not all the lymph vessels lie in the same plane, as shown in the drawing. It will be seen that the primary plexus is represented by an almost complete loop lying nearest to the circular muscle coat. From it branches have been given off extending inward toward the villi. In the older embryos and in the new-born and adult pigs, these branches remain as the connecting vessels between the mucosal and submucosal plexuses. In two places in the section, these branches have been themselves branched to form lymph-vessels running along at the base of the villi. This is an important step

in the development of the plexus in the mucosa, for it shows the way in which this plexus is formed. In embryos 9, 10, and 11 cm. long, the branching of the vessels has increased and numerous branches have united to form a close-meshed plexus at the bases of the villi. The vessels of this mucosal plexus are of smaller size than those of the preceding plexus and remain so through the ascending series. The plexus remains a close-meshed one throughout, which was seen not to be the case in the submucosal plexus.

The central lymph vessels of the villi of the intestine develop from and almost simultaneously with the secondary lymphatic plexus of

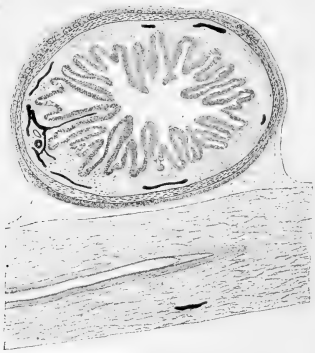

Fig. 12.—Composite section made from four adjacent sections to show the central villus, from the small intestines of a pig embryo 9 cm. long.

the mucosa. From a study of injections, they, like the plexuses of which we have spoken, seem to be the result of the peripheral extension of the lymphatics throughout the wall of the intestine, the villi being the peripheral limit of this extension. Repeated injections in the younger embryos have shown no lymph vessels in the villi. In an embryo 8.7 cm. long, as we have seen, a beginning plexus in the mucosa has been formed and in following through this series a few short branches have been found which have extended into the bases of the villi. In embryo 9 cm. long, the central lymph vessels of the villi have been injected, and they appear as straight

ducts with bulbous rounded ends extending from the mucosal plexus of the center of the villi. Fig. 12, combining four consecutive sections of a series from an embryo 9 cm. long, shows such a vessel.

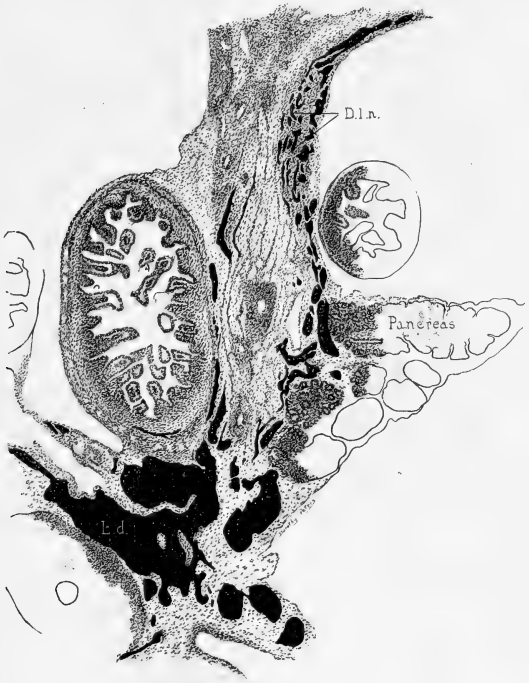

FIG. 13.—Section of the mesentery and intestine of an embryo pig 9.5 cm. long. This is an incomplete injection. It shows the size of the mesenteric vessels, their course along the artery and a tertiary lymph node. D. l. n., developing lymph node.

We see here the branches of the primary plexus near the circular muscle coat, those of the secondary plexus near the base of the villi, while connecting the two plexuses are vessels from which the secondary plexus is derived. From the secondary plexus the central lymph

duct is seen extending into the villus. The artery and vein are shown in the drawing.

There are, then, in the mucous membrame of an embryo 9 cm. long all the essential structures which go to make up the lymphatic plexuses as found in the mucosa and submucosa of the adult, and it is by the growth of these parts that the latter are derived.

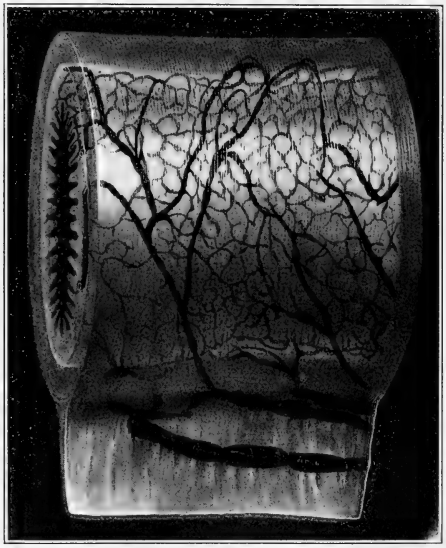

Fig. 14.—Cleared specimen of a loop of the small intestine from an embryo pig 16 cm. long to show the mesenteric vessels with their valves, the large meshed submucosal plexus, the deeper, closer-meshed mucosal plexus, and the central lacteals.

In Fig. 13 is shown an incomplete injection of the duodenum to show the very large ducts at the root of the mesentery, the fact that the vessels follow the artery in the mesentery, and thirdly, that there are beginning tertiary lymph nodes along the ducts in the mesentery. In Figs. 14 and 15 are shown injected lymphatics in

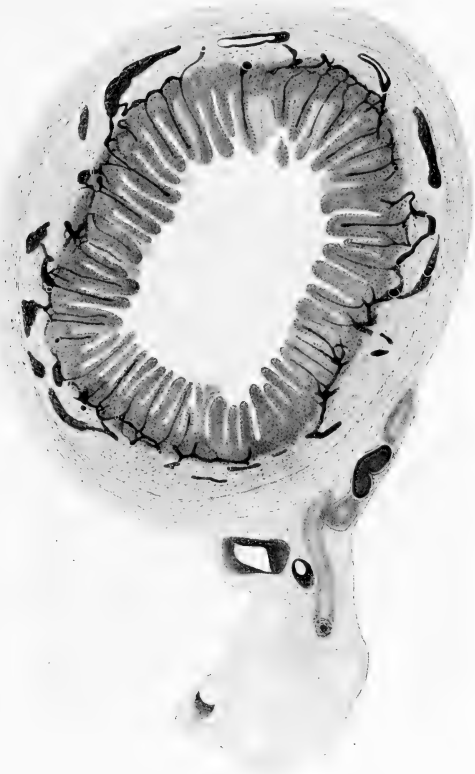

Fig. 15.—Thick section from the same specimen as Fig. 14, to show the exact position of the two plexuses.

the intestine of a pig 16 cm. long. Here the characteristics of the adult system are all laid down.

In the gross specimen of Fig. 14, the characteristic submucosal plexus is well seen. The vessels are no longer capillaries, but are collecting trunks, as shown by their size and the obvious valves. The well known "collarettes" indicate the position of these valves.

The plexus of these vessels is wider meshed, and shows clearly the primitive units, in contrast with the finer meshed mucosal plexus of capillaries. This mucosal plexus gives rise to the central lacteals, as is seen in both Figs. 14 and 15.

The development of the third lymphatic plexus of the intestine of the pig, namely that confined to the muscularis and serosa, has been the most difficult to study. It apparently develops later than the plexuses in the mucous membrane and an injection of what could be definitely termed a plexus has not been obtained in embryos under 10 cm. in length. In embryos up to 9 cm. in length no injected lym-

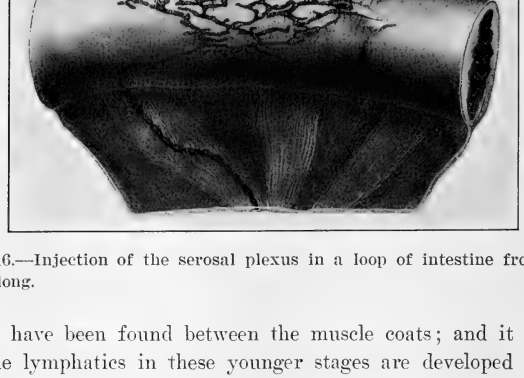

Fig. 16.—Injection of the serosal plexus in a loop of intestine from a pig 20 cm. long.

phatics have been found between the muscle coats; and it appears that the lymphatics in these younger stages are developed only in the mucous membrane.

In following the lymphatics through the wall of the intestine, it was found, as previously stated that the lymph ducts may pass in the serum some distance around the wall of the intestine before penetrating the muscle layers. Branches may be given off from the lymph ducts, while lying in this layer and by the intercommunication of these branches the beginning of a subperitoneal plexus may be formed. Such vessels have been found as early as 10 cm. In older embryos, as for example at 20 cm., it is easy to demonstrate

a serosal plexus. In Fig. 16, from an embryo 20 cm. long, the serosal plexus was injected by introducing the needle directly into the wall of the intestine. Sections of this specimen proved that the lymphatics are in the serous covering of the bowel.

The origin of this plexus is then similar to that of the plexuses in the mucous membrane; that is, it is by the growth and extension of lymphatic vessels through the serosa, these vessels being derived from the primary lymph ducts. The mode of origin is indicated in embryos younger than 10 cm., in which short, blunt-ended lymph

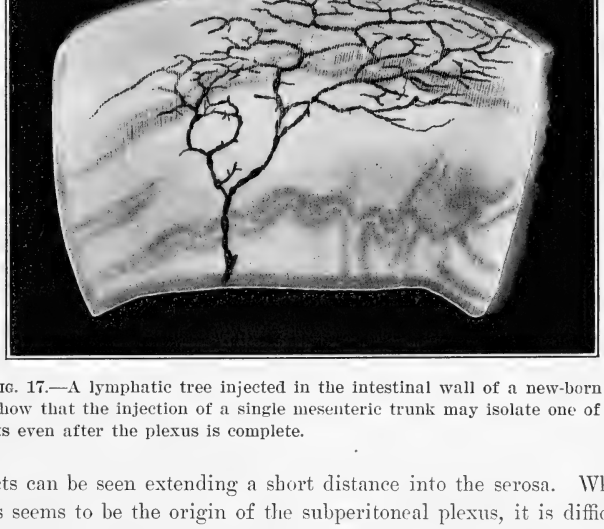

FIG. 17.—A lymphatic tree injected in the intestinal wall of a new-born pig to show that the injection of a single mesenteric trunk may isolate one of the units even after the plexus is complete.

ducts can be seen extending a short distance into the serosa. While this seems to be the origin of the subperitoneal plexus, it is difficult to say that another process does not enter into its formation, that is a growth outward of lymphatics from the primary plexus in the submucosa. In embryos 10.3 cm. long, serial sections of the intestine show a few lymphatic vessels extending from the primary plexus through the circular muscle coat toward the serosa and from the

series we can feel fairly certain that these are not vessels coming into the intestine. In embryos 16 cm. long, such branches are more numerous and can be traced for some distance between the muscle layers before penetrating the longitudinal coat, to enter the serosa. Such vessels extend into the serosa and may help to form the plexus there. They form the connecting vessels between the plexuses in the mucous membrane and the serosa.

From the preceding study it may be seen that the evolution of the lymphatic system of the intestine is from the center to the periphery. The retroperitoneal sac is the origin of the lymph vessels of the intestine; by repeated injections it has been shown that this sac is the best place from which to inject the intestinal vessels, and that corresponding with the growth of the embryo an increasing zone of lymphatics can be injected. From the sac vessels grow out into the mesentery making a plexus which extends to the wall of the gut. The primary mesenteric lymphatics enter the mesentery with the artery. From the mesenteric plexus a series of vessels enters the wall of the intestine with the branches of the mesenteric artery. These lymphatic vessels penetrate to the submucosa and form there a series of loops extending around the wall of the gut. These loops represent lymphatic units, which soon become united into a complete but coarse-meshed plexus. From this submuosal plexus, the mucosal plexus of smaller vessels develops. The mucosal plexus is fine meshed. From the mucosal plexus, the lacteals grow into the villi. The serosal plexus develops late from the lymphatic trunks as they are entering the bowel wall. The retroperitoneal sac, which is the origin of the lymphatic vessels of the intestine, is the anlage of the retroperitoneal, preaortic chain of lymph nodes. The first evidences of the formation of the nodes from the sac occur as early as 3 cm., when the sac begins to be bridged by connective tissue bands. The preaortic nodes are all primary ones for the intestine; the mesenteric nodes develop along the course of the lymphatic ducts and form the secondary group. Thus, the primary nodes, are those that come from the primitive sacs, secondary and tertiary nodes, etc., develop along the course of the vessels.

ON THE PRENATAL GROWTH OF THE HUMAN BODY AND THE RELATIVE GROWTH OF THE VARIOUS ORGANS AND PARTS.

BY

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WITH 4 FIGURES AND 6 TABLES.

Although numerous observations on various phases of the growth of the human embryo and fetus are scattered throughout the anatomical literature, they have never been collected and presented so as to give a comprehensive view of the subject. It is the purpose of this paper to present, in addition to the data already available, the results of an extensive series of original observations. These observations were made primarily in order to fill some of the existing gaps in our knowledge regarding this subject, particularly concerning the rate of growth during the earlier months. It is now possible to describe (though imperfectly and still subject to correction by further data) the general course of prenatal growth in the human body, and in its various organs and parts.

The material used for these observations includes 43 specimens from my collection of human embryos and fetuses. The specimens range all the way from 6 mm. up to the full-term fetus. Upon 32 of these specimens, the observations include the total volume, and the volume of the head, trunk, extremities, and of each of the principal organs of the body. For supplementary data concerning human embryos of the first month, the volumes of seven of the His-Ziegler models were measured.

For obvious reasons, the volume rather than the weight was chosen for measurement in the case of the models. In the small embryos

also the volume may be determined, where it is difficult or impossible to ascertain the weight. Even where the organs are large enough to be dissected out and weighed, one does not like to sacrifice valuable specimens for this purpose, if it can be avoided. On the other hand, it is comparatively easy, though somewhat tedious, to measure the volumes of embryos which have been cut into serial sections. The sections must first be drawn to a definite scale of enlargement. Then one may proceed in either of two ways. In the first embryo measured (11 mm.) a rough model was constructed by Born's wax-plate method, and the volumes of the body and of the various organs and parts were measured by water displacement.[1] An easier method, which is equally accurate, was used with other small embryos. In the enlarged drawings of the sections, the areas of the body and of the various organs were measured by means of a planimeter. The volumes desired were then easily calculated by multiplying the areas (corrected for magnification) by the thickness of the sections. From the third month onward, it was found possible to measure directly the volumes desired by means of water displacement in graduated glass cylinders of various sizes. In some fresh specimens the volume and specific gravity were determined by weighing successively in air and suspended in water.

Certain sources of error must be recognized in the use of these methods. First is the effect of the reagents used. Most of the specimens used had been fixed and preserved in 5 per cent to 10 per cent formalin solution. It is well known that in general formalin causes a certain amount of swelling or expansion of tissues. In one fetus of the 5th month in which this point was carefully observed, the swelling amounted to nearly 13 per cent of the total volume, after three months in a 10 per cent formalin solution. Furthermore, it is stated that the swelling is not equal in all of the various organs, though the amount of difference and the conditions of occurrence are not yet fully known. Alcohol, on the other hand, causes shrinkage.

[1]This embryo was studied by Bonnot and Seevers (6) under my direction. I am also under obligations to J. A. Watkins, M. L. Clint, and R. Lhamon for assistance in making a part of the observations used in this paper.

For the specimens sectioned, it must also be remembered that the process of dehydration and embedding in paraffin causes a shrinkage of at least 20 per cent, or more than enough to counterbalance the swelling due to the formalin fixation. It is improbable, however, that the errors from these sources are large enough to affect materially the main conclusions concerning growth, especially concerning the relative growth of the various parts.

In the following pages there will be considered briefly: first the prenatal growth of the body as a whole, then the relative growth of its principal parts, and finally the relative growth of most of the individual organs. For the organs and parts, it has been found more convenient and useful to record the relative size, expressed in percentage of the entire body. From these data, the absolute size of any part can easily be calculated, if desired (that of the whole body being given).

1. Growth of the Body as a Whole.

In Table I, a list is given of the 43 specimens upon which my own observations were made. In the first column, the catalog numbers (in my collection) are indicated. In estimating the age of the specimens, Mall's rule was used for the first four months and Hasse's rule for the last five months. In the fifth month, a compromise was used between figures derived from Mall's method and those from Hasse's.

In Table II, some observations upon the volumes of the His-Ziegler models are recorded. The embryos corresponding to these models have been figured and described in detail by His (23), who gives no data concerning their weight or volume, however.

While a considerable amount of data has accumulated concerning the growth of the fetus from the 4th to the 10th month, very few observations have been made upon the earlier embryos. In fact up to this time no data have been published which allow any accurate conclusions concerning growth in the human embryo during the first three months. My own observations include, in addition to the seven His-Ziegler models, eighteen embryos within this period. Two of these embryos (6 mm., 7.3 mm.) are of the 1st month, six of the 2d month; and ten of the 3d month. Four of these embryos (6 mm.,

11 mm., 17 mm., and 31 mm.) were measured from sections, the remainder by the direct method described (7.3 mm. by weighing).

The data obtained from these specimens form the basis for the figures given for the first three months in Table III. The volume of the human ovum (the diameter being assumed to be .2 mm., as usually stated) is about .000004 cc., which, assuming the specific gravity to be 1.0, corresponds to a weight of .000004 g. The 7.3 mm. embryo (volume .026 cc.) was probably somewhat shrunken by the alcohol and embedding process so that the volume of .041 cc. obtained from the His model of a 7.5 mm. embryo is perhaps nearer the true size at the end of the first month, corresponding to a weight of about .04 g.

Thus we obtain for the relative monthly growth rate[2] of the 1st month, 9999; for the 2d month, 74; for the 3d month, 11. Fehling (16), whose figures are often quoted, gave no estimate for the 1st month, but (without reliable data) estimated the relative monthly growth rate for the 2d, 3d, and 4th months at 3, 4, and 5 respectively, the greatest relative growth being in the 4th month. My observations, however, prove beyond doubt the conclusion of Mühlmann (36), recently emphasized by Minot (34), that the relative growth in the human embryo is by far the greatest during the 1st month, declining rapidly at first, then more slowly hroughout succeeding months.

Even the large number, 9999, representing an increase of nearly one million per cent, is in reality too small for the relative growth of the human embryo during the 1st month. For as a matter of fact, not the entire ovum, but only a portion of it, actually goes to form the embryo. The remainder is concerned with the formation of the membranes, etc. Since it is not known what proportion of the ovum goes for each of these purposes, the problem may be approached in another way. Table IIIa shows the weight of embryo plus membranes and enclosed fluids, at the end of the 1st, 2d and 3d months,

[2]The relative growth rate is the ratio of the gain during a given period to the weight at the beginning of the period. and is the most accurate index of the rate of growth. It indicates the increase in a unit of weight during the given unit of time. Thus while the total amount of gain in absolute weight increases steadily for each prenatal month, the gain per gram of body weight (as shown by the relative growth rate) is constantly decreasing.

according to observations by Waldeyer (44) and Daffner (10). This gives the enormous figure of 574,999 for the relative growth during the 1st month, corresponding to an increase of over 57 million per cent. This number is undoubtedly too high, however, since the fluids enclosed in the membranes and making up a considerable proportion of the total weight, can hardly be fairly considered as products of embryonic growth, in the ordinary sense of the term. The true relative growth for the 1st month therefore lies somewhere between 1 million and 50 million per cent.

From the foregoing, it appears that the relative growth of the human embryo is enormous in the 1st month, declining thereafter, at first very rapidly, then more and more slowly. The next question

TABLE IIIa.

Growth of the Human Embryo plus Membranes and Enclosed Fluids.

	Weight at Beginning of Month. (a.)	Weight at End of Month. (b.)	Relative Growth for Month. $\left(\frac{b-a}{a}\right)$
1st Month.	(Ovum .000004 g.)	2.3 g. (Waldeyer.)	574999.
2d "	2.3 g.	25 g. (Daffner.)	9.9
3d "	25 g.	100 g.	3.9

which naturally arises concerns the growth *within* the 1st month. Some light is thrown upon this question by the observations on the volumes of the His models, recorded in Table II.

First it may be noted that the yolk sac is relatively large in the early embryos. In the 2 mm. embryo, it makes up more than three-fourths of the total volume. In the 2.6 mm. embryo, the yolk sac remains at about the same absolute size, but owing to the increase in the size of the embryo proper, it here forms less than two-thirds of the total volume. In my 6 mm. specimen (No. 176) the volume of the yolk sac was .0056 cc., forming a little more than one-third of the total volume, the embryo proper measuring .0098 cc. According to Mall (29) the diameter of the yolk sac is approximately 1 mm. at the age of 1 week, increasing 1 mm. each week up to the 6th. It is therefore evident that although the growth of the yolk sac has

been relatively very great during the first two weeks, its later growth is much less rapid.

As to the embryo proper, the actual volume at the end of the 2d week (2.2 mm.) is seen to be .000781 cc. As the volume of the ovum at the beginning is about .000004 cc., this corresponds to an increase of 195 times in volume during the first half of the 1st month. During the second half of the 1st month, the embryo proper increases from .000781 to about .04 cc., or about 50 times. It is therefore evident that the growth of the human embryo is relatively more rapid during the first half of the first month than during the second half. The difference would appear still greater, if the growth of the yolk sac, membranes, etc., was taken into account.[3]

From what we know of the development in lower animals, as Donaldson (11) has pointed out, there is probably no increase in volume during the early segmentation stages of the ovum; so that the increase must be all the more rapid when it actually begins.

In addition to the observations in Tables I and II, a considerable amount of data concerning the prenatal growth of the whole body from the 4th to the 10th months has already been published. Ahlfeld (1), Fehling (16), Legou (25), Faucon (15), Michaelis (35) and others have recorded the weight of fetuses whose age was estimated from menstrual histories. Curves of absolute growth for the prenatal period, based upon these data, are shown in Fig. 1, (curves 1, 2, 4, 5). No curves are shown for the data of Faucon and others which do not differ materially from those given. From all the data available, I have ventured to construct a normal curve (Fig. 1, curve 3), which is intended to represent the absolute prenatal growth, according to our present knowledge. As will be seen, it does not differ greatly from that based upon the data of Fehling (who utilizes also observations from Hecker and Schroeder). Ahlfeld's figures seem entirely too high for the average weight at the

[3]Daffner (10) gives the weight of a fresh "ovum" of fourteen days as 0.82 g., which is more than 200,000 times the weight of the ovum at the beginning. During the second half of the third month, however (accepting Waldeyer's observations of the 2.3 g. for the weight at the end of the first month), the embryo plus membranes, etc., increases only about three times in weight.

corresponding ages, in spite of his statement that "Nur solche Kinder verwendet werden deren Mutter den Tag der Conception genau anzugeben wussten." Hennig (19) has published a curve of growth in fetal weight, but without the data upon which it is based. His curve shows a marked increase in the growth rate in the 6th and 8th months, followed by retardations in the 7th and 9th months. Donaldson (12) believes that a new phase of growth in the human fetus begins with the 6th month, where the curve of absolute growth begins to rise more rapidly. A study of the growth *rate,* however, as expressed by the figures for the relative monthly growth rate in Table III (or corresponding figures in Fehling's table) reveals no evidence of any marked change at this particular time.

All of the data being considered, it seems most probable that the normal curve of fetal growth is fairly regular, though the uncertainty regarding the age of specimens and the degree of individual variation makes it very difficult to determine this curve accurately. The curve as drawn (curve 3, Fig. 1) is fairly regular, corresponding roughly to the formula $y = x^4$, or

$$\text{Weight (g)} = \left(\frac{\text{Age (days)}}{37}\right)^4$$

From this formula, the weight may be calculated approximately from the age, or *vice versa,* for any time beyond the first month. By some such growth formula the age should be determined more accurately than by the length (which theoretically should vary as the cube root of the volume, or weight).[4] The majority of previous investigators have concluded that for determining the age, the length is a more reliable criterion than the weight; probably because the skeleton, which determines the length, is thought to be less variable than the soft parts, which make up most of the weight. This is still an open question, however.

[4]Roberts (40) has worked out a rule, assuming that the weight increases as the cube of the age; but this results in figures somewhat too high for the average weight at the various months. This is also the case with the formula: Weight (g) = 50 (months — 2)^2 recently proposed by Tuttle (42).

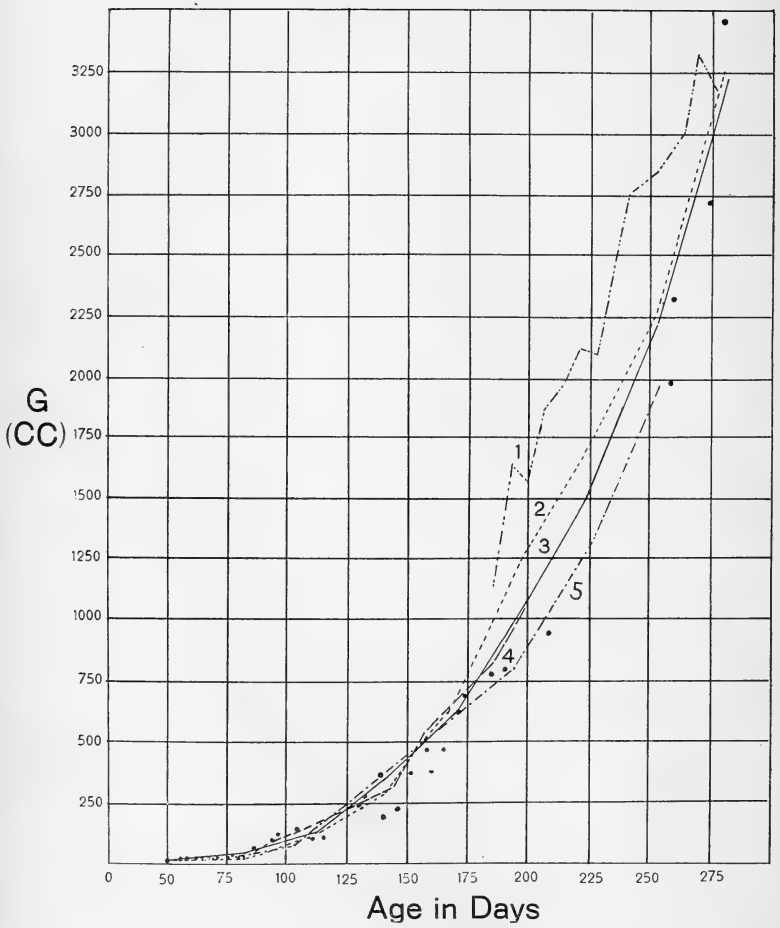

FIG. 1. Curves of absolute prenatal growth. Curve No. 1, data from Ahlfeld; 2, from Fehling; 4, from Legou; 5, from Michælis. Curve No. 3 represents the normal curve of growth (weight) constructed by the author, based on Table III, from all data available. The dots represent the total body volume in cubic centimeters of the specimens studied, the age being estimated from their lengths.

Although the growth rate at the end of the fetal period is far less than at the beginning, it is still very rapid as compared with the growth rate after birth. If the relative monthly growth rate of the last fetal month (.45) were maintained during the first year after birth, the weight of the body at the end of the first year would be over 250 kilograms! This marked diminution in the growth rate after birth indicates that the prenatal conditions are far more favorable to growth than the postnatal.

As may be seen by the dots in Fig. 1, the volume in cc. of the fetal specimens studied, the age being estimated from their length, corresponds roughly to the curves of growth in weight, where the age was determined from menstrual history. We should expect the curve of growth in volume to differ slightly from that of weight, on account of slight changes in the specific gravity of the fetus. In the earlier months, the specific gravity of the embryo is very little over 1, though in later fetuses it reaches 1.04 or 1.05.

2. Relative Growth of the Principal Parts of the Body.

Since growth is not uniform in the various parts of the body, these must be separately considered. No data have been published showing the relative size of the head, trunk and extremities in the various prenatal months, although it is well known that the head is at first relatively large and the extremities small. My own observations on the relative growth of the various parts in 32 specimens are included in Table IV. The relative size of the head was also observed in 5 of the His models (Table II).

The growth of the various parts in the specimens observed is illustrated graphically by the curves of relative growth shown in Fig. 2.[5]

The unbroken lines connect points corresponding to the observations on the 32 specimens in Table IV. The dotted line at the beginning of the "Head" curve indicates approximately the relations found in the His models representing embryos in the latter part of the 1st

[5]It must be borne in mind that the curves of relative growth merely indicate whether the part is growing more or less rapidly than the average rate of the body as a whole, whose absolute growth curve is shown in Fig. 1.

month. The dotted lines on the right indicate, for convenience of comparison, the relative growth of the corresponding parts between birth and adult life, based chiefly upon observations by Meeh (31).

The method followed in dividing the body was to separate the head from the trunk by a plane passing just below the mandible anteriorly, and just below the cranium posteriorly. The neck is therefore included in the trunk. The upper extremities were separated from the trunk by an approximately sagittal plane through the shoulder joint, and the lower extremities by an oblique plane through the hip joint, parallel to Poupart's ligament. As it is impossible to pass these planes always in exactly the same way, the measurements on different specimens are not exactly comparable to each other, though the error is comparatively small. There is also a certain loss of blood, etc., especially in the case of the fresh specimens.

Head.

As may be seen in Table II, the head in the His models of embryos in the latter half of the 1st month forms from 34 per cent to 39 per cent of the entire body volume. Table IV and the "Head" curve in Fig. 2 show that the head reaches its maximum relative size, about 45 per cent of the total body volume, during the 2d month. Thereafter it declines gradually in relative size, forming only about 26 per cent or 27 per cent of the total body at birth.

His (23) from a study of the profile areas in embryos of the 1st and 2d months, concluded that the head is at first relatively small, increasing from about 30 per cent in the latter part of the 1st month to 56 per cent of the total body at the end of the 2d month. He thought that the head and trunk during this period are in a sort of race for supremacy, first one being larger, then the other. Profile areas, however, lacking the third dimension, are not necessarily in the same proportion as the volumes. His calculates the profile areas of the head in his embryos 4 mm., 5 mm. and 7.5 mm. to be 32.3 per cent, 30.7 per cent and 30.6 per cent of the total body; but I find in his models of these same embryos the volume of the head to be respectively 34.9 per cent, 38.7 per cent and 36.6 per cent of the

total volume. In no specimen examined by me does the head exceed the trunk in size, though it sometimes approaches it closely.

The steady decrease in the relative size of the head from the 2d month onward is in part due to a corresponding decrease in the relative size of the brain (which will be described later). As the brain throughout bears a fairly constant ratio to the volume of the head (somewhat less than half), however, it would appear that the facial portion of the head must also decrease in relative size at about the same rate as the brain. This does not agree with the conclusion of Merkel (32), who found the facial portion of the head of about the same relative size in a series of fetuses of different ages, excepting the youngest (3d month), where the face was relatively larger.

In the adult, according to Meeh's (31) observations, the head forms from 6 per cent to 11 per cent of the total body volume; or, according to Harless (18), 6 per cent to 9 per cent of the total body weight.

Trunk.

In Fig. 2, the curve of the relative growth of the trunk is not represented in the 1st month. Since, however, the extremities are very small at this time, it is evident that the trunk must be relatively very large. When the head forms 35 per cent of the body, the trunk would necessarily form nearly 65 per cent. At the beginning of the 2d month, as is shown by the curve, the trunk has decreased in relative size, so that it forms about 50 per cent of the total body volume. From the 2d month onward, the trunk continues to decrease (somewhat irregularly) in relative size. During the first half of the fetal period, the curve of relative growth of the trunk descends nearly parallel with that of the head. The curves diverge in the second half of the fetal period, however, that of the trunk remaining on the whole nearly horizontal, fluctuating between 40 per cent and 45 per cent.

Meeh (31) separated the trunk from the lower extremities by a horizontal section at the level of the perineum, which apparently adds about 6 per cent to the relative size of the trunk. The average in 8 adults measured by him is about 54 per cent, which would cor-

respond to approximately 48 per cent of the total volume by my method. Between birth and adult life the trunk therefore apparently increases slightly in relative size.

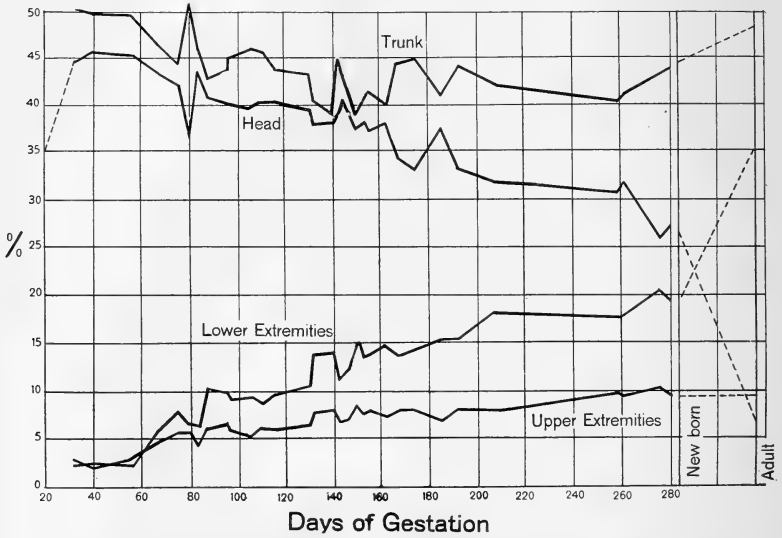

Fig. 2. Curves showing relative prenatal growth of the various parts of the body, in specimens studied, the size being expressed in percentage of the total body volume (Table IV). The dotted lines at the right represent the postnatal growth of the corresponding parts (based on data from Meeh).

The Extremities.

The extremities are seen to be at first relatively very small, each forming between 2 per cent and 3 per cent of the total body volume during the 2d month. The upper extremities are usually slightly larger than the lower until early in the 3d month. Then both begin to increase in relative size, the lower more rapidly. The increase in the relative size of the extremities during the first half of the fetal period counterbalances the relative decrease of the head and trunk during that period, as shown by the curves in Fig. 2. In the second

half of the fetal period, the extremities continue to increase in relative size, but more slowly; the increase of both counterbalancing the continued decrease in the relative size of the head, the trunk remaining nearly unchanged. At birth, the upper extremities form about 10 per cent of the whole body, the lower about 20 per cent. In the adult (judging from the data of Meeh and Harless), the upper extremities have increased but slightly, if at all; while the lower extremities have increased to about 35 per cent, or nearly twice the relative size at birth.

In general, it may be said that the period of maximum relative growth passes somewhat wave-like over the body from the head toward the foot. The head, as we have seen, reaches its maximum relative size in the 2d month. In the trunk, the upper portion, including the thorax and the upper abdominal viscera, is relatively largest during the earlier half of the fetal life. The lower part of the abdomen becomes more prominent toward the end of the fetal period, due chiefly to the rapid expansion of the intestines at this time. The pelvis and lower extremities do not reach their greatest relative size until early adult life, although the upper extremities have reached their maximum relative size at birth.

It may also be noted that the organs lying dorsal to the body axis (brain and spinal cord) grow at first far more rapidly than the organs ventral to the body axis. The volume of the brain and spinal cord together at the beginning of the 2d month is nearly 3 times as great as the combined volume of the organs lying ventral to the body axis. At birth, they are about equal. In the adult, the ventral organs are 6 times as large as the brain and spinal cord. The significance of the rapid growth of the brain and spinal cord in determining the marked flexure of the body in the early embryo has already been pointed out by Merkel (32) and by Keibel (Normentafel zur Entwicklungsgeschichte des Schweines, 1897).

3. Growth of the Individual Organs.

As the growth rate of the whole body is the resultant of the growth rate of the various parts, so the growth of the various parts depends

in turn upon the growth of their component organs. The relative size of the principal organs in the specimens examined is given in Table IV. In Table V the average relative size of the principal organs is given for the various lunar months. In this table, all the available data published in the literature have been utilized, measurements on about 800 embryonic, fetal and newborn specimens being used.

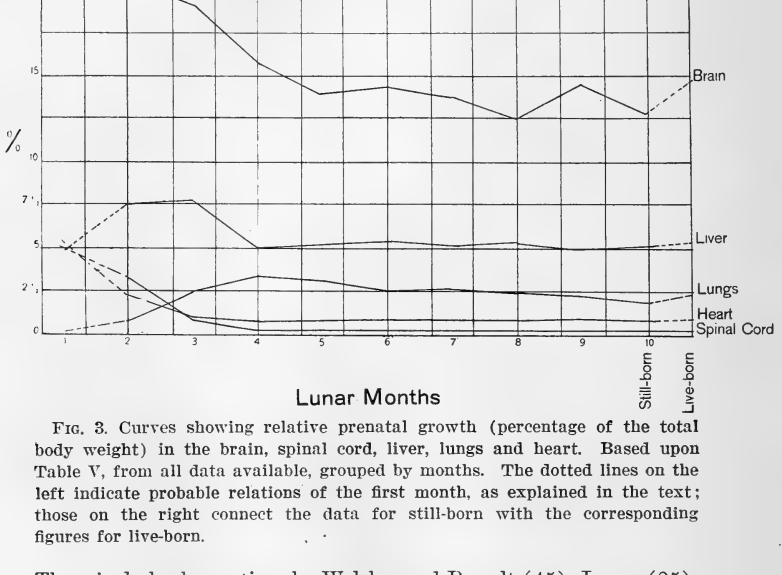

Fig. 3. Curves showing relative prenatal growth (percentage of the total body weight) in the brain, spinal cord, liver, lungs and heart. Based upon Table V, from all data available, grouped by months. The dotted lines on the left indicate probable relations of the first month, as explained in the text; those on the right connect the data for still-born with the corresponding figures for live-born.

These include observations by Welcker and Brandt (45), Legou (25), Faucon (15), Arnovljevic (3), Brandt (8), Anderson (2), Boyd (7), Lomer (28), Meeh (31), Liman (26), Thoma (41), Oppenheimer (38), Mühlmann (36), Collin and Lucien (9), and Beneke (4). A few cases, clearly either abnormal or erroneous, were excluded. In calculating the averages in Table V, the percentage of the total body (weight) was reckoned separately for each specimen, then

the average percentage taken for all the cases in each lunar month. This is more accurate but more tedious than to divide the sum of the weights of an individual organ by the sum of the corresponding body weights. The latter method was used only in the case of the data by Boyd and Oppenheimer and the lung observations by Schmitt, Devergie and Elsässer (cited by Liman). In these cases, the individual data are not available, but the number of observations is so large that the probability of error is reduced. The figures in parenthesis fol-

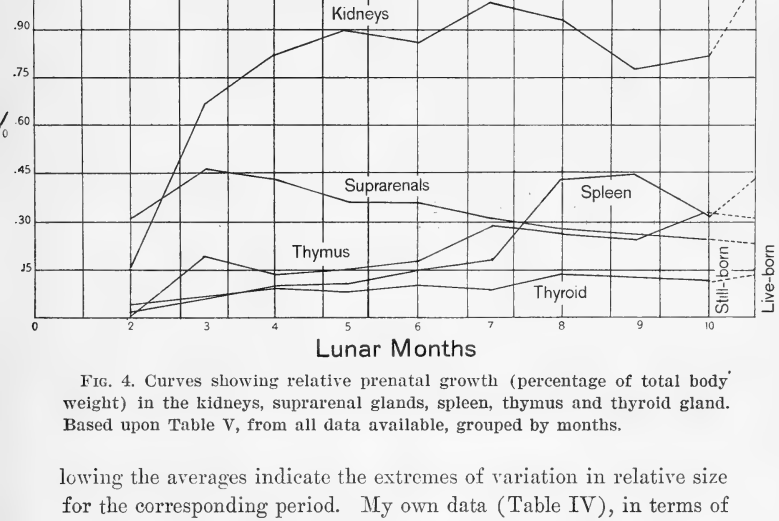

Fig. 4. Curves showing relative prenatal growth (percentage of total body weight) in the kidneys, suprarenal glands, spleen, thymus and thyroid gland. Based upon Table V, from all data available, grouped by months.

lowing the averages indicate the extremes of variation in relative size for the corresponding period. My own data (Table IV), in terms of volume (cc.) have been added unchanged to the others in terms of weight (g.), although strictly considered they are subject to a slight correction on this account.

In Figs. 3 and 4, curves are shown illustrating graphically the relative prenatal growth rate for some of the principal organs. Table VI shows the relative size of the various organs by lunar months, the

sexes being separated, and also the right and left in the case of the paired organs. Data from the literature were utilized here as in Table V.

The Brain.

Although subject to considerable individual variation (cf. Tables IV and V) the relative size of the brain, when the average for lunar months is taken (Table V), gives a fairly regular curve of growth, as shown in Fig. 3. No data for the 1st month are available, but it is very probable that, as in the case of the whole head, the maximum relative size is not reached until the 2d month. Then it is seen to form slightly more than 20 per cent of the entire body. From this time onward, it decreases in relative size. The increase at the 9th month is probably accidental, due to the small number of observations. The decrease is most rapid in the first half of the fetal period, the relative size remaining fairly constant in the latter half, as noted by Legou, (25). The brain reaches an average of about 12.8 per cent in the still-born fetus (120 cases). In those born living, the average appears to be somewhat higher, being about 14.6 per cent (90 cases). The reason for this increase in relative size in the live-born, which is found in all of the organs (excepting pancreas and suprarenal glands), is not clear. In the case of the lungs, it is evidently due chiefly to a larger influx of blood, and this may perhaps in part account for the difference observed in the other organs.

After birth, as is well known, the decrease in the relative size of the brain continues, reaching about 2 per cent in the adult. Vierordt (43) gives an estimate of 12.29 per cent of the total body weight for the brain of the new-born, and 2.16 per cent for the adult. It may be noted, however, that Vierordt's estimates (which are widely used) are not calculated from individual data, and are therefore not free from the possibility of error.

Spinal Cord.

The spinal cord attains its maximum relative size earlier than the brain. In an embryo of the fifth week (11 mm.), it forms 4.85 per cent of the entire body, and in models of earlier stages appears even

larger. As seen in Table IV and V, and also by the curve in Fig. 3, the spinal cord declines rapidly in relative size during the 2d and 3d months, then more slowly throughout the remainder of the fetal period. At birth, it forms only about .15 per cent of the entire body (average of five cases, including two of Bischoff). It is thus evident that the prenatal decline in relative size is more marked in the spinal cord than in the brain. In other words, prenatal growth is relatively more rapid in the brain than in the spinal cord, especially in the earlier part of the fetal period. After birth, the relations are changed; so that the postnatal growth of the spinal cord is relatively more rapid than that of the brain, as pointed out by Donaldson (11) and Bonnot and Seevers (6). Vierordt gives .18 per cent of the total body weight for the spinal cord in the newborn, and .06 per cent for the adult.

Eyeballs.

As is well known, the eyeballs are relatively large at birth, forming, according to Vierordt, .24 per cent of the entire body weight, as compared to .02 per cent in the adult. Welcker and Brandt (45) cite 2 newborn in which the eyeballs formed .20 per cent and .38 per cent, respectively, of the entire body weight; in one fetus (6th month) they formed .71 per cent and in another (3d month), .53 per cent. I have made no systematic observations on the eyeballs, but in 3 fetuses of about the 6th month (Nos. 210, 211 and 218), the eyeballs were weighed and formed .45 per cent, .40 per cent and .39 per cent, respectively, of the entire body weight. It is thus evident that they are relatively larger in the fetus than at the birth; and they are probably still larger in the embryo.

Thyroid Gland.

Although subject to considerable individual variation (cf. Tables IV and V) the thyroid gland in general increases slowly but steadily in relative size during the prenatal period, as shown by the curve in Fig. 4 (data from Table V). In an embryo of 2 months (3.1 cm.), it formed .035 per cent of the total body, increasing to .111 per cent

(average of 26 full-term still-born) or .125 per cent (average of 11 born alive). Vierordt gives .16 per cent of the total body weight for the thyroid gland in the new-born, decreasing to .05 per cent in the adult.

Thymus.

In the youngest specimen measured (end of 2d month), the thymus formed only .008 per cent of the entire body. As may be seen in Tables IV and V, it is subject to extreme fluctuations in relative size, being in this respect one of the most variable of all the organs. When the average of a considerable number of specimens is taken, however, as shown by the curve in Fig. 4 (from data in Table V), the increase in the relative size of the thymus is evident. The average of all observations available gives .326 per cent of the total body for the thymus in 124 full-term still-born, and .313 per cent for 101 born alive. According to Vierordt, the thymus decreases from .26 per cent of total body weight in the newborn to .04 per cent in the adult.

Heart.

In the early embryo, the heart is relatively large. In the youngest specimen directly measured (5th week, 11 mm.), the heart formed 3.64 per cent of the total body volume. On the His-Ziegler model of a 4 week embryo (A, 7.5 mm.), I have taken measurements from which the heart is estimated to form more than 5 per cent of the total body volume (as indicated by the dotted line at the beginning of the curve of relative growth for the heart in Fig. 3). From this curve, and from the data in Tables IV and V, it is evident that the heart decreases rapidly in relative size, dropping to .85 per cent in the 3d month. It continues fairly uniform in relative size from the 4th month onward, usually averaging between .7 per cent and .8 per cent for each month. In 165 full-term still-born the average was .70 per cent; and in 164 born alive it was .77 per cent. According to the estimate of Vierordt, the heart forms .76 per cent of the total weight in the newborn, and .46 per cent in the adult.

Lungs.

The lungs (Fig. 3, Tables IV and V) are relatively small at first. They increase steadily in relative size, reaching at the maximum an average of 3.29 per cent of the total body weight during the 4th month. From this time on, they decline slowly (with considerable variations) in relative size throughout the fetal period.[6]

In the full-term still-born (289 cases) the lungs averaged 1.71 per cent of the total body weight. In the live-born (202 cases) the average was 2.18 per cent, the difference doubtless being due chiefly to the increased blood supply to the lungs when respiration begins.

When respiration begins, the lungs expand to two or three times their original volume. It is doubtful, however, whether there is any postnatal increase in the relative weight of the lungs, excepting the immediate increase when respiration begins. Vierordt gives 1.75 per cent of the total body weight for the lungs in the newborn (still-born?) and 1.50 per cent for the adult. The adult lungs vary exceedingly in air and blood content, however, so that it is very difficult to determine their normal relative size (volume and weight).

As to the comparison between right and left lungs, His (24) found the anlage of the right lung larger than that of the left from the very beginning, and attributed it to the asymmetry of the heart and mesentery. In the youngest specimen observed by me (11 mm.), there was no appreciable difference in size between the two lungs (Table IV). Thereafter, however, the right lung appeared constantly larger than the left, averaging about 20 per cent larger throughout the fetal period. In the newborn, the difference appeared somewhat larger, being 25 per cent to 30 per cent. The ratio between the right and the left lung is subject to considerable individual variation. It is, however, apparently not correlated with any corresponding variation in the size of the heart or thymus, but varies independently of these.

In the adult, according to most of the text-books of anatomy, the right lung averages only 10 per cent larger than the left. Data com-

[6]Legou erroneously concluded that the lungs remain of about the same relative size throughout prenatal life. The remarkably large relative size of the lungs during the middle period of fetal life seems to have escaped all previous observers.

piled by Vierordt, however, show that this figure is open to question and that the ratio is quite variable. If the difference is due to the asymmetry of the heart, we should naturally expect it to be less in the adult, where the heart is relatively smaller.

Liver.

In the youngest specimen in which the liver was measured (11 mm., Table IV), it formed 4.85 per cent of the total body volume. At the beginning in the 1st month it is of course relatively smaller. As indicated by the curve in Fig. 3 (Table V), it increases to its maximum relative size during the 2d and 3d months. At this time individual specimens may reach 10 per cent (cf. Table IV and V), the average being about 7.5 per cent of the total body. During the 4th month, however, the liver drops in relative size to an average of a little more than 5 per cent. This average is maintained throughout the succeeding fetal months, although there is considerable individual variation. In 145 full-term still-born cases, the average was 5.05 per cent; while in 101 live-born it was 5.23 per cent. Vierordt estimates that the liver forms 4.57 per cent of the total body weight in the new-born and 2.75 per cent in the adult.

Pancreas.

The pancreas is at first relatively small (cf. Tables IV and V), forming in a specimen of the 6th week (1.7 cm.) .032 per cent of the entire body volume.[7] From the 4th month onward, the pancreas remains fairly constant in relative size, averaging about .1 per cent of the entire body. In 37 full-term still-born, the average was .105 per cent. In 90 born alive the average was .145 per cent of the total body weight. Vierordt gives .11 per cent of the total body weight for the pancreas in the newborn, and .15 per cent for the adult.

Spleen.

As indicated by the curve of growth in Fig. 4 (also Tables 4 and

[7]The case recorded by Welcker (45), in which the pancreas formed .45 per cent of the total body weight, is either erroneous or an abnormality.

5), the spleen is at first relatively small, but increases slowly to an average of .176 per cent of the whole body in the 7th month. About this time it appears to increase rapidly in relative size, averaging over .4 per cent in the 8th and 9th months. In the full-term still-born (143 cases) the spleen averaged .32 per cent of the total body weight, and in the live-born (101 cases) .43 per cent. Vierordt gives .34 per cent of the total body weight for the spleen in the new-born, and .25 per cent for the adult. The fetal spleen, as in postnatal life, is subject to extreme individual variations in relative size.

Stomach and Intestines.

Only a few observations upon the prenatal growth of the alimentary canal are available, chiefly (besides my own) those of Arnovljevic (3) and Brandt (8). The data upon stomach and intestines (including mesentery) are included in Tables IV and V. The data for stomach and intestines in Tables IV and V include contents. No data are available for the empty stomach before the 4th month, but since the contents are usually slight at this time, the figures given for the stomach plus the contents are probably only a little larger than they would be for stomach alone. It seems that the empty stomach is relatively somewhat larger at an early period than later. It varies irregularly in relative size, the average per cent of the entire body in the different months varying from .16 per cent to .39 per cent. A larger series would doubtless give more uniform figures. The figures for stomach with contents are at first but little larger than those for the empty stomach; but in the later fetal months the contents (chiefly mucous) become relatively much larger in amount. In the full-term fetus the empty stomach averaged .20 per cent of the entire body weight (7 cases), while the stomach plus contents formed .49 per cent (8 cases).

The intestine is relatively small in the early embryo, not being at the 5th week very much larger than the stomach. It grows very rapidly, however, so that in the full-term fetus the weight of the intestines (either filled or empty) averages more than six times that of the stomach. As in the case of the stomach, the contents of the in-

testine are at first small in amount, but increase gradually, amounting on the average in the full-term fetus to about twice the weight (or volume) of the empty intestines (plus mesentery). Vierordt estimates that the (empty) stomach and intestine together form 2.1 per cent of the entire body weight in the newborn, which is considerably higher than the figures just given (1.23 plus .20 equals 1.43 per cent). For the adult he gives 2.06 per cent.

For the entire alimentary canal (empty), from the end of the pharynx to the anus, a few data are available. Welcker and Brandt (45) cite 4 observations; fetus 3 mo., 2.49 per cent of the total body weight; 6 mo., 3.01 per cent; newborn, 3.15 per cent, and 2.45 per cent. Mühlmann obtained a much higher figure for the empty canal in 2 newborn,—6.7 per cent and 7 per cent respectively. In a series extending through childhood up to the adult, he found the relative weight of the alimentary canal gradually diminishing to an average of about 3 per cent of the total body weight in the adult.

On account of swallowed air and accumulated gas, in addition to the fecal contents, the volume occupied by the intestines in postnatal life is relatively much greater than in the full-term fetus,—on the average probably twice as great.

Kidneys and Wolffian Bodies.

Beginning with the 2d month, the kidneys increase in relative size, at the first rapidly, then more slowly, together forming an average of about 1 per cent of the total body in the 7th month (Fig. 4, Tables IV and V). They apparently decrease slightly in relative size during the 8th and 9th months, and in the full-term still-born (144 cases) the average was only .82 per cent of the total body. In the live-born, however, the average (101 cases) was 1.05 per cent. Vierordt estimates for the kidneys in the newborn .75 per cent; and for the adult, .46 per cent.

When the right and left kidneys are compared in size (Tables IV and VI, it is evident that in the majority of cases the left kidney is slightly larger than the right. As shown in Table VI, this is true on the average in both sexes for every month from the 2d onward, except the 2d (2 cases only), 7th (female cases), and 10th (stillborn).

In the adult, it is well known that the left kidney is larger than the right in the great majority of cases (cf. data by Thoma). Beneke (4) from observations on only a few cases concluded that at birth the kidneys are approximately equal in size, no difference being appreciable until after the 3d month (postnatal). The data collected by me, however, demonstrate that the predominance of the left kidney extends back to the early embryonic months. As to the cause of the smaller size of the right kidney, it is probable that its growth is retarded by the greater pressure of the liver on the right side. This conclusion is strengthened by the fact that (as will be shown later) the right suprarenal gland is also usually smaller than the left.

The Wolffian bodies are relatively large in the early embryo, forming .60 per cent of the total body volume in an embryo of the 5th week (11 mm.), in which the renal anlages are just appearing. As the kidneys enlarge, the Wolffian bodies become not only relatively but absolutely smaller, as shown by measurements on the following three specimens:

EMBRYO NO.	ACTUAL VOLUME OF WOLFFIAN BODIES.	% OF TOTAL BODY.
60 (1.1 cm.)	. 000734 cc.	. 601
58 (1.7 cm.)	. 00055	. 124
57 (3.1 cm.)	. 00045	. 0212

Suprarenal Glands.

As is evident in Fig. 4, the curve of growth of the suprarenal glands is quite different from that of the kidneys. During the 2d month, when they first become definitely outlined, the suprarenal glands form about .3 per cent of the total body volume (Tables IV and V). They increase rapidly to a maximum of about .46 per cent in the 3d month, decreasing steadily thereafter in relative size. In the full-term still-born, they form an average of .246 per cent of the total body weight (108 cases), and in the live-born .229 per cent (101 cases). Vierordt gives .23 per cent for the suprarenals in the newborn, and .01 per cent in the adult.

As in the case of the kidneys, the left suprarenal gland is usually larger than the right. As may be seen in Table VI, where right and left are given separately, the left averaged larger for both sexes in every month except the 2d, 4th (female), and 6th (female), in which the right averaged larger, and in the 3d month, in which they were equal.

For a comparison of the right and left suprarenal glands in the adult, I have been able to find no data; but suspect that the left will be found the larger here also.

Reproductive Organs.

Only a few observations upon the prenatal sex glands are available. In addition to the data in Table IV, I find the testis in embryo No. 147 (2.3 cm.) to form .160 per cent of the total body volume; and in No. 122 (3.9 cm.) .057 per cent. Welcker and Brandt (45) cite a case (3d mo.) in which the testis formed .10 per cent of the total body weight; and another (6th mo.) in which it formed .06 per cent.

In an embryo of the 5th week (1.1 cm.), before the sex could be determined with certainty, the anlage of the sexual gland formed .085 per cent of the total body volume. Beginning with the 2d month and extending up to the 10th mo., the relative size of the testis, in per cent of the entire body, forms the following series:— .16, .056, .10, .040, .045, .060, .080. In the female, from the 2d to the 7th mo., the series for the relative size of the ovary (per cent of entire body) is as follows:—.112, .036, .035, .026, .022.

In both sexes it therefore appears that the sexual gland is relatively larger in the embryo than in the later fetal stages, and that the testis is much larger than the ovary at corresponding stages. According to Vierordt, in the newborn the testis and ovary are about equal in size, forming .026 per cent of the entire body weight. For the adult he gives .080 per cent for the testis and .012 per cent for the ovary.

Skeleton, Musculature and Skin.

While the skeleton, musculature and skin were not observed in the present investigation (except in one specimen), it is perhaps worth

while to mention briefly the data available, which are presented in the following table:—

	Embryo 3d mo. (Welcker)	Fetus No. 210 6th mo.	Fetus 6th mo. (Bischoff)	Newborn (Bischoff)	Newborn (Bischoff)	Average Newborn (Vierordt)	Average Adult (Vierordt)
Body Weight..	12.51 g.	400.8 g.	491 g.	2360.5 g.	2915. g.	3100. g.	66200 g.
Skeleton and ligaments....	46.2 %	19.0 %	20.57 %	18.03 %	16.0 %	13.7 ? %	17.48 %
Musculature...		22.75 %	22.71 %	23.30 %	24.03 %	25.05 %	43.4 %
Skin.....	8.39%	12.9 %	14.97 %	20.33 %	15.34 %	19.73 %	17.77 %
Subcutaneous fat					13.91 %		

It therefore appears that in the fetus the skeleton forms a somewhat larger percentage of the total body weight than later. The musculature, on the other hand, increases in relative amount with age. The skin also increases, on account of the accumulation of subcutaneous fat. In the adult, the skin, exclusive of subcutaneous tissue, forms only about 6 per cent of the entire body weight (average of 6 normal adults, by Welcker and Brandt).

Variations According to Sex.

In Table VI, the relative size of several organs in the various months, grouped according to sex, is shown. The columns "No." give the number of cases (including my own and those already recorded in the literature), while "per cent" indicates the percentage of the entire body (chiefly weight, except in my own cases). The average weight of the entire body in each group is given at the foot of each "per cent" column.

It will be noted that the average weight of the entire body is larger for the male in each month, excepting the 5th and 9th months. In the 9th month, the number of cases (5) is too small to be significant, and the figures for the 5th month may be accidental. The conclusion is therefore evident that the male fetus is heavier than the female fetus of corresponding age throughout the fetal period. We know, of course, that this is true in the newborn. The conclusion that it is true for the entire fetal period is strengthened when it is remem-

bered that the age of the fetuses observed was determined in some cases by their length, the same rule being used for both sexes. Since the body length of the male at term averages greater than that of the female, the same thing is probably true for the fetus, at least in the later months. If allowance were made for this in grouping the fetuses by months, the difference in body weight between male and female would be even more pronounced.

In most of the viscera observed, on the other hand, the organs are, as a rule, relatively heavier in the female.[8] This is the case with the brain, heart, liver, spleen and suprarenal glands; while the thymus, lungs and kidneys are usually relatively heavier in the male. For the other organs, insufficient data are available.

The brain averages larger in the female in every month, excepting the 4th, 5th and 8th. No data are available for the female in the 9th month, however; and none for the male in the 2d. So that, after all, the preponderance of relative weight in favor of the female is but slight, and perhaps without significance.

In the thymus, the relative size averages greater in the male for every month recorded, except the 10th. Here the averages are equal for those born alive, but slightly larger for the female in the still-born.

The heart averages relatively larger in the male for the 4th, 8th and 9th months; equal in male and female for the 7th; and is larger in the female for the remaining months. Here also the significance of the difference is questionable, though well marked in the newborn.

The lungs, both right and left, average relatively larger in the male, excepting in the 3d month (2 cases only), 9th month (no data for female), and 10th month. In the 10th month, the averages are nearly equal in the still-born, but are decidedly larger for the female in the live-born.

The liver averages relatively larger in the female for every month from the 3d onward. The only exception is the 9th month, in which no data are available for the female.

[8]This general conclusion was reached by Loisel (27) from a study of Legou's cases. The additional data now available do not confirm most of his conclusions, however.

The spleen averages relatively larger in the female for each month from the 4th onward, excepting the 4th and 9th, in which the male is larger and in the 9th, where data for the female are wanting.

The kidneys are almost constantly relatively heavier in the male. In the 4th month, however, the kidneys average larger in the female, and also in the full-term still-born (only 1 female). No data are available for the female in the 9th month.

The suprarenal glands, unlike the kidneys, are usually relatively larger in the female. The only exception is the 6th month, in which the left suprarenal only averaged larger in the male. No data are available for the 9th month, or for the female in the full-term still-born.

Comparison with other Species.

A comparison of the growth in the human body with that in the lower animals should be of value in enabling us to judge as to which phenomena are common to various animals (and therefore probably more fundamental in significance) and which are peculiar to the human species.

It is possible at present to make such a comparison only to a very limited extent, owing to the lack of data concerning growth in the lower animals, particularly prenatal growth.

We may consider first the rate of prenatal growth in the body as a whole. It is a matter of common observation that in forms with eggs of the holoblastic type of cleavage there is during the early stages of segmentation a period during which the cells divide actively, with little or no increase in volume. In fishes and amphibia, this initial period is longer than in the higher vertebrates. In the frog embryo, Davenport (10 a) has shown that after hatching the growth rate increases up to the 10th day (with coincident increase in the percentage of water), after which it decreases rapidly. Even during the initial period, while the segmenting ovum as a whole remains nearly stationary in size, the actual amount of protoplasm is increasing rapidly at the expense of the yolk material.

His (21) in 1868 concluded that the relative growth in the chick is greatest at the beginning, a conclusion supported to a certain extent

by the observations of Falck (14) on the weight of the chick embryo at various stages. The data by Welcker (45) also indicate that in the chick the relative growth rate diminishes steadily from the 9th day of incubation to the time of hatching. As Minot (34) has pointed out, an inspection of Keibel's Normentafeln also demonstrates the more rapid relative growth in the earlier embryonic stages. Fischel (17) states that this is likewise true for duck embryos. We may therefore conclude that in the bird embryo the relative growth rate is most rapid at the beginning, and decreases with age. Preyer (39) gives a curve of growth showing the increase in the weight of chick embryos, based on 42 observations by Potts. Growth is far more rapid than in the human embryo of the same age, since the chick reaches 30 grams in weight within 21 days. But on account of the great individual variations a much larger series of observations is necessary before it is possible to construct an accurate curve of growth for comparison with that of the human embryo.

For mammalian embryos also, the data available are not very extensive. Fehling (16) has shown that in rabbit embryos from the 15th day onward the relative growth rate decreases, at first rapidly, then more slowly. Minot (34) has confirmed this result. For earlier embryos apparently no data are available. As in the case of the chick, however, it is easy to show that the rate of growth is in general far more rapid in the rabbit embryo than in the human embryo of the same age. Assuming the diameter of the mature rabbit ovum to be .116 mm. (Marshall), its volume would be about .0000008 cc., and its corresponding weight about .0000008 g., or about one-fifth that of the human ovum. At the end of about 30 days, the rabbit embryo has reached full-term, with an average weight of 38.35 g. (Fehling), which is nearly 50 million times the weight of the ovum! In the same length of time, as we have seen, the human embryo has increased in size only about 10 thousand times. The human organism reaches finally a larger size than the rabbit or chick, in spite of the lower growth rate, because growth continues for a much longer period of time (Minot).[9]

[9]Curves of growth by Donaldson (12) show apparently a slower prenatal growth in the white rat than in man. But for these curves the body

The only other mammalian form upon which specific data concerning prenatal growth are available (so far as I know) is the guinea pig. A few observations by Hensen (20) show that, in general, the relative growth is more rapid during the earlier stages (from 16th day) than in later fetuses. There are, however, some irregularities and evidently great individual variations.

Concerning the relative size and growth of the various organs and parts in embryonic life, exact data are still scarce. Certain facts, however, are already generally known, or can be easily observed from specimens or published figures. In all vertebrates, from the fishes upward, the embryonic head is relatively large, especially in the earlier stages. There is considerable variation in the extent to which this is true in different forms, however. It appears most strikingly developed in the amniota; less so, as a rule, in amphibia and fishes. The head is perhaps relatively largest in the embryos of birds, where it may form more than half the entire body. Among mammals there is also variation in different species; e. g., the head of the pig embryo is relatively much smaller than that of the rabbit or human embryo.

The extremities in all forms appear relatively small in the embryo, gradually increasing to the relative size of the adult.

Correlated with the size of the head, we find the brain always relatively larger in the vertebrate embryo. It is almost always largest at a comparatively early stage, diminishing thereafter in relative size throughout prenatal and postnatal life up to the adult stage. In the chick, a few observations are recorded by Welcker and Brandt (45) indicating that at the 9th day of incubation the brain forms 28.2 per cent of the body; at the 10th day, about 14 per cent; 11th day, 13 per cent; 13th day, 9 per cent; 17th day, 5 per cent; newly hatched 3 per cent; adult, less than .5 per cent. Similarly in the dog, shrew, salamander and stickleback, observations indicate

weight and span of life in man and white rat have been reduced to the same basis. The actual growth rate in the guinea pig and rabbit has been shown by Minot (33) to be about 25 times as great as in the human body. Oppel (37) points out that animals of greatly diverse adult size are much less different in size at corresponding early embryonal stages.

that the brain is relatively larger in the embryo or newborn than in the adult.

For the spinal cord, however, this rule is apparently not constant. In the chick and stickleback, the spinal cord in the adult diminishes in relative size; but in the shrew and (to a slight extent) in the dog, it apparently increases from newborn to adult.

The eyeballs are in all the animals just mentioned (excepting the salamander?) relatively smaller in the adult. In the chick embryo of the 11th day of incubation (Welcker and Brandt), the eyeballs form nearly 25 per cent of the entire body. In the newborn chick, they have decreased to about 3 per cent, and in the adult to .3 per cent or .4 per cent.

The thyroid and thymus glands in the dog and shrew appear to remain of about the same relative weight in the adult as in the newborn. For the spleen, this applies to the chick as well as to the dog and shrew.

The heart appears relatively smaller in the adult stickleback, chick and dog; but larger in the salamander and shrew (?). In the chick, dog and shrew, the lungs are relatively smaller in the adult than in the embryo or newborn.

The alimentary canal is relatively larger in the adult stickleback, dog and shrew; but smaller in the salamander and chick. The liver is relatively much smaller in the adult shrew, slightly smaller in the adult stickleback, chick and dog; but much larger in the adult salamander than in the embryo.

The kidneys appear relatively larger in the adult stickleback and salamander, but smaller in the shrew, dog and chick (slightly). The suprarenal glands are relatively slightly larger in the adult shrew, (no data on other forms). The reproductive glands are relatively larger in the adult chick, but smaller in the dog and shrew.

If we compare the foregoing data with the course of growth in the human body, two facts stand out clearly: In the first place, it is evident that, although the growth rate of the body as a whole varies greatly in different animals, it is greatest in the early embryo (at least in birds and mammals). In the second place, it is evident that in vertebrates in general the prenatal growth is relatively greater

in the head region, including also individually the brain, eyeballs, and tongue.[10]

Beyond this it is perhaps unsafe to generalize, on account of the scanty data available; but it seems likely that the viscera in general (circulatory, respiratory, alimentary and genito-urinary) are as a rule relatively smaller in the adult than in the earlier stages of the higher vertebrates. If this is true, it follows that the remainder of the body (chiefly locomotor apparatus) must be relatively greater in the adult, which appears to be true in the chick, dog and shrew, as well as in the human species.

Significance of the Growth Changes.

It is the purpose of the present paper to present facts concerning growth, rather than to speculate upon their significance.[11] It may be worth while, however, to point out that growth can be considered with reference to (1) its immediate causes, or (2) its physiological, ontogenetic or phylogenetic significance. Concerning (2) we may safely say that the size of an organ or part, like its position, form, and structure, may depend upon, or be related to, the present, past and future. The present refers to the physiological relations which the part bears to the existing organism, an increase or decrease in function being generally correlated with a corresponding increase or decrease in size. The past refers to the conditions associated with the ontogenetic[12] or phylogenetic history (of *e. g.,* Wolffian bodies) of the individual.

The future refers to changes in an organ which take place in anticipation of physiological needs which will arise at a later period in the life cycle of the organism. Preyer's (39) statement that "Im embryonalen Leben diejenigen Theile am schnellsten wachsen welche

[10]The prenatal tongue is relatively larger in the chick, dog and shrew (Welcker and Brandt).

[11]For a detailed consideration of the principles of embryonic growth, cf. His (22).

[12]It is noted that, in general, organs which arise as infoldings (brain, spinal cord) are relatively large at the beginning and decrease later, while the converse is true of organs which arise as outgrowths (glands, lungs, etc.).

am frühesten nach der Geburt in Function treten" is an inexact expression of this relation.

If we attempt to go beyond these general relations and to analyze the phenomena of growth in order to determine the more direct and immediate factors, we meet with the greatest difficulties. The problem appears at present too complicated for solution. The conditions determining the growth rate of an organ or organism (aside from heat, light and other external factors) may, however, be traced back to the cells and grouped under two general headings: (1) specific physico-chemical differences in the protoplasm, chiefly determined (in the beginning) by heredity, which affect metabolism and thereby the growth rate; and (2) conditions within the organism which affect the quality and quantity of available food and oxygen supply for the cells, or which affect the removal of their waste products of metabolism.

Concerning the first group, the intrinsic differences in protoplasm, we know very little.[13] The second group of conditions is more easily accessible to investigation, however, and we may expect that much light will be thrown upon this phase of the problem of growth by experimental methods.

Summary.

The more important conclusions concerning prenatal growth may be summarized as follows:—

1. The human ovum increases more than 10,000 times in size during the 1st month, the embryo proper attaining a weight of about .04 g. The increase for the succeeding months (relative monthly growth rate) is expressed by the figures 74, 11, 1.75, .82, .67, .50, .47 and .45. The curve of absolute growth after the 1st month corresponds approximately to the formula:

[13]The tissues of early embryos are known to be very rich in water, and it has been suggested that this may favor the chemical changes in the rapid growth characteristic of that period. The converse, however, is probably nearer the truth. Minot (34) believes that the relative abundance of nuclear material at this period accounts for the greater intensity of growth and that the decreasing growth rate results from an increase in the amount and differentiation of the cytoplasm.

$$\text{Weight (g)} = \left(\frac{\text{(Age (days)}}{37}\right)^4$$

2. The head attains its maximum relative size, about 45 per cent of the total body weight, during the 2d month, thereafter decreasing to about 26 per cent at birth. A relatively large embryonic head is characteristic for vertebrates in general.

3. The trunk is relatively larger in the 1st month (about 65 per cent of the whole body) decreasing to between 40 per cent and 45 per cent in the later fetal months.

4. The extremities increase gradually in relative size from the beginning, the upper extremities forming at birth about 10 per cent of the entire body, and the lower about 20 per cent.

5. The brain curve of relative growth is nearly parallel with that for the head, reaching a maximum of about 20 per cent in the 2d month and decreasing thereafter to an average of 13 per cent or 14 per cent of the whole body at birth.

6. The spinal cord is relatively largest (about 5 per cent of the entire body) in the 1st month, decreasing at first rapidly, then more slowly to about .15 per cent at birth.

7. The curve of relative growth of the heart is close to that of the spinal cord during the 1st, 2d and 3d months, remaining near an average of .7 per cent thereafter.

8. The liver increases to a maximum of 7.5 per cent (average) of the entire body during the 2d and 3d months, remaining fairly constant between 5 per cent and 6 per cent during the remainder of the fetal period.

9. The lungs increase gradually in relative size to a maximum average of about 3.3 per cent in the 4th month, decreasing thereafter to an average of about 2 per cent of the entire body at birth.

10. The spleen, thymus and thyroid gland increase from the beginning more or less regularly in relative size, averaging about .4 per cent, .3 per cent, and .12 per cent, respectively, of the total body weight at birth.

11. The kidneys increase at first rapidly, then more slowly to a maximum of about 1.0 per cent in the 7th month. Later they de-

crease slightly in relative size, but average 1.05 per cent in the live-born.

12. The suprarenal glands increase rapidly to a maximum relative size of about .45 per cent of the whole body in the 3d month, decreasing steadily thereafter to about .24 per cent at birth.

13. In the case of the paired organs, the larger size of the right lung, left kidney and left suprarenal gland is established early in the fetal period.

14. At full-term, almost all of the viscera (excepting the thymus and suprarenal glands) average relatively greater in the live-born than in the still-born.

15. The fetal viscera appear as a rule to be relatively heavier in the female (excepting the thymus, lungs and kidneys).

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TABLE I.

MEASUREMENTS OF SPECIMENS OBSERVED.

Catalog No. of Embryo.	Sex.	Length in cm.		Est. Age. Days.	Volume.	Fixation.
		Crown-Rump.	Total.			
176	–	0.6 cm.	—	25	.01544 cc.	Formalin.
220	–	0.73	—	27	.026	Alcohol–Formalin.
60	–	1.1	—	33	.0976	Alcohol.
58	f.	1.7	—	41	.3788	Formalin.
147	m.	2.3	—	48	1.3	"
99	f.	2.6	—	51	3.0	Alcohol–Formalin.
224	–	3.0	—	55	2.29	Fresh.
57	f.	3.1	—	56	1.693	Formalin.
158	–	3.5	—	59	5.6	Alcohol.
51	m.	3.5	—	59	6.0	Formalin.
122	m.	3.9	—	62	4.1	"
185	–	4.5	6.5	67	8.8	"
121	m.	4.6	—	68	5.0	"
115	m.	5.0	—	71	10.0	"
194	f.	5.6	—	75	14.75	"
148	m.	5.8	—	76	15.5	"
197	f.	6.2	9.0	79	15.5	"
123	m.	6.8	9.2	82	24.0	"
128	f.	7.5	12.	87	54	Alcohol.
181	m.	9.	13.7	95	80	"
129	m.	9.5	14.5	97	108	"
130	f.	10.5	16.	105	122	"
143	m.	11.	—	110	95	Formalin.
162	m.	11.5	—	115	97	"
186	m.	13.	20.3	130	243	"
199	f.	13.2	23.	132	257	"
191	f.	14.	22.5	140	370	"
211	m.	15.	21.5	140	180	Fresh.
218	m.	15.5	23.	146	214	"
195	m.	16.	25.5	152	375	Formalin.
154	f.	17.	26.	158	464	Alcohol.
210	f.	17.5	26.5	160	383	Fresh.
172	m.	18.	27.5	165	460	Formalin.
89	f.	20.	28.5	170	605	"
171	m.	21.	31.	174	690	"
192	f.	20.	33.	185	775	"
219	f.	23.	34.	192	791	Fresh.
193	m.	26.	37.	209	941	Formalin.
208	m.	31.5	46.	258	1981	Fresh.
201	m.	30.	46.5	260	2310	Formalin.
234	m.	31.	49.	274	2727	Fresh.
202	m.	32.	50.	280	3470	Formalin.
198	m.	36.	54.	302	3830	"

TABLE II.

Observations on Volume of His-Ziegler Models.

Number.	1 (SR.)	4 (M.)	3 (BB.)	5 (Lr.)	6 (α.)	7 (R)	8 (A.)
Embryo length.	2.2 mm.	2.6 mm.	3.2 mm.	4.2 mm.	4 mm.	5 mm.	7.5 mm.
Age His:	14 da.	....	20 da.	....	23 da.	....	....
Mall's Rule.....	15 da.	16 da.	18 da.	20 da.	20 da.	22 da.	28 da.
Total volume of model.....	208 cc.	236 cc.	*95cc.	*218 cc.	86cc.	124 cc.	328cc.
Magnification of model.....	40 diam.	40 diam.	40 diam.	40 diam.	20 diam.	20 diam.	20 diam.
Corresponding (actual) volume of embryo.....	.00325cc	.003687cc	.001484cc	.003406cc	.01075 cc.	.0155 cc.	.041 cc.
Actual volume of embryo proper.....	.000781cc	.001281cc	.001484cc	.003406cc	.01075 cc.	.0155 cc.	.041 cc.
Actual volume of yolk sac.....	.002469cc	.002406cc	....	....	....	....	....
Head = % of Total body.....	....	....	37.9%	33.9%	34.9%	38.7%	36.6%

*In Nos. 3 and 5, the data of the volume of the models are somewhat uncertain since in each a portion of the ventral body wall is deficient in the model. Correction was made for this by adding 5 cc. to the volume observed for No. 3, and 9 cc. to that for No. 5.

TABLE III.

Prenatal Growth of Human Body by Months.

Lunar Month.	Weight at Beginning of Month. (a.)	Weight at end of Month. (b.)	Relative Monthly Growth. $\left(\frac{b-a}{a}\right)$
I.	.000004 g.	.04 g.	9999.
II.	.04	3.0	74.
III.	3.0	36	11.
IV.	36.	120.	2.33
V.	120.	330.	1.75
VI.	330.	600.	.82
VII.	600.	1000.	.67
VIII.	1000.	1500.	.50
IX.	1500.	2200.	.47
X.	2200.	3200.	.45

TABLE IV.

Relative Size of Various Fetal Organs (in Percentage of Total Body Volume) in Specimens Observed.

No. and Sex.	60 (—)	58 (f)	57 (f)	185 (f)	148 (m)	197 (f)
Crown-rump length	1.1 cm.	1.7 cm.	3.1 cm.	4.5 cm.	5.8 cm.	6.2 cm.
Volume of body	.122 cc.	.4735 cc.	2.117 cc.	8.8 cc.	15.5cc.	15.5 cc.
Head	44.42%	45.76%	45.4%	43.18%	41.94%	36.8 %
Upper extremities	2.91	2.13	2.64	4.67	5.87	5.75
Lower extremities	2.43	2.26	2.21	5.68	7.61	6.58
Trunk	50.12	49.86	49.76	46.47	44.58	50.9
Brain	20.26	22.35	18.81	22.72	18.58	....
Spinal cord	4.85	3.43	1.53	1.25	.774	....
Thyroid gland	....	....	.035	....	....	....
Thymus	....	....	.008	....	....	....
Heart	3.64	1.71	1.32	1.14	1.06	1.03
Right lung	.18	.276	.777	1.7	1.26	1.74
Left lung	.18	.21	.605	1.48	1.03	1.23
Liver	4.85	6.91	10.56	7.39	6.45	8.71
Spleen	....	.019	.0088	....	....	....
Pancreas	....	.032	.0533	....	....	....
Right kidney	....	.044	.115	.255	.516	.29
Left kidney	....	.042	.105	.255	.548	.355
Right suprarenal	....	.169	.141	.255	.31	.258
Left suprarenal	....	.172	.131	.255	.31	.258
Stomach	....	.56	....	.17	.452	.58
Intestines	....	.72	....	2.05	2.0	2.71
Sex glands	.0852	.112	.0357	....	....	....

Note. To correct for shrinkage in the first three embryos (which were embedded in paraffin), 25 per cent was added to the observed body volumes given in Table I.

TABLE IV (Continued).

Relative Size of Various Fetal Organs (in Percentage of Total Body Volume) in Specimens Observed.

No. and Sex.	123 (m)	128 (f)	181 (m)	129 (m)	130 (f)	143 (m)
Crown-rump length	6.8 cm.	7.5 cm.	9 cm.	9.5 cm.	10.5 cm.	11. cm.
Volume of body.....	24. cc.	54. cc.	80. cc.	108 cc.	122 cc.	95. cc.
Head.....	43.75%	40.74%	40. %	39 81%	39.35%	40. %
Upper extremities....	4.17	6.11	6.88	6.02	5.33	6.11
Lower extremities....	6.25	10.2	9.75	9.07	9.43	8.42
Trunk.....	45.83	42.96	43.38	45.09	45.9	45.47
Brain.....	15.0	20.37	12.5	18.52	21.31	20.95
Spinal cord.....	.458	.278	.25	.14	.164	.21
Thyroid gland.....	....	.11	.063	.083	.102	.095
Thymus.....	....	.1	.075	.102	.148	.105
Heart.....	.833	.926	1.125	1.39	1.19	.842
Right lung.....	1.21	2.96	1.63	2.13	1.07	1.58
Left lung.....	1.00	2.22	1.44	1.85	.82	1.16
Liver.....	7.08	5.56	5.25	7.04	7.38	5.05
Spleen.....	....	.096	.081	.074	.131	.053
Pancreas.....	....	.11	.094	.102	.123	.095
Right kidney.....	.292	.377	.4	.46	.475	.295
Left kidney.....	.271	.35	.369	.509	.484	.316
Right suprarenal.....	.188	.17	.288	.204	.295	.158
Left suprarenal.....	.188	.15	.275	.204	.344	.168
Stomach.....	.375	.33	.25	.26	.41	.21
Intestines.....	2.29	2.78	1.0	3.33	3.36	2.84
Sex glands.....	....	....	....	....	....	....

TABLE IV (Continued).

Relative Size of Various Fetal Organs (in Percentage of Total Body Volume) in Specimens Observed.

No. and Sex.	162 (m)	186 (m)	199 (f)	191 (f)	211 (m)	218 (m)
Crown-rump length	11.5cm.	13 cm.	13 cm.	14 cm.	15.0 cm.	15.5cm.
Volume of body.....	97 cc.	243 cc.	290 cc.	370 cc.	180.4cc.	214 cc.
Head.....	40.21%	39.5 %	37.93%	39.19%	38.83%	40.32%
Upper extremities....	6.19	6.67	7.93	8.3	6.71	7.24
Lower extremities....	9.79	10.7	13.79	14.05	11.14	12.13
Trunk.....	43.81	43.13	40.34	38.46	45.12	(42.)
Brain.....	18.56	13.16	13.79	16.22	16.94	16.29
Spinal cord.....	.263	.198	.203	.232	.336	.235
Thyroid gland.....	.052	.045	.062	.132	.080	.053
Thymus.....	.103	.09	.107	.243	.125	.117
Heart.....	.618	.76	.876	.89	.67	.67
Right lung.....	1.85	1.23	1.07	1.54	1.60	1.32
Left lung.....	1.57	1.07	.872	1.30	1.25	1.08
Liver.....	4.95	4.01	3.28	5.76	6.30	4.70
Spleen.....	.052	.07	.1	.26	.077	.095
Pancreas.....	.103	.082	.107	.108	.058	.118
Right kidney.....	.34	.33	.35	.62	.413	.58
Left kidney.....	.36	.41	.379	.73	.401	.57
Right suprarenal.....	.154	.164	.19	.216	.224	.18
Left suprarenal.....	.206	.185	.224	.27	.247	.188
Stomach.....	.32	.206	.328	.405	.28	.96
Intestines.....	2.58	2.18	3.1	3.05	2.86	2.68
Sex glands.....	—..	....	.035	....	(.040)	(.045)

TABLE IV (Continued).

Relative Size of Various Fetal Organs (in Percentage of Total Body Volume) in Specimens Observed.

No. and Sex.	195 (m)	154 (f)	210 (f)	172 (m)	89 (f)	192 (f)
Crown-rump length	16 cm.	17 cm.	17.5 cm.	18 cm.	20 cm.	20 cm.
Volume of body	375 cc.	464 cc.	383 cc.	460 cc.	605 cc.	775 cc.
Head	37.33%	38.79%	37.13%	38.04%	34.05%	37.42%
Upper extremities	8.53	7.76	7.91	7.39	8.1	8.0
Lower extremities	15.28	13.36	13.81	14.78	13.72	14.19
Trunk	38.86	40.04	41.07	39.78	44.13	40.65
Brain	15.47	15.95	13.76	15.54	16.53	16.65
Spinal cord	.187	.155	.226	.184	.198	.271
Thyroid gland	.053	.069	.061	.148	.107	.132
Thymus	.2	.179	.171	.113	.165	.232
Heart	1.07	1.08	.83	1.195	1.37	1.42
Right lung	1.27	1.25	1.59	.87	1.12	1.61
Left lung	1.04	1.08	1.42	.71	.88	1.2
Liver	5.87	4.74	5.81	5.4	6.94	6.19
Spleen	.051	.11	.106	.076	.088	.194
Pancreas	.107	.097	.068	.098	.099	.101
Right kidney	.427	.302	.378	.54	.446	.465
Left kidney	.453	.28	.386	.51	.479	.490
Right suprarenal	.373	.172	.154	.196	.182	.155
Left suprarenal	.400	.205	.186	.217	.198	.232
Stomach	.28	.194	.52	.196	.529	.606
Intestines	2.59	3.02	2.34	2.83	3.8	4.26
Sex glands	....	....	(.026)	....	....	....

TABLE IV (Continued).

RELATIVE SIZE OF VARIOUS FETAL ORGANS (IN PERCENTAGE OF TOTAL BODY VOLUME) IN SPECIMENS OBSERVED.

No. and Sex.	171 (m)	219 (f)	193 (m)	208 (m)	201 (m)	234 (m)	202 (m)	198 (m)
Crown-rump length.....	21 cm.	23 cm.	26 cm.	31.5 cm.	30 cm.	31 cm.	35 cm.	36c m.
Volume of body.....	680 cc.	791.8cc.	941 cc.	1981.4 cc.	2310 cc.	2727.1 cc.	3470 cc.	3830 cc.
Head.....	33.09%	33.0 %	31.88%	30.9 %	31.86%	25.8 %	27.09%	26.9 %
Upper extremities.....	6.91	8.20	7.95	9.56	9.52	10.18	9.65	9.14
Lower extremities.....	15.15	15.32	18.17	17.56	17.49	20.16	19.31	19.84
Trunk.....	44.85	44.0	42.0	41.40	41.13	40.12	43.95	44.12
Brain.....	17.65	(16.0)	14.88	14.5	13.91	13.12	11.10	9.48
Spinal cord....	.199	.230	.191	....	.143	....	.075	.091
Thyroid gland	.072	.043	.084	.065	.065	.054	.110	.065
Thymus.....	.221	.256	.425	.311	.212	.66	.320	.149
Heart.....	1.03	.87	1.06	.779	1.08	.59	.72	....
Right lung.....	1.4	1.36	1.51	.988	.909	....	.778	....
Left lung.....	1.1	1.03	1.16	.79	.688	....	.576	....
Liver.....	4.85	4.74	6.38	4.35	2.0	4.84	4.61	....
Spleen.....	.078	.227	.159	.201	.108	.24	.188	....
Pancreas.....	.074	.060	.069	.067	.054	.12	....	....
Right kidney..	.338	.468	.499	.885	.286	.37	.231	....
Left kidney....	.368	.484	.478		.26	.41	.228	....
Right suprarenal.....	.147	.136	.117	.231	.087	.118	....	....
Left suprarenal.....	.176	.160	.128		.087	.138	....	....
Stomach.....	.22	.519	.213	....	.273	2.82	....	....
Intestines.....	3.24	3.77	3,08	....	3.98		....	....
Sex glands.....	....	(.022)	....	.080	....	....	....	....

TABLE V.—Relative Size of Fetal Organs in the Various Months.

	Second Month.		Third Month.		Fourth Month.		Fifth Month.		Sixth Month.	
	No.	% of Total Body.	No.	% of Total Body.	No.	% of Total Body.	No.	% of Total Body.	No.	% of Total Body.
Brain.....	3	20.47 (18.81–22.35)	5	19.15 (15.–22.72)	30	15.72 (10.–23.7)	53	13.96 (9.58–23.03)	49	14.30 (9.33–20.8)
Spinal cord	3	3.27 (1.53–4.85)	4	.82 (.458–1.25)	5	.21 (.14–.278)	4	.25 (.198–.263)	7	.22 (.155–.336)
Thyroid gland ...	1	.035	—	.....	5	.091 (.063–.11)	4	.073 (.052–.132)	5	.094 (.053–.148)
Thymus...	1	.008	1	.19	28	.134 (.039–.41)	50	.149 (.053–.41)	45	.175 (.041–.39)
Heart.....	3	2.22 (1.32–3.64)	7	.85 (.55–1.14)	38	.685 (.40–1.39)	60	.65 (.34–1.33)	63	.73 (.45–1.37)
Lungs.....	3	.743 (.36–1.38)	7	2.52 (1.36–3.18)	37	3.29 (1.55–5.18)	61	3.09 (1.70–5.14)	64	2.54 (1.28–4.16)
Liver.....	3	7.44 (4.85–10.56)	7	7.61 (5.–10.5)	39	5.08 (2.7–8.1)	59	5.27 (3.28–7.93)	64	5.39 (2.68–8.69)
Spleen.....	2	.014 (.009–.019)	—	.....	33	.092 (.02–.46)	52	.107 (.035–.26)	60	.142 (.051–.36)
Pancreas...	2	.043 (.032–.053)	—	.....	5	.105 (.063–.11)	4	.100 (.082–.108)	5	.094 (.068–.107)
Stomach (empty).	—	.....	—	.....	2	.25 (.23–.27)	4	.29 (.17–.45)	5	.29 (.19–.45)
Stomach (+ contents)...	1	.56	4	.39 (.17–.56)	7	.33 (.21–.46)	8	.42 (.21–.65)	10	.45 (.19–.96)
Intestines (empty).	—	.....	—	.....	2	1.33 (1.2–1.45)	4	1.24 (.58–1.67)	4	.75 (.43–1.0)
Intestines (+ contents)...	1	.72	4	2.26 (2.–2.71)	7	2.55 (1.–3.36)	8	2.57 (2.–3.1)	10	2.68 (1.96–3.14)
Kidneys...	2	.153 (.086–2.20)	5	.659 (.46–1.06)	36	.815 (.40–2.33)	60	.891 (.53–2.81)	64	.854 (.49–1.29)
Supra-renals,..	2	.306 (.272–.341)	5	.458 (.27–.62)	30	.43 (.20–1.46)	49	.362 (.175–.86	44	.36 (.13–.773)
Avg. body weight ..	3	.904 g.	7	14.96 g.	38	80.88 g.	62	234 g.	67	413.1 g.

TABLE V (Continued).—Relative Size of Fetal Organs in the Various Months.

	Seventh Month.		Eighth Month.		Ninth Month.		Tenth Month. (Still-born.)		Tenth Month. (Live-born.)	
	No.	% of Total Body.	No.	% of Total Body.	No.	% of Total Body.	No.	% of Total Body.	No.	% of Total Body.
Brain	26	13.77 (10.94–17.65)	57	12.46 (11.69–15.5)*	3	14.48 (12.73–15.7)	120	12.78 (9.23–16.1)*	90	14.59 (....)*
Spinal cord	3	.23 (.199–.271)	1	.19	..	.....	5	.144 (.075–.21)	...	.....
Thyroid gland	4	.089 (.043–.132)	7	.129 (.055–.22)	..	.....	26	.111 (.041–.33)	11	.125 (.089–.16)
Thymus	19	.285 (.11–.42)	60	.259 (.10–.65)*	6	.24 (.20–.36)	124	.326 (.149–1.21)*	101	.313 (.141–.53)*
Heart	36	.83 (.48–1.45)	65	.71 (.60–1.13)*	15	.78 (.40–1.56)	165	.70 (.45–1.21)*	164	.77 (.45–1.44)*
Lungs	36	2.58 (.98–6.31)	67	2.40 (1.39–3.45)*	13	2.14 (1.63–3.51)	289	1.71 (.061–3.49)*	202	2.18 (1.02–3.65)*
Liver	36	5.31 (3.39–7.8)	66	5.28 (2.56–7.8)*	8	4.93 (3.8–6.67)	145	5.05 (2.0–6.85)*	101	5.23 (3.03–7.48)*
Spleen	32	.176 (.043–.51)	66	.428 (.039–.44)*	7	.44 (.26–.74)	143	.32 (.108–.73)*	101	.431 (.08–.39)*
Pancreas	4	.084 (.06–.101)	49	.129 (....)*	..	.....	87	.105 (.067–.15)*	90	.145 (....)*
Stomach (empty)	8	.39 (.22–.59)	5	.25 (.17–.49)	3	.16 (.13–.20)	7	.20 (.14–.23)	...	.....
Stomach (+ contents)	12	.51 (.22–.65)	6	.40 (.21–.82)	3	.30 (.26–.37)	8	.49 (.26–1.39)	...	.....
Intestines (empty)	8	1.27 (.85–2.30)	5	1.38 (.83–2.0)	3	.98 (.65–1.5)	7	1.23 (.77–1.75)	...	.....
Intestines (+ contents)	12	3.08 (1.69–4.26)	6	3.35 (1.74–4.55)	3	3.36 (3.02–3.52)	8	3.53 (2.78–3.76)	...	.....
Kidneys	35	.987 (.47–2.13)	65	.93 (.40–1.30)*	6	.77 (.43–1.07)	144	.82 (.40–1.49)*	101	1.05 (.85–1.83)*
Supra-renals	17	.31 (.143–.44)	58	.28 (.17–.458)*	..	.....	108	.246 (.11–.52)*	101	.229 (.11–.36)*
Avg. body weight	37	748 g.	68	1196 g.	16	1609 g.	177	3046 g.	153	2590 g.

*Individual variations not available in Boyd's cases, which include 48 in the 8th month, 83 in the 10th month (still-born), and 90 in the 10th month (live-born); in Oppenheimer's 23 cases (10 month, still-born), and in 155 observations on the lungs (10th month) by Schmitt, Devergie and Elsasser.

TABLE VI.

Relative Size of Fetal Organs im the Various Months by Sexes.

	Second Month.				Third Month.				Fourth Month.				Fifth Month.				Sixth Month.			
	Male.		Female.		Male.		Female.		Male.		Female.		Male.		Female.		Male.		Female.	
	No.	Per Cent	No.	Per Cent	No.	Per Cent	No.	Per Cent	No.	Per Cent	No.	Per Cent	No.	Per Cent	No.	Per Cent	No.	Per Cent	No.	Per Cent
Brain	..	....	2	20.52	3	17.96	1	22.72	15	15.41	9	15.15	26	14.40	23	13.58	26	14.23	22	14.33
Thymus	..	....	1	.00017	1	.19	..	....	13	.151	9	.135	24	.150	22	.140	23	.181	22	.169
Heart	..	....	2	1.51	3	.84	2	1.09	15	.74	9	.704	30	.638	24	.675	37	.70	25	.78
Right lung	..	....	2	.53	2	1.24	2	1.72	16	1.80	9	1.68	31	1.71	24	1.53	36	1.44	25	1.32
Left lung	..	....	2	.41	2	1.02	2	1.36	16	1.50	9	1.45	31	1.47	24	1.30	36	1.21	25	1.08
Liver	..	....	2	8.73	3	7.22	2	8.05	16	5.47	9	6.09	28	5.16	25	5.29	38	5.34	25	5.46
Spleen	..	....	2	.014	..	....	..	....	15	.107	8	.073	26	.107	22	.108	36	.136	23	.150
Right kidney	..	....	2	.08	2	.404	2	.273	16	.384	8	.388	30	.439	24	.439	37	.425	25	.420
Left kidney	..	....	2	.074	2	.42	2	.305	16	.386	9	.405	29	.473	25	.443	37	.429	25	.424
Rt. suprarenal	..	....	2	.155	2	.249	2	.257	14	.191	9	.287	24	.154	21	.177	21	.179	21	.181
Lt. suprarenal	..	....	2	.151	2	.249	2	.257	14	.197	9	.268	24	.173	22	.189	20	.192	22	.178
Av'ge body weight	..	....	2	1.4 g.	3	17.34 g.	2	12.15 g.	16	106.2 g.	9	86.9 g.	31	226 g.	25	253 g.	40	418g.	25	402 g.

TABLE VI (Continued).

RELATIVE SIZE OF FETAL ORGANS IN THE VARIOUS MONTHS BY SEXES.

	SEVENTH MONTH.				EIGHTH MONTH.				NINTH MONTH.				TENTH MONTH. (Still-born.)				TENTH MONTH. (Live-born.)			
	Male.		Female.		Male.		Female.		Male.		Female.		Male.		Female.		Male.		Female.	
	No.	Per Cent	No.	Per Cent	No.	Per Cent	No.	Per Cent	No.	Per Cent	No.	Per Cent	No.	Per Cent	No.	Per Cent	No.	Per Cent	No.	Per Cent
Brain	15	13.32	11	14.37	33	12.61	24	12.24	3	14.48	..	....	71	12.91	49	12.59	45	14.41	45	14.77
Thymus	10	.290	9	.279	31	.253	23	.224	..	....	..	....	65	.296	35	.309	45	.31	45	.31
Heart	20	.83	16	.83	34	.71	25	.69	5	.83	4	.81	80	.69	61	.73	74	.70	79	.83
Right lung	20	1.43	16	1.38	34	1.30	25	1.29	3	1.33	..	....	69	.98	48	.99	45	1.28	45	1.76
Left lung	20	1.25	16	1.07	34	1.15	25	1.07	3	.99	..	....	69	.79	48	.78	45	1.05	45	1.27
Liver	20	5.05	16	5.64	34	5.21	25	5.55	3	4.09	..	....	71	4.81	50	5.46	45	4.94	45	5.64
Spleen	17	.191	15	.158	34	.404	25	.524	3	.27	..	....	70	.27	50	.37	45	.41	45	.50
Right kidney	19	.530	16	.453	6	.517	5	.441	3	.49	..	....	9	.367	1	.56	45	1.07	45	1.00
Left kidney	19	.535	16	.441	6	.520	5	.456	3	.50	..	....	9	.341	1	.50				
Rt. suprarenal	9	.139	8	.166	2	.130	2	.174	..	....	..	....	2	.101	..	....	45	.21	45	.24
Lt. suprarenal	9	.148	8	.170	2	.154	2	.176	..	....	..	....	2	.111	..	....				
Av'ge body weight.	21	833 g.	16	637 g.	34	1248 g.	26	1106 g.	5	1526 g.	5	1626 g.	89	3067 g.	64	2963 g.	74	2725 g.	79	2464 g.

THE CHONDROCRANIUM OF AN EMBRYO PIG, SUS SCROFA.

A Contribution to the Morphology of the Mammalian Skull.

BY

CHARLES SEARING MEAD.

With 11 Text Figures and 4 Plates.

CONTENTS.

Introduction.

The study of the chondrocranium of Sus is of value not only in assisting us to understand the structure of the adult skull in this form, but also on account of its bearing on the general morphology of the mammalian cranium. Owing to the generalized dentition and the structure of the feet, Sus has been placed relatively low in the ungulate series. Hence, we would expect many primitive characters to be retained in its cartilaginous cranium, and, indeed, this is the fact, for a number of reptilian characters are present. This chondrocranium is also valuable for comparison with those of primates and insectivores.

The Sus embryo studied was 30 mm. long (head-rump measurement), and the length of its head 12 mm. The head was cut transversely into 795 sections, each 0.015 mm. thick. The cartilage in all the even-numbered sections was drawn with the aid of a projection apparatus, the drawings being enlargements of 25 diameters. In making the reconstruction, Born's wax plate method was used.

For comparison I have had Ziegler's wax models of the chondrocrania of man, Gallus, Lacerta and Rana. In addition, Professor Eugen Fischer, of the University of Freiburg, loaned me his reconstructions of the Semnopithecus, Macacus, Tarsius and Talpa skulls, and the series of sections from which his Talpa reconstruction was made. Also a reconstruction of the primordial cranium of Lepus, prepared by Dr. Max Voit, has been of service.

Of the models and reconstructions used for comparison, the one of Talpa resembled more closely that of Sus than did any of the others, and, hence, has been referred to most frequently in the comparisons.

The literature dealing with the development of the mammalian skull is very extensive. The publications of Parker, Spöndli, Kölliker and Decker, on the chondrocrania of the mammals, are the principal works belonging to the old school, in which the skulls were prepared principally by the maceration method. The introduction of Born's wax plate method of reconstruction has made possible not only a more exact study of the embryonic cranium itself, but has also enabled one to study the surrounding tissues as well. A more fundamental view of the skull is thus obtained. Among the more recent papers on the embryonic skull may be mentioned Gaupp's "Die Entwickelung des Kopfskelettes" in Hertwig's Handbuch (1905 b), valuable on account of its general survey; also his extensive contribution on the development of the skull of Echidna (1908). The papers of Fischer on Talpa (1901 b) and the apes (1903) and a forthcoming paper of Voit on Lepus (1909) likewise contain valuable results.

This investigation was undertaken at the suggestion of Professor Ernst Gaupp. The work was conducted in the laboratory of the Comparative Anatomical Institute of Freiburg in Baden.

I will here take the opportunity to express to Professor Gaupp my sincere appreciation not only for the use of his extensive series of sections, but also for his very valuable help and suggestions. Dr. Max Voit I wish to thank for the use of his Lepus reconstruction and for the assistance which he has rendered me. And for the loan of the reconstructions of the Semnopithecus, Macacus, Tarsius and Talpa skulls, and also for the series of sections from which the Talpa reconstruction was made I wish to express to Professor Eugen Fischer my sincere thanks.

The Skull as a Whole.

The form of the primordial cranium of the pig represents well the generalized mammalian type of chondrocranium. No part is markedly underdeveloped and no part is greatly overdeveloped at the expense of the surrounding portions.

As a whole, the chondrocranium at this stage of development, *i. e.*, in an embryo 30 mm. long, resembles a pear in shape, with the small end anterior, forming the nasal region, and the large end posterior. The large brain-box is widely open dorsally, a feature characteristic of the amniotes. Later this region of the skull is completed by the formation of the large roofing membrane bones, the frontals and parietals. Ventrally the general contour is completed by the mandibular, hyoid and branchial arches. The brain-case is large and extends forward over the posterior half of the nasal capsules, forcing the fenestræ cribrosæ from their primitive vertical position into a horizontal one. Its side walls are formed by a broad continuous plate of cartilage on each side, thus standing in sharp contrast to the condition in similar embryos of man and the primates, in which this region is very rudimentary. The nasal capsules are of moderate length, not long as in Talpa, nor short as in man. Taken as a whole, the chondrocranium is complete except for some minor details. None of the cartilaginous bones (*Ersatzknochen,* Gaupp) have as yet begun to ossify. Some of the membrane bones have not yet appeared and the others are only in the very early stages of their formation; consequently they have not been included in my reconstruction (Pls. I-IV). With the exception of the mandibular, hyoid and branchial

arches (the visceral skeleton), the different parts of the skull are not separated by sutures, the cartilage composing the neural cranium forming one continuous unit. Kölliker has divided the skull into the posterior part, the *pars chordalis,* through which the notochord runs, and the *pars prechordalis,* situated anterior to this. Each of these divisions Gegenbaur has again divided into two regions, which Gaupp has named, from behind forward, as follows: *regio occipitalis, regio otica, regio orbitotemporalis* and *regio ethmoidalis.* For descriptive purposes it will be convenient to follow these divisions, except that the basal portions of the two posterior regions will first be considered together, since they form a fairly complete unit, the *planum basale* (Gaupp).

Planum Basale.

The planum basale comprises the basal portions of the two posterior regions of the skull. It extends from the foramen magnum forward to the posterior border of the hypophysial fossa and is perforated for nearly its entire length by the notochord. Its anterior three-fifths, *i. e.,* the portion belonging to the regio otica, is narrow and rod-like (Pl. I and Figs. 1, 3, 4 and 5), while its posterior portion is spread out into a broad quadrangular plate, the basilar part of the occipital region.

The anterior portion of the basal plate in Sus differs strikingly from that in other mammals on account of its narrow, rod-like shape and also because of the character of its union with the ear-capsules. Here it is separated from the ear-capsules except for a thin connecting lamella of cartilage, while in the other mammals that I have examined it is firmly united with the capsules. In the other mammals, also, the planum basale forms a broad plate of cartilage, which passes over without sharp demarcation into the broad basal cartilage of the orbitotemporal region. Tarsius offers an exception to this statement, since in it the portion between the cochlear spheres is even narrower than in Sus. This, however, is due to the extremely large size of the ear-capsules, which approach the middle line and compress the cartilaginous plate which lies between them.

Back of the auditory bullæ the planum basale spreads out into

a broad quadrangular plate, limited posteriorly by the foramen magnum, anteriorly by the *foramen jugulare* or *fissura metotica* and the auditory bullæ, while laterally it passes over into the *pars lateralis, regio occipitalis.* On the boundary between the basal plate and the

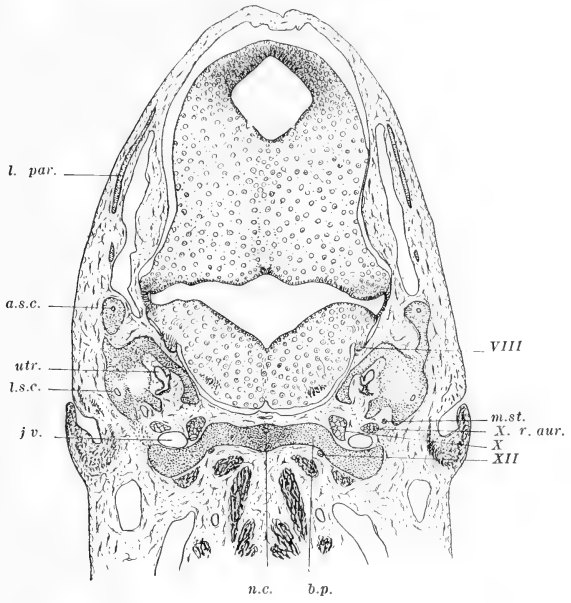

Fig. 1. Section showing the broad basal plate *b.p.*, the notochord *n.c.* near its dorsal surface, and the large auricular branch of the vagus nerve *X r.aur.* × 10.

VIII, nervus acusticus; *m.st.*, stapedial muscle; *j.v.*, jugular vein; *l.s.c.*, lateral semicircular canal; *utr.*, utriculus; *a.s.c.*, anterior semicircular canal; *l. par.*, lamina parietalis; *X*, nervus vagus; *XII*, nervus hypoglossus.

pars lateralis the *processus paroccipitalis* arises and extends forward. The basal plate is perforated just median to this process by the *foramen nervi hypoglossi* (Pl. I, *f. hyp.*). Three branches of the hypoglossus nerve have been demonstrated in a number of mammalian

embryos, but the anterior one disappears and the other two usually leave the cranial cavity through a single foramen, the foramen hypoglossi (foramen spinoöccipitale). Voit found that in Lepus embryos two foramina hypoglossi are present on each side, and these may persist even in the adult. Fischer described in an embryo of *Semnopithecus pruinosus* two hypoglossal foramina on the left side and three on the right. On the other hand, his reconstruction of *Semnopithecus maurus* shows but a single foramen on each side. This shows what variations may occur within the limits of a single genus or even species.

Back of the hypoglossal foramen the basal plate passes, without demarcation, into the lateral walls of the occipital region.

The union which occurs between the cochlear part of the auditory capsule and the basal plate is later destroyed by the absorption of the lamella of cartilage mentioned above. At this stage this lamella is perforated on each side by a foramen (Pls. I and II, *, and Fig. 4) filled with tissue resembling precartilaginous tissue (*Vorknorpel*). It is probably the homolog of the fissura basicochlearis posterior, which Noordenbos (1905) has described in embryos (14 mm.) of Talpa. However this may be, this lamina fails to ossify so that in the adult the foramen lacerum anterius and the foramen jugulare are united by a broad slit median to the auditory bulla.

In a Tarsius embryo of a somewhat later stage this lamina of cartilage is not present and the ear-capsules are separated from the planum basale for nearly their entire length by a large fissure, the *fissura basicochlearis*. In Talpa the large foramen piercing the basis cranii median to the trabecula alicochlearis is not the foramen lacerum anterius, as Fischer called it, but the fissura basicochlearis anterior (Noordenbos). It is located internally to the foramen caroticum. No nerves nor blood vessels pass through it, as was determined by examining the series of sections from which Fischer's reconstruction was made; it is filled only with connective tissue.

The foramen for the facial nerve, which in the reptiles pierces the basis cranii anteriorly and ventrally to the ear-capsule, has here migrated around to the antero-dorsal side of the cochlear portion of the ear and lost completely its connection with the basal plate. This will be considered in greater detail in connection with the otic region.

Looking now at the inside of the skull, it is seen that the base rises gradually and in an almost straight line from the foramen magnum to the dorsum ephippii. From side to side the curve on the inner wall of the brain-case is nearly uniform, thus closely simulating the half of a cylinder.

On each side of the anterior end of the basal plate there is a large triangular opening (Pl. I, *for. abduc.*). This is bounded anteriorly by the widely projecting *processus clinoideus posterior,* and posterolaterally by the cochlear portion of the ear-capsule. It is filled mostly with indifferent tissue, but serves also for the passage of the nervus abducens. This is especially noteworthy since such a foramen has not been described in any mammal. In the chondrocranium of Semnopitheeus Fischer found the nervus abducens passing through a groove, which was closed by a band of connective tissue, but no distinct foramen was present. A similar groove has also been described in some human embryos. At a later stage in Sus the lateral boundary of the foramen, *i. e.,* the rod of cartilage, connecting the lateral end of the processus clinoideus posterior with the front end of the ear-capsule, is absorbed, leaving a condition similar to that in the Semnopitheeus embryo. At this stage the processus clinoideus posterior is relatively much larger than in the adult. This foramen I do not hold to be the exact homolog of the foramen for the nervus abducens in the reptiles, although it is similarly located, but rather I am inclined to look upon it as a secondary formation which includes more than the original or true reptilian foramen abducens, and is formed by a secondary fusion of the processus clinoideus posterior with the cochlear capsule. The nervus abducens (Figs. 7 and 8) leaves the cranial cavity through the large foramen just mentioned, and, continuing forward, passes dorsal to the ala temporalis and through the *fissura orbitalis superior,* in company with the oculomotor and trochlear nerves and the first two branches of the trigeminal nerve.

The course of the notochord through the skull in Sus embryos has been described and figured by Parker and Kölliker. In his stage three (length 1 1/3 inch) Parker (1874, page 310) says that "the notochord has retired from the posterior clinoid wall and has been

buried in cartilage; it still lies, however, nearest the upper surface of the investing mass." And in his figure to which he refers (Pl. XXXVIII, Fig. 2) he indicates the notochord as following a nearly straight course which lies a little above the middle of the basal plate. Kölliker (1879, page 444, ff.) in his studies on a pig embryo 32 mm. long, noted accurately the course of the notochord through the cartilage, and also its enlargements, which he called the occipital and sphenoidal swellings.

Fig. 2. Sagittal section showing the course of the notochord through the base of the skull and its two connections with the pharynx. Line AB would represent the section from which Fig. 5 was taken.

In the specimen, from which my reconstruction was made, the notochord follows much the same course as it did in the Sus individuals which Kölliker studied. But, whereas in his the notochord was continuous throughout, in mine its course is broken near the middle of its passage through the basal plate, and each part leaves this and gains a connection with the dorsal wall of the pharynx (Fig. 2). The occipital and sphenoidal swellings are present, but are not as prominent as Kölliker figured them. Anteriorly the notochord leaves the basal cartilage and ends in the perichondrium lining the floor of the hypophysial fossa.

Regio Occipitalis.

The regio occipitalis forms a complete cartilaginous ring, the lateral parts being connected by a dorsal band of cartilage, the *tectum posterius* or *synoticum* (Pls. I-III, *tectum post.*). In the remainder of the skull a roofing over is present only in the anterior half of the regio ethmoidalis. In the central portion of the skull the roof of the brain-box is formed only by the membrane bones, and at no time is it covered over by the chondrocranium.

The basal portion of the regio occipitalis has already been described. It is broad and rectangular in shape and passes without demarcation into the pars lateralis. Near its lateral border and postero-internal to the foramen jugulare, it is perforated by the foramen nervi hypoglossi, already referred to. At this stage the foramen is filled mostly with connective tissue. The three ventral roots of the nervus hypoglossus, corresponding to the roots passing through the three foramina in Lacerta embryos, unite inside the cavum cranii and so pass through this foramen as a single nerve. No indication of the dorsal roots of this nerve could be found. These three roots are the nerves which were taken into the skull during the phylogenetic history when the three cervical vertebræ were added to the amphibian skull to complete the occipital segment of the amniotic skull. Before entering the brain each of these three roots splits up into a number of rootlets (4-6), as in man, which enter the brain singly.

The paroccipital processes, just lateral to the foramina jugulares and hypoglossi, are remarkably large even at this early stage, an indication of the great length they attain in the adult pig. Each is rounded and blunt and extends forward so as to hide the greater part of the foramen jugulare.

The occipital condyles are large, but as yet they are only slightly convex and scarcely project out from the general surface of the skull. They face downward, backward and outward and bridge over the boundary between the pars basalis and the pars lateralis. They are well separated, forming two distinct atlanto-occipital articular surfaces, although the capsule surrounding them is continuous from side to side in both the embryo and the adult.

The lateral and dorsal portions of the occipital region can be divided into two parts, a ventral third extending upwards as far as the dorsal border of the foramen magnum, and a dorsal two-thirds extending up over the brain and forming the tectum posterius. The ventral third forms a nearly flat rectangular plate (pars lateralis, regio occipitalis) with its outer face directed backward and outward. Posteriorly it forms the greater part of the lateral border of the foramen magnum; anteriorly it is separated from the auditory capsules throughout nearly its entire length by a slit, which probably represents merely a place where the capsule has not united with the rest of the cranium. The dorsal two-thirds, the tectum posterius, is shaped somewhat like a pair of saddle bags, curving nearly uniformly from side to side. Ventrally it is bounded by a membrane, the membrana atlanto-occipitalis dorsalis. The dorsal part, *i. e.,* the band connecting the saddle bags is narrower than the lateral portions and is placed perpendicularly, that is, with its outer face looking posteriorly. This is the usual position in the mammals. In man, owing to the great development of the brain, the tectum has been shunted backward and downward, so that its outer face looks ventralward. Its free dorsal border is nearly straight, but in the mid-dorsal line and above the tectum there is a free nodule of cartilage (Pls. I and III, *pr. asc. tect. post.*). This may possibly be the homolog of the *processus ascendens* of the tectum posterius of the reptiles. The lateral portions of the tectum are perforated by several small foramina, which serve for the passage of blood-vessels.

Anteriorly the tectum posterius has three connections: two with the postero-dorsal part of the auditory capsule, between which lies the *foramen petroso-occipitale,* and one, by which it is continued forward into the parietal plate.

Regio Otica.

In comparison with the simple occipital region, the otic region presents a very complex and highly differentiated portion of the chondrocranium. It extends from the posterior borders of the ear capsules, forward to the sella turcica, and includes the laminæ parietales, the ear-capsules and the anterior three-fifths of the basal

plate. The shape of the otic region is that of a tube with walls of varying thickness. Its internal surface is that of an elliptical cylinder, while externally its surface would correspond with that of a cylinder which is oval in cross-section, with the larger side ventral; the axes of the cylinder coincide and the major axes lie in the sagittal plane. The crescentic spaces situated between the two cylinders are occupied by the auditory capsules.

Dorsally the otic ring is incomplete, no part of the chondrocranium roofing over the brain except in the occipital region, as already stated. The lateral walls are formed by the fenestrated and nearly vertical *laminæ parietales* and the vestibular portions of the otic capsules. The basal portion of the otic region is formed by the planum basale and the ventro-internal portions of the auditory capsules. It might here be mentioned that in the animals below the mammals and birds the otic capsules take no part in the formation of the floor of the brain cavity but are located laterally and form a large portion of the sides of the brain-case. As Gaupp says, the large brain of the mammals has overgrown the capsules and crowded them more and more ventralward. This condition reaches its highest stage in man, where the ear-capsules lie almost wholly in the floor of the brain cavity, and the foramen magnum, and even the tectum posterius, have been shoved around to the ventral side. Lateral and ventral to the capsules are the cartilaginous forerunners of the ear-bones, together with the proximal ends of the mandibular, hyoid and branchial arches.

Dorsally the auditory capsules are bounded by the thin, slightly-curved lamina parietalis (Pl. III) which forms the greater part of the lateral wall of the brain-case in this region. Whereas throughout most of the head the chondrocranium is almost completely formed, in the region of the lamina parietalis it still consists largely of precartilaginous tissue, thus leaving several large foramina and fissures. Later these are reduced in size or closed completely. Especially noteworthy is the large fissure cutting into the lamina from above and behind (*fis. lam. par.*) It has been mentioned by several authors, but no special term has been applied to it, and so I propose the term *fissura laminæ parietalis.* It is even larger in the recon-

struction of another pig's head, the drawings of which I have had access to. In this head it cuts clear through the lamina, dividing it into two parts and uniting with the fenestra spheno-parietalis. Spöndli (1846) and Kölliker (1879) have indicated it plainly in their drawings of the pig embryo, where it lies just in front of their "squama occipitalis." In Decker's drawings (1883) of a pig embryo much older than mine, he has placed this fissure further back, and with the apex directed toward the posterior part of the ear-capsules. According to his conception, this fissure separates the lamina parietalis (cartilago parietalis) from the tectum posterius (squama occipitalis). In his drawings of the sheep embryo, the fissure is further forward and points in the same direction as it does in his Sus drawings. In Bos, according to his figure, it is absent. How far it is present in the other mammals, I am not able to say. It is probable that it occurs in most mammals during the formation of the lamina parietalis.

Anteriorly the lamina parietalis passes, without demarcation, into the broad *commissura orbito-parietalis* and thence into the *ala orbitalis*. The lamina parietalis with the commissura orbito-parietalis together constitute the homolog of the narrow reptilian tænia marginalis (Gaupp, 1900). This broad, plate-like condition may be secondarily developed in the mammals, or it may be an inheritance from their prereptilian ancestors; that is, it may have come from the solid side-wall of the amphibian skull. From his studies on the development of the skull of Echidna, Gaupp considers the broad plate-like form of this commissure a primitive condition.

Posteriorly the dorsal part of the lamina ends freely, while that below the fissura laminæ parietalis is continued into the tectum posterius. Ventrally it is connected with the anterior and posterior corners of the vestibular portions of the ear-capsules, while between these two connectives lies the large crescentic *foramen jugulare spurium*. In some forms (*cf.* Talpa) this serves for the passage of large veins going from the sinus transversus to the vena jugularis externa, but in the specimen of Sus from which my reconstruction was made only a very small vein passes through this foramen. The remainder of the foramen is filled with connective tissue. A similar

vein traverses the foramen petroso-occipitale. There is no indication of the presence of a processus opercularis, such as Fischer described in Talpa.

Posteriorly the auditory capsules are united with the lateral parts of the regio occipitalis by two small bridges of cartilage, between which lies a long narrow slit filled with precartilaginous tissue (*Vorknorpel*). This slit is probably the homolog of the dorsal part of the reptilian fissura metotica. Above the upper bridge lies the foramen petroso-occipitale, formed, as Fischer says (1901 b), by a bridge of cartilage dividing the original foramen jugulare spurium into two parts. In the reconstruction of a younger Sus embryo neither of the bridges forming the foramen petroso-occipitale are present, nor is the one which closes anteriorly the foramen jugulare spurium, so that the auditory capsules are entirely free from the side-walls of the cranium.

The *foramen jugulare* (Pls. I and III) is a large irregular opening posterior to the cochlear portion of the auditory capsule and ventral to the pars vestibularis. Externally it looks forward, downward and outward; internally, toward the foramen magnum. It serves for the exit of the glossopharyngeus-vagus group of nerves and the internal jugular vein. The paroccipital process extends forward and under the foramen, so that it is not seen when the skull is viewed directly from beneath. Laterally it has a large opening into the *fenestra cochlearis* (*rotunda*). Postero-dorsally the foramen is continued as a narrow slit, which is later closed as the vestibular portions of the ear-capsules fuse more firmly with the exoccipital elements. The thin lamina of cartilage lying between the cochlear part of the auditory capsule and the basal plate has already been described (p. 172). The auditory capsules are free anteriorly, with the exception of two narrow bars of cartilage, one connecting the upper part of the pars vestibularis with the lamina parietalis and the other going from the pars conchlearis to the outer end of the processus clinoideus posterior and forming the foramen above mentioned through which passes the nervus abducens. In front of the capsules lie the large *fenestræ spheno-parietales*. No trabecula alicochlearis, such as occurs in Lepus and Talpa, is present in Sus, nor

is there a special foramen for the carotid artery, which here enters the cavum cranii through the large slit between the processus clinoideus posterior and the processus alaris.

Auditory Capsules.

The position of the auditory capsules has already been described. Their demarcation from the rest of the cranium is sharp and distinct. The general shape of each capsule is that of an ovoid body, the long axis of which is obliquely placed in relation to the long axis of the skull. The smaller end is located anteriorly and is directed downward and inward; the larger end is situated posteriorly and points upward and outward.

The auditory capsules are divided into two parts, the *pars cochlearis,* containing the sacculus and the cochlea, and the *pars vestibularis,* in which the utriculus and semi-circular canals are located. The nearly spherical pars cochlearis (Pl. II) occupies the antero-median two-fifths, while the larger pars vestibularis (Pl. III), resembling a three-sided pyramid in shape, completes the postero-lateral three-fifths. The median faces of the two parts form together a surface, which is concave in a transverse direction and which conforms to the general internal surface of the cavum cranii.

The pars vestibularis presents an outer surface which is that of an equilateral triangle, one side of which is next to the pars cochlearis and the mandibular and hyoid arches, one next to the lamina parietalis (foramen jugulare spurium), while the third borders on the lateral parts of the regio occipitalis. This surface is arched in an antero-posterior direction, the convexity being outward. Its edges are formed by the ridges of the semi-circular canals (*prominentiæ semi-circularæ*), those of the posterior and lateral canals being the most prominent. That of the lateral canal takes part in the formation of the crista parotica and the processus perioticus superior (Pl. III). In the central area between the ridges are a number of small pits (8-10), some of which penetrate the wall and open into the fossa subarcuata. They persist throughout life and serve for the passage of small veins which carry blood to the sinus transversus. The ampullæ form no distinct swellings on the outside of the capsules, such as Fischer described in Talpa.

The median surface of the pars vestibularis is also triangular in shape. The surface is irregular, although in general character it is concave, and it is perforated by three foramina. The smallest of these is located in the postero-dorsal corner; it is long and narrow and opens into the posterior semi-circular canal. At this stage it is filled with connective tissue; later it disappears. On the postero-ventral side is another long slit-like aperture, the *foramen endolymphaticum,* or *aquæductus vestibuli* (Pl. I, *for. endolym.*). It runs parallel to the sinus utriculi superior, which lies beneath it within the capsule, and serves for the exit, into the cavum cranii, of the ductus endolymphaticus. In its position and shape the foramen is almost identical with the corresponding foramen in Talpa and Semnopithecus, although it is larger. In a Lacerta embryo it is oval. This oval shape is attained in older specimens of Sus and is due to the thickening of the walls of the capsule and to their surrounding more and more the oblique duct, thus constricting the opening and bringing the internal aperture further back. On the posterior face of the pars vestibularis, a short distance below and back of the foramen endolymphaticum, is a small oval pit which communicates internally with the fossa subarcuata. It is filled with precartilaginous tissue and is probably later closed with cartilage.

At about the middle of the median surface of the pars vestibularis lies the third and largest of the three cavities, the *fossa subarcuata* (*fos. subarc.*) or *floccularis.* It is roughly oval in shape and extends to the outer wall of the ear-capsule. Several foramina which perforate this wall (see above) place it in communication with the outside of the skull. Its posterior portion is deeper than the anterior and forms a pocket which extends between the sinus utriculi and the outer wall of the capsule and communicates with the small oval pit above mentioned. In the embryonic Talpa the fossa is very shallow, while in the adult it is deep. In the human embryo and also in the Lacerta embryos, it is likewise shallow. From this Fischer concluded (1901 b) that the deep pit is a secondary acquisition. Its presence and size seem to be dependent, to a certain degree, upon the development of the flocculus, since it is occupied by this; but still not entirely, since a shallow pit is present in

Lacerta, which has no flocculus. In man its depth varies with age—in the embryonic stages (Hertwig's model) it is only a shallow pit; in children from two to ten years of age, it is a deep pit, com-

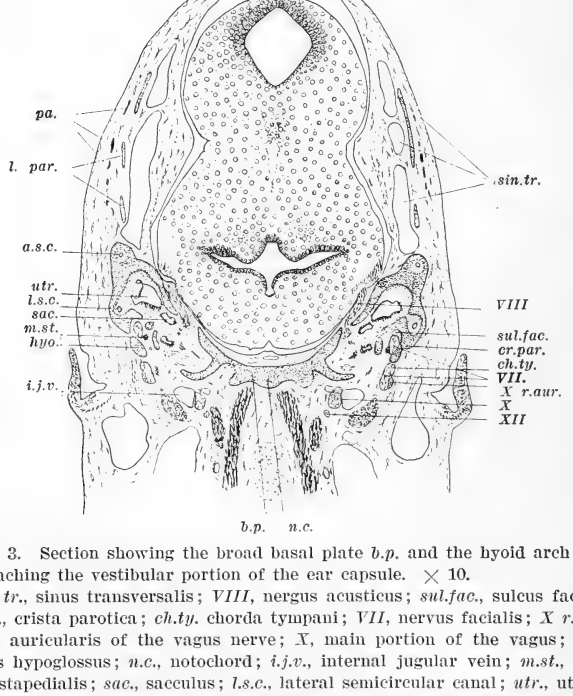

FIG. 3. Section showing the broad basal plate *b.p.* and the hyoid arch *hyo.* approaching the vestibular portion of the ear capsule. × 10.

sin. tr., sinus transversalis; *VIII*, nergus acusticus; *sul.fac.*, sulcus facilis; *cr.par.*, crista parotica; *ch.ty.* chorda tympani; *VII*, nervus facialis; *X r.aur.*, ramus auricularis of the vagus nerve; *X*, main portion of the vagus; *XII*, nervus hypoglossus; *n.c.*, notochord; *i.j.v.*, internal jugular vein; *m.st.*, musculus stapedialis; *sac.*, sacculus; *l.s.c.*, lateral semicircular canal; *utr.*, utriculus; *a.s.c.*, anterior semicircular canal; *l.par.*, lamina parietalis; *pa.*, anlagen of parietal bone.

parable to that in the adult dog, while in adults it becomes again only a shallow depression. Skulls of adult Lepus show a deep pit, as do also the embryos, and the same is true of Macacus and Semnopithecus. In the adults of Sus the pit is shallow. From the con-

flicting evidence at hand, *i. e.,* the shape and depth of the fossa, I do not think we are in a position to say which is the primary and which the secondary condition. Before this question can be determined, it will be necessary to study more closely the relations of the surrounding elements.

Externally on the antero-ventral face of the pars vestibularis is a deep groove, the *sulcus facialis,* the anterior two-thirds of which is occupied by the nervus facialis, while the stapedial muscle is located in its posterior half, the two running side by side for a short distance, with the muscle on the median side (Fig. 3). The latter, in cross-section, is about one-sixth the size of the nerve. The position and shape of the groove is similar to that in Lepus, Semnopithecus and human chondrocrania, but differs from that in Talpa, where its posterior portion is enlarged to form an oval pit which is placed more on the outer side of the lateral semi-circular canal. Its large size in Talpa is due not only to the large size of the stapedial muscle but also to the digastric muscle, which has its origin in this cavity. In Sus this latter muscle arises on the processus pareccipitalis. The lateral wall of this groove is formed by the *crista parotica* (Pl. III and Fig. 3, *cr. par.*), which is located on the antero-ventral face of the prominentia semi-circularis lateralis. At the middle of its length is attached, by connective tissue, the hyoid arch (Reichert's cartilage). To the middle of the crista the processus brevis of the incus is attached by a ligament. At the anterior end of the crista is a prominence which later develops into the *processus perioticus superior* (*tegmen tympani*) (Pl. III). There has been considerable confusion over the homologies of the processus perioticus superior and the crista parotica. Gaupp (1900) described and figured a process in a Lacerta embryo, which he called the crista parotica. This he homologized with the processus perioticus superior (tegmen tympani) of mammals, and Fischer (1901 b) supported him. It remained for van Kampen (1905, p. 345) to show that the processus perioticus superior is a new structure in the mammals, and that the processus facialis is the homolog of the reptilian crista parotica.

Antero-ventrally and somewhat internal to the pars vestibularis lies the balloon-shaped pars cochlearis (Pl. II and Fig. 4, *coch.*).

The two parts are separated externally by the fenestra vestibuli and the fenestra cochleæ (fenestræ ovalis et rotunda), internally by the foramen acusticum, and dorsally by the foramen nervi facialis. The median face of the pars cochlearis is flattened so that it conforms

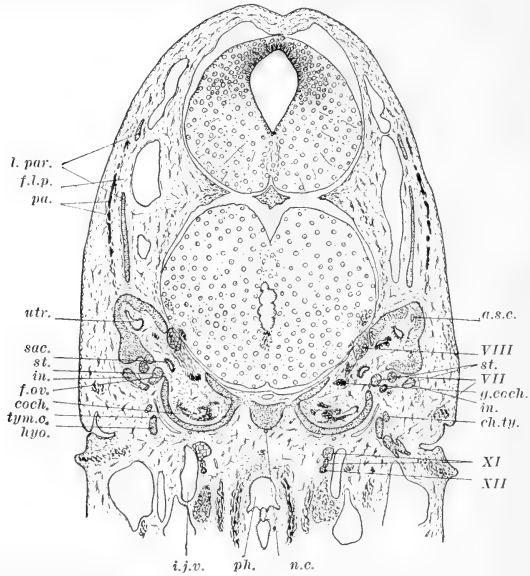

Fig. 4. Section showing the notochord *n.c.* beneath the basal plate and the thin lamella of cartilage between the latter and the cochlear portion of the ear. × 10.

a.s.c., anterior semicircular canal; *VIII*, nervus acusticus; *VII*, portions of nervus facialis; *g.coch.*, ganglion cochleare; *ch.ty.*, chorda tympani; *X*, nervus vagus; *XII*, nervus hypoglossus; *ph.*, pharynx; *i.j.v.*, internal jugular vein; *hyo.*, cornu hyale; *tym.c.*, tympanic cavity; *coch.*, cochlea; *f.ov.*, foramen ovale; *in.*, incus; *st.*, stapes; *sac.*, sacculus; *utr.*, utriculus; *pa.*, anlagen of parietal bone; *f.l.p.*, fissura laminæ parietalis; *l.par.*, lamina parietalis.

better to the inner surface of the cranium, while the rest of its surface is hemispherical in shape. Compared with Talpa, the pars cochlearis is relatively bigger and the pars vestibularis relatively smaller. In the latter form the cochlear portion occupies only about

a quarter of the bulk of the entire capsule. This condition corresponds with the great development of the cochlea in the adult pig (nearly four turns). The inner portion of the pars cochlearis is a single cavity, but it is provided with a ridge, (Fig. 5, *coch.*) largest next to the foramen acusticum, which runs spirally once around the inside. This represents the beginning of the cochlear formation.

Sound-Conducting Apparatus.

The ear-bones, or rather their cartilaginous forerunners, will now be considered, together with Meckel's cartilage and the hyoid arch, which are connected with the ear-bones at an early stage in their development. The ear-bones are located on the outer side of the pars cochlearis and in front of the pars vestibularis.

The Meckelian cartilages (Pls. III and IV, *Meck. cart.*) extend forward in the mandibles until they meet and form a firm mandibular symphysis. Throughout their whole length, except just before they unite, they are nearly circular in cross-section, although their diameters vary at different points. They do not continue in a direct course from the malleolar portion to the symphysis, but each is sigmoidally curved, the middle half sloping ventralward more sharply than the end portions. Each cartilage forms at its posterior end the irregular expansion which is later transformed into the malleus, while between this and the pars vestibularis is the triradiate incudal cartilage. Ventral to the mallear and incudal *Anlagen* is the posterior end of the slender rod-shaped hyoid arch. In Sus this portion of the arch (Reichert's cartilage) ends freely at this stage. Whether or not there is a connection between the crista parotica and Reichert's cartilage at an earlier stage, I cannot say. In the adult there is no indication of an osseous connection with the periotic; in connection with this the crista parotica is feebly developed, and the hyoid arch is supported secondarily by the auditory bulla, which surrounds and generally fuses with it. On the other hand, in most mammals the tympanohyal (proximal end of the hyoid arch) is continued directly into the crista parotica, which then forms a prominent ridge external to the sulcus facialis (*cf.* Cervus, Homo, Semnopithecus and Lepus). Anteriorly the *hyoid arches* (Pls. III and IV) extend

diagonally downward and forward, and, converging, end freely just dorsal to the anterior end of the corpus hyoideum. They do not unite with this till later. The same is true in Homo and Lepus embryos. From this circumstance Kölliker (Gaupp, 1905 b, p. 836) thinks

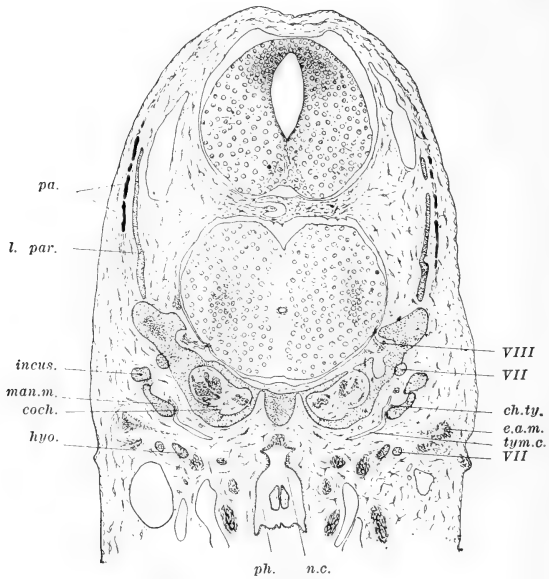

FIG. 5. Section showing the notochord leaving the pharynx. × 10.

VIII, nervus acusticus; *VII*, nervus facialis; *ch.ty.*, chorda tympani; *e.a.m.*, external auditory meatus; *tym.c.*, tympanic cavity; *ph.*, pharynx; *hyo.*, cornu hyale; *n.c.*, notochord; *coch.*, ridge which forms the inner part of the cochlea; *man. m.*, manubrium malleus; *l. par.*, lamina parietalis; *pa.*, anlagen of parietal bone.

that the hyoid arches (hyalia) take no part in the formation of the *corpus hyoideum*. The corpus hyoideum is broadly U-shaped with the opening anterior. This opening does not show well in the figure on account of the angle at which it was drawn. Attached at each side of the base of the U and curving diagonally outward and then

backward are the long *cornua branchialia I,* the homologs of the cornua majora in human anatomy.

The cartilaginous forerunners of the ear-bones, as said above, lie outside the general surface of the primordial cranium, the *malleus* and *incus* outside and the *stapes* within, its plate lying in the fenestra vestibuli. This opening into the inner ear, the fenestra ovalis of human anatomists, is here large and triangular, with one side horizontal and the opposite angle directed ventralward. It is only about one-third filled by the stapedial plate (Fig. 4, *st.*). Later the plate fits snugly, due, in part, to the growth of the stapes, and in part to the constriction in the size of the fenestra. In the adult the opening is so small that the plate of the stapes will not pass through it. The stapes is a thick oval ring of cartilage, with the long axis lying in an antero-posterior direction. Its median side is somewhat the heavier and is flattened to form the stapedial plate. Between the plate and the edge of the fenestra vestibuli is a layer of denser connective tissue, the *Anlage* of the ligamentum annulare stapedis (Fig. 4). There is no stapedial artery, although its earlier presence is indicated by the shape and arrangement of the cells within the stapedial foramen. The musculus stapedius (Fig. 3, *m. st.*) is inserted on the postero-lateral part of the stapedial ring; from thence it runs backward in the narrow sulcus facialis.

Immediately in front of the insertion of the musculus stapedius the stapes articulates with the crus longum incudis by a dense layer of tissue, the *Anlage* of the *processus lenticularis.* This is plainly a part of the incus, a further proof that the os lenticularis of the earlier anatomists in not a distinct bone. From its attachment with the stapes, the crus longus curves outward and upward to the corpus incudis. This is short and thick and extends forward a short distance to where it ends in a saddle-shaped articular surface, the two forwardly-projecting points of which are medianly and laterally placed. The crus breve incudis extends posteriorly from the corpus to the crista parotica, to which it is attached by the ligamentum incudis posterius.

The malleus articulates with the above-mentioned articular surface of the incus and is continued forward, without interruption, into

the long rod-like Meckelian cartilage. Malleus and Meckel's cartilage form, therefore, at this stage, one continuous piece of cartilage. On the posterior part or malleus proper, one can distinguish several processes, the most prominent of which is the *manubrium mallei* (Pls. III and IV, *man. malleus*), a long slender process, which curves downward and forward along the inner surface of the future tympanum. Where the forward curvature begins is a slight ventral enlargement, the external part of which forms the *processus lateralis,* while to the inner part is attached the musculus tensor tympani. Later this inner part forms a prominent process, the processus muscularis. The head of the malleus bears two other prominent processes, one of which extends dorsalward and the other backward. They are both short and rounded and form the two prominences of the saddle-shaped articular surface, by which the malleus articulates with the incus. There is as yet no indication of the *processus anterior* (*Folii*). This arises later in ontogeny as a membrane bone and then fuses with the malleus.

The size of the cartilages, which later form the ear-bones, is noteworthy. The length of the embryo (head-rump measurement), from which the reconstruction was made, was 30 mm. and that of the head 12 mm. and yet some of the parts of the ear-bones had reached 1/4 to 2/5 the size of these elements in the adult. The length of the incus from the upper part of the head to the end of the processus longus was 11/25 that of the adult, while the head of the malleus was 1/3 the adult size. At birth the ear-bones have reached practically their full size. This early attainment of their full size would indicate that they are derived from elements which reached a considerably larger size in their adult ancestors. This furnishes another point strengthening the hypothesis that the incus is the homolog of the quadrate and the malleus of the articular.

Nerve Foramina in the Region of the Ear-Capsules.

On the median side of the otic capsule, just in front of the point where the pars cochlearis and pars vestibularis unite, is a large shallow pit, the *meatus acusticus internus.* It is perforated by two openings, the posterior of which is several times as large as the other

and serves for the passage of the nervus acusticus (Pl. I). This opening is roughly dumb-bell-shaped, with its axis placed in a plane lying almost vertical. Its lower portion serves for the passage of the ramus cochlearis, nervus acusticus, while the ramus vestibularis passes through the upper portion. These nerves are distributed over the inner part of the ear-capsule and need not concern us further.

The anterior opening in the meatus acusticus internus is for the passage of the nervus facialis. Anteriorly it is bounded by a slender rod of cartilage (*r. for. fac.*), which extends from the anterior part of the pars vestibularis to the dorsal part of the pars cochlearis. This short stretch, the only place where the facial nerve is completely surrounded by cartilage, represents the canalis facialis and its opening is the *hiatus canalis facialis* of Vrolik. He believed (Vrolik, 1873) that the hiatus was the homolog of the external opening of the foramen facialis in the lower vertebrates. Directly in front of the foramen is the ganglion geniculi, which is continued forward by the nervus petrosus superficialis major; the main stem of the facial nerve curves outward and backward onto the outer side of the auditory capsule.

In Talpa, Fischer (1901 b) found a double bridge from the vestibular to the cochlear part of the auditory capsule, or, as he expressed it, a broad connection which is perforated by an opening through which passes the nervus petrosus superficialis major. He found the same condition also in one of Hertwig's models of the human ear-capsule. He rightly identified this opening as the hiatus canalis facialis, but from the evidence quoted he concluded that this condition is constant in the mammals (p. 504) and that the hiatus is only a perforation of the cartilaginous wall of the primitive facial canal. Van Kampen (1905, p. 387) supports him in this belief. I am inclined to uphold Vrolik's opinion and, therefore, look upon the cartilaginous bridge over the facial canal distal to the hiatus as being altogether secondary, a fact supported by its total absence in the crania of the Sauropsida and Amphibia and by its usual absence in mammalian chondrocrania. Where such a secondary bridge occurs in the embryo, we always find it well developed in the osseous skull of the adult. Hence, I believe that this cartilaginous bridge is merely

the forerunner of the later well-developed osseous roof, the tegmen tympani, a structure which occurs for the first time in the mammals.

Comparing the hiatus in Sus and in mammals generally with the foramen nervus facialis in the Sauropsida and Amphibia, we are struck with a great difference in two important points; in the first place, the hiatus in the mammals lies *in* the ear-capsule; and secondly, it is situated *within the cranial cavity* in the adult, so that the nervus petrosus runs for a short distance through the cranial cavity after it leaves the hiatus. Gaupp explains these two points in connection with the changes which the mammalian cranial cavity has undergone, as follows (1900, p. 509): "The intercapsular position of the foramen facialis is a result of the growth of the cochlea. In the amphibians it lies in the boundary between the ear-capsule and the solid basal plate; in the lizards the most anterior part of the capsule which contains the cochlea is ventral to this foramen; in the mammals we find this foramen on the dorsal edge of the ear-capsule; a portion of the capsule is not only ventral to, but is also in front of, the foramen. These changes are comprehensible, when one recognizes that in the mammals the ductus cochlearis has grown into that part of the skull which, in the lizards, forms the lateral part of the anterior half of the basal plate. One also notices a reduction in the size of the pars vestibularis. The bridge, therefore, which roofs over the first portion of the facial canal in the mammals, has its homolog in the lower vertebrates in the prefacial commissure. That one cannot speak of this foramen in the mammals as being basicapsular but intercapsular, is due to the fact that that part of the cranium which forms the cochlear part of the capsule in the mammals is, in the reptiles, an undivided part of the basal plate."

Let us come back now to the facial nerve. As stated above, immediately in front of the primary portion of the facial canal it gives off a branch, the nervus petrosus superficialis major (ramus palatinus) which passes out of the hiatus canalis facialis. The petrosus passes through the ganglion geniculi, which also covers a part of the main trunk of the facial nerve, then goes forward and downward, passing close under the processus alaris (Fig. 7, *n. p. s. m.*) and

then forward into the ganglion spheno-palatinum. From the hiatus, the facial nerve curves outward and downward and enters the sulcus facialis, which it follows backward through the tympanic cavity (Figs. 3 and 4), passing over the stapes and medianly to Reichert's cartilage.

Vrolik says (*cf.* van Kampen, p. 388, note) that the sulcus facialis forms a completely closed canal in Sus embryos. He must be mistaken in this, for in my reconstruction the sulcus is open for its full length, and even in the adult animal only the anterior half forms a closed channel. After crossing the proximal end of Reichert's cartilage, the facial nerve divides, the anterior branch going forward as the *chorda tympani,* while the main stem, the motor portion of the facial nerve, curves forward and follows the outer side of the cornu hyale to a point opposite the anterior end of the ear-capsule, where it divides into a number of small branches. The chorda tympani (Figs. 3, 4, 5 and 8,*ch. ty.*), after leaving the facial nerve, swings around to the lateral side of Reichert's cartilage, passes between the manubrium mallei and the crus longum incudis, and continues forward on the median side of Meckel's cartilage to where it joins the ramus lingualis, nervi trigemini, in the submaxillary ganglion. At this stage of the embryo, the tympanic cavity has not attained its final large size, so that the chorda tympani does not run through the cavity proper but through the adjacent tissues, which are later absorbed as the cavity enlarges.

In the postero-lateral wall of the pars cochlearis is a large opening, the fenestra cochleæ or rotunda (Pl. III). Posteriorly it communicates by a broad opening with the foramen jugulare, while laterally it opens into the space later occupied by the tympanic cavity. In its relations to the surrounding parts, it is almost identical with the fenestra cochleæ of Lacerta, if one considers that the anterior end of the fissura metotica (recessus scalæ tympani) has been closed.

Comparing the region around the tympanic cavity in the embryo with the same in the adult, we notice in the 30 mm. embryo a complete absence of all the parts later represented by membrane bones, *e. g.,* the processus anterior mallei, the squamosum and the osseous

bulla with its adjoining foramina (foramen Glaseri and foramen stylomastoideum); and an incomp'ete development of certain of the cartilage formations, namely, the tegmen tympani and the distal two-thirds of the canalis facialis. The connection of the malleus with Meckel's cartilage still persists in the embryo at this stage.

Regio Orbitotemporalis.

The orbitotemporal or sphenoidal region may conveniently be divided into the median unpaired basal, and the lateral paired, portions. The lateral portions are composed, on each side, of two wings, the posterior or ala temporalis, which ends freely, and the anterior or ala orbitalis, which is attached to the nasal capsule and the lamina parietalis.

This region in the embryo differs more from that in the adult skull than any other, with the exception of the cranial roof and the ear-bones.

In the hinder part of the basilar cartilage is the *hypophysial fossa*. This marks the anterior extent of the notochord. It is shallow posteriorly and deeper anteriorly, where it is roofed over for one-sixth of its length by the prominent backwardly-projecting tuberculum ephippii. At a later stage the anterior wall of the fossa slopes backward, the partial roofing over just mentioned not being present. But meanwhile the dorsum ephippii and the processus clinoideus posterior have grown forward and now roof it over *from behind* for fully one-half of its length.

The alar processes, to be fully described later, are narrow and extend directly outward from the anterior half of the floor of the hypophysial fossa.

From the anterior edge of the hypophysial fossa to the nasal septum the basilar cartilage continues in the same general direction as the basal plate. The basal cartilage is continued backward into the p'anum basale and forward into the nasal septum, with no sharp boundary setting it off from either. It may be said to extend from the dorsum sellæ to the posterior ends of the nasal capsules. Its breadth is nearly uniform, while its height gradually increases from a point in the region under the sella turcica, where it presents

almost the character of a horizontal septum, to its front end, where it passes insensibly into the hinder end of the nasal septum. In this it differs markedly from its homolog in Semnopithecus, Homo and Lepus, in which the height is nearly uniform. In Talpa the posterior end of the nasal septum rises almost perpendicularly above the floor of the cavum cranii, whereas in Sus the crista galli runs forward horizontally. The explanation for this difference lies in the marked ventral flexion, which the facial part of the skull in Sus has undergone in relation to the neural portion (Pl. III).

The basal portion of the regio orbitotemporalis, which lies in front of the sella turcica, is higher than wide and so forms a vertical plate, a true *interorbital septum.* Near its anterior end, where it joins the nasal septum, it is 2½ times as high as broad (Fig. 6).

In Semnopithecus and Macacus, Fischer (1903) also found an interorbital septum. This very primitive character of the mammals is also shared by the reptiles and birds, which possess this *tropibasic* or keeled type of skull. As Gaupp has plainly shown (1900), this keel was developed as a result of the increased size of the eyes forcing the lateral walls of the brain-case more and more together till only a vertical plate remained between the eyes. In the mammals this keel has been obscured by the subsequent development of other parts, namely: (1) the increase in the size of the brain, which presses upon it from above and from the rear; (2) the formation of the secondary palate and the backward prolongation of the narial tube (ductus nasopharyngeus), which thus limit its ventral extent; and (3) the backward shifting of the posterior wall of the nasal capsule in connection with the development of the ethmoturbinals, thus increasing the extent of the nasal septum at the expense of the interorbital septum.

The lateral parts or alæ of the orbitotemporal region are dissimilar in size, shape, direction and location. Posteriorly is the small single-rooted *ala temporalis,* extending directly outward from the side of the basal cartilage and lying ventral to the general wall of the cranial cavity. Anteriorly is the double-rooted *ala orbitalis,* extending diagonally outward and upward and expanding into a broad plate, which forms the antero-lateral wall of the brain cavity. The

ala temporalis and ala orbitalis are respectively the homologs of the ala magna and the ala parva in human anatomy. The latter names are often unsuitable when applied to the other mammals, since the size relation of the alæ is so frequently reversed, as is the case in Sus.

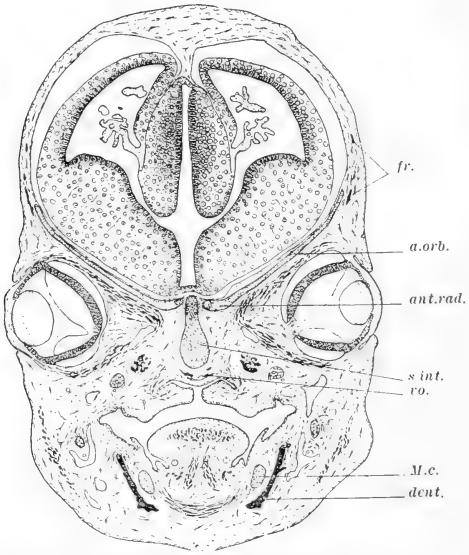

Fig. 6. Section just behind the nasal capsules, showing the interorbital septum *s. int.* × 10.

a.orb., ala orbitalis; *ant.rad.*, anterior radix of the ala orbitalis; *M.c.*, Meckel's cartilage; *dent.*, dentary bone; *fr.*, frontal; *vo.*, vomer.

The posterior wing or *ala temporalis* is divided into two parts, the proximal processus alaris and the more laterally situated ascending part or ala temporalis proper. The *processus alaris* (Fig. 7, *pr. al.*) extends directly outward from the basal cartilage opposite the anterior end of the sella turcica and serves to connect the ala temporalis with the base of the skull. In shape it is like a short triangular rod. The ascending part of the ala temporalis resembles

a small thick plate, triangular in shape, with the broadest side facing toward the rear and the opposite angle directed antero-ventrally; to its median corner the processus alaris is attached. Its antero-dorsal surface is hollowed out for the reception of the ganglion semilunare (Gasseri). Anteriorly the ala is bounded by the fissura orbitalis superior and posteriorly by the foramen lacerum. Through the former there pass the oculomotor, trochlear and abducens nerves, as well as the first two branches of the trigeminal. Through the foramen lacerum the third branch of the trigeminal nerve and the internal carotid artery pass. No foramina perforate either the processus alaris or the ascending part of the ala temporalis, nor are there any notches in their borders for the passage of blood-vessels or nerves. Contrasting the ala temporalis of Sus with that of some other mammals, many differences are noted. In the first place, in Talpa the processus alaris seems more like a broadened-out portion of the basal cartilage than like a rod, as in Homo, Echidna and Sus, in which forms it is well differentiated from the basal cartilage. In Sus the ala temporalis is small, a character which may be due, in part, to the young stage from which my reconstruction was made. The absence of foramina or fissures in its border has been noted above. In Talpa there is a broad connection with the otic capsule through the tænia alicochlearis, while in Sus there is no indication of such a rod.

In earlier stages of some mammals (Homo, Felis, Canis, and Ursus *cf.* Winzca, 1896) the ascending part of the ala temporalis is formed first as a separate piece of cartilage and then later unites with the processus alaris. No indication of such a separate formation has yet been described in Sus and yet I venture to predict that such a condition will be found, since in my series of sections the middle of each processus alaris is constituted of less dense cartilage than the alæ or the median rod (Fig. 7, *pr. al.*), indicating that it may have been formed subsequently to these.

Gaupp holds (1902) that the cavum cranii in the mammals is not the equivalent of that in the reptiles, but that it has been increased by the addition on each side of an accessory cavity (*Nebenraum*), the *cavum epiptericum.* This additional cavity in the mam-

mals lies, in the reptiles, on the outside of the brain-case above the processus basipterygoideus (ala temporalis). A large number of facts support this view, among which I will only briefly refer to

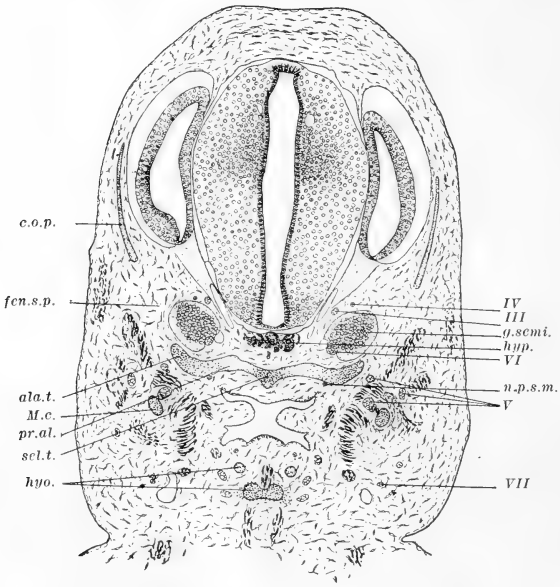

Fig. 7. Section through the region of the ala temporalis. × 10.

III, nervus oculomotorius; *IV*, nervus trochlearis; *g.semi*, ganglion semilunare; *hyp.*, hypophysis; *VI*, nervus abducens; *n.p.s.m.*, nervus petrosus superficialis major; *V*, branches of trigeminal nerve, together with the chorda tympani; *VII*, nervus facialis; *hyo.*, corpus hyoideus and cornu hyale; *M. c.*, Meckel's cartilage; *sel.t.*, sella turcica; *pr.al.*, processus alaris; *ala t.*, ala temporalis; *fen. s. p.*, fenestra spheno-parietalis; *c.o.p.*, commissura orbito-parietalis.

the following: (1) the long course within the mammalian brain cavity of the oculomotor, trochlear, abducens and first two branches of the trigeminal nerves, which perforate the dura mater at approximately the same points as those at which they leave the cranial

cavity in the reptiles (Fig. 7); (2) the location of the ganglion semilunare (Gasseri) outside the cranial cavity in the reptiles and within it in the mammals; (3) the formation of a part of the side wall of the brain-case (ala temporalis) in the chondrocrania of the mammals at some distance outside the general surface of the brain-box; (4) the vestiges of the primitive side wall which are still present in the mammals; (5) the homology of the ala temporalis with the reptilian processus basipterygoideus. For a full discussion of these points I would refer to Gaupp's papers of 1900 and 1902. Sus offers another vestige of the primitive side wall which is not mentioned by Gaupp, namely the connective between the front end of the ear-capsule (pars cochlearis) and the lateral end of the processus clinoideus posterior, forming the secondary foramen for the nervus abducens. Among the vestiges which he mentions and which are not present in Sus are the *tænia interclinoidea,* often present in the primates, and the *tænia clino-orbitalis* of Echidna. The separate pterygoid cartilage pieces which have been described in Talpa, Lepus, Tarsius and the apes, and which Parker mentions in an older stage of Sus (external pterygoid plates), are here only in the precartilaginous condition.

The character of the *ala orbitalis,* or anterior wing of the orbito-temporal region, is that of the generalized mammalian type. The two roots, which are pressed close together in Talpa, are here well separated by the large foramen opticum, and, therefore, the two roots can rightly be called *tænia proöptica* and *tænia metaptica.* Between the two optic foramina the median basal cartilage shows a shallow transverse furrow, the *sulcus chiasmatis.* A small process on the ventral side of the posterior root serves for the attachment of some of the eye muscles. The two roots soon join outwardly enclosing the optic foramen and, expanding, form the ala orbitalis (Frontalplatte of Spöndli), the homolog of the reptilian *planum supraseptale.* This unites anteriorly with the nasal capsule and posteriorly with the lamina parietalis. The latter connection, the *commissura orbito-parietalis,* is broad (Pl. III) and similar to the corresponding commissure in Echidna. This is, in all probability, a primitive condition, showing an approach to the solid side wall of the ancestors of

the mammals. The highly fenestrated character of the side wall in the reptilian chondrocrania is undoubtedly a secondary acquisition,

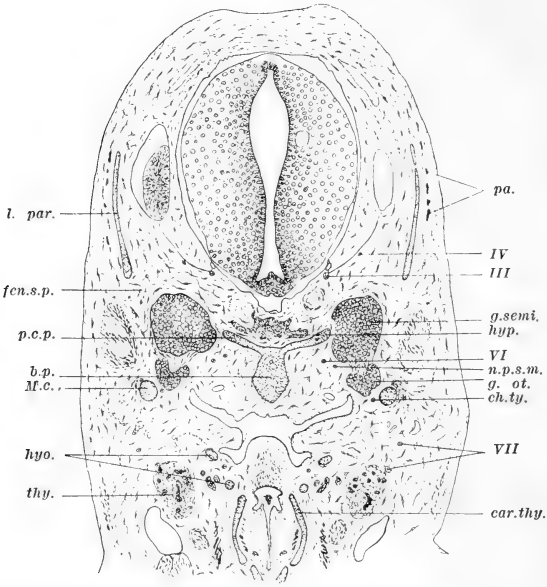

Fig. 8. Section through the anterior portion of the otic region just in front of the ear-capsules, showing a vestige of the primitive side wall of the brain, the processus clinoideus posterior *p.c.p.*, and the deep narrow anterior portion of the basal plate *b.p.* The outer ends of the processi clinoidei posteriores are connected with the ear-capsules. × 10.

IV, nervus trochlearis; *III*, nervus oculomotorius; *g.semi.*, ganglion semilunare (Gasseri); *hyp.*, hypophysis; *VI.*, nervus abducens; *n.p.s.m.*, nervus petrosus superficialis major (palatine branch of the facial); *g.ot.*, ganglion oticum; *ch.ty.*, chorda tympani; *VII*, nervus facialis, motor branches; *car.thy.*, thyroid cartilage; *thy.*, thyroid gland; *hyo.*, hyoid arches, cornu hyale above and cornu branchiale I below; *M. c.*, Meckel's cartilage; *fen.s.p.*, fenestra spheno-parietalis; *l.par.*, lamina parietalis; *pa.*, anlagen of parietal bone.

and not at all in the line of mammalian evolution. In Lepus and Talpa this commissure is only a narrow bridge of cartilage, while in

the primates (Macacus, Semnopithecus and Homo) it is merely indicated by a posteriorly-projecting point on the outer part of the ala. Tarsius shows a stage intermediate between that of the primates and of the other mammals, in that this posterior process is in the form of a rod which extends almost to the ear-capsule. It forms the dorsal border of the foramen spheno-parietale, the largest opening in the side wall of the primordial cranium.

The *commissura orbitonasalis* (cartilago spheno-ethmoidalis, or commissura spheno-frontalis of Spöndli) is a narrow bar connecting the ala orbitalis with the dorso-lateral portion of the nasal capsule, and enclosing beneath it and between the ala and the capsule the large oval *fissura orbitonasalis*. This fissure, which is at this stage filled mainly with undifferentiated tissue, is later nearly closed by the backward migration of the nasal capsule and the forward growth of the ala orbitalis, leaving only a small opening for the passage of the nervus ethmoidalis.

Regio Ethmoidalis.

The nasal region of the embryonic skull approaches more closely in form the corresponding region in the adult skull than does any other portion of the chondrocranium. The chief difference is in the excessive lengthening in the face of the adult, a condition developed mainly after birth. The shape of the nasal capsules as a whole is that of two closely appressed cylinders, whose diameters are greater in their middle portions than toward their ends, where they taper almost to points. An oblique slice has been removed from their postero-dorsal portions along the plane where the cribriform plate is later developed. The total length of the nasal capsules in Sus embryos at this stage is equal to 1/3 that of the entire skull, in Talpa embryos it equals 2/5, while in those of Semnopithecus it is only equal to about 1/5 of the cranial length. In this character the Sus embryo occupies an intermediate position, showing neither excessive lengthening nor shortening.

Let us turn our attention now to some of the details in the different parts of the nasal capsules.

Along the mid-dorsal line, in front of the foramina cribrosa and

on the roof of the capsules, there extends a well developed furrow, the *sulcus supraseptalis* (Fig. 9). In many animals with a freely movable snout (Erinaceus, Nasua, Talpa) this furrow lodges the ligamentum suspensorium (Spurgat), which extends forward from the region at the anterior end of the suture between the nasal bones. I can find no mention in the literature of such a ligament having been found in Sus, either in adults or in embryos, but my series

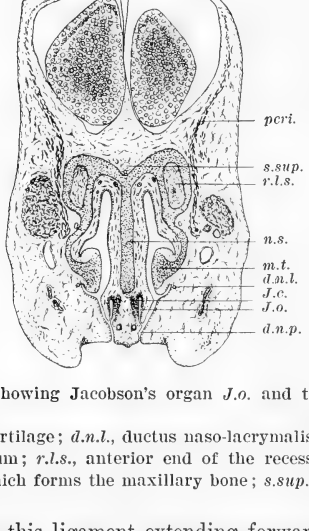

Fig. 9. Section showing Jacobson's organ *J.o.* and the naso-palatine duct *d.n.p.* × 10.

J.c., Jacobson's cartilage; *d.n.l.*, ductus naso-lacrymalis; *m.t.*, maxilloturbinal; *n.s.*, nasal septum; *r.l.s.*, anterior end of the recessus laterale superior; *peri.*, periosteum which forms the maxillary bone; *s.sup.*, sulcus supraseptalis.

of sections shows this ligament extending forward from the region, where the anterior ends of the nasal bones are later located to the point where the prenasal bone is developed. Throughout its whole length the sulcus supraseptalis lodges a blood-vessel deep in its furrow. Anteriorly this vessel lies beneath the ligamentum suspensorium. In some embryos (Macacus, Semnopithecus, Tarsius, Talpa) the sulcus is only present in the anterior part of the tectum nasi, while in others (Homo, Lepus, Bos, Sus) it extends throughout

its whole length. Whether this difference is due to the difference in the stages of development of the nasal capsules in the several embryos or to more fundamental causes, I am, at present, not able to say.

The nasal septum, as stated above, forms the anterior continuation of the interorbital septum or basal cartilage of the orbitotemporal region, the one passing gradually into the other. The nasal septum undergoes no sudden increase in height nor diminution in width immediately in front of the basilar cartilage of the orbitotemporal region, a feature so striking in Talpa, but present also, although to a less degree, in Lepus, Homo and Semnopithecus. With the exception of its anterior quarter, it is thickest near its lower border (Fig. 6). Its greatest height is in the middle region (Fig. 9) opposite the anterior ends of the fenestræ cribrosæ, from which place it tapers both anteriorly and posteriorly. Posterior to this highest point its dorsal edge forms the crista galli, while anteriorly it splits, the two dorsal parts then curving upward and outward to form the tectum nasi. The extreme tip of the chondrocranium is formed by the two capsules which project a short distance in front of the septum.

In the anterior part of the nose, a short distance within the nasal opening there is a thin place in the septum. This was noticed by Parker in Sus embryos, while Fischer found that in Talpa no cartilage was formed at this point, but that the two chambers were connected by an opening in the cartilaginous septum filled only with connective tissue. Gaupp found a similar *fenestra septi* in Echidna. Spurgat (1896) considers this one of the adaptations in connection with the flexible snout, the absorption of a part of the cartilage a short distance back from the end of the nose leaving the distal portion more movable. In Erinaceus, Nasua, Lutra and Canis, there is also, to a greater or less extent, an absorption of the zone of cartilage lying between the anterior ends of the membrane bones (nasals and premaxillaries) and the tip of the nose.

The *processus maxillaris posterior,* which in a number of forms, Decker wrongly named processus uncinatus, is also present here. It is probably the homolog of the processus maxillaris posterior of

the reptiles, as Gaupp has suggested (1905 b). On the side of the nasal capsule, above and posterior to this process, is a large shallow pit hollowed out for the large eyes (Pl. III).

The *paries nasi,* in the narrower sense, includes, in the mammals, only that portion of the side wall of the nasal capsule which is anterior to the processus maxillaris posterior, since in the reptiles the portion back of this process forms the planum antorbitale. The

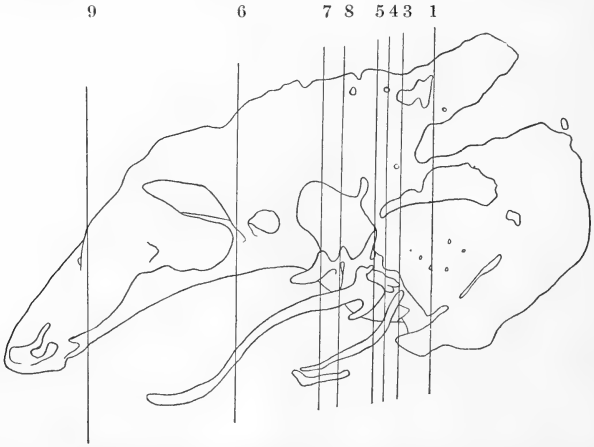

Fig. 10. Diagram to show the location in the head of the sections from which the various text figures were taken. The numerals refer to the corresponding figures.

posterior third of the paries nasi is swollen on account of the large size of the recessus lateralis (see below). The anterior two-thirds is much flattened and where it joins the posterior third there is a large shallow pit. At the upper portion of this pit there is, on each side, a small foramen, the *foramen epiphaniale* (Gaupp), for a blood-vessel, and the ramus lateralis nasi of the nervus ethmoidalis.

At the dorso-lateral part of the nasal capsule is the commissura spheno-ethmoidalis, which connects it with the ala orbitalis. In the reptiles a line joining the corresponding place with the processus

maxillaris posterior would separate the paries nasi from the planum antorbitale. The same is true also of the mammals, although here the planum antorbitale is usually oblique instead of transverse. The change to the mammalian condition has probably been brought about in the following manner:

In the streptostylic reptiles the posterior part of the nasal capsule is free from the septum. In the evolution into the nasal capsule of the mammals, the posterior part of the capsule of the reptiles has been expanded by the backward rotation of the posterior wall (reptilian planum antorbitale), the pivot being the more solid lateral side. This will be made more evident upon reference to Fig. 11. This explains how a part of the interorbital septum in the reptiles has been converted into the posterior portion of the nasal septum in the mammals, and also how the ethmoturbinal region of the latter has been derived.

The *solum nasi* is as yet incomplete. The anterior connection between the septum and the side wall by the *lamina transversalis anterior* is narrow and the posterior part is quite free. The *lamina transversalis posterior* is formed by an inrolling of the latero-ventral part of the planum antorbitale. At this stage the infolded ventral border forms a narrow horizontal shelf extending along the posterior quarter of the capsule and is entirely free from the septum; moreover, it has not yet been turned upward to form the *paraseptal lamella.* From the lamina transversalis anterior there extends backward the great *fenestra basalis,* bounded medianly by the lower border of the septum and laterally by the lower border of the side wall. In connection with the lamina transversalis anterior, a strip of precartilaginous tissue runs backward median to Jacobson's organ, but it ends freely before reaching the lamina transversalis posterior. This is the *Anlage* of the *cartilago paraseptalis.* Later this whole region is enclosed by membrane bones (maxilla, palatine, vomer) so that it then lies within the nasal cavity.

Forward, in the region of the external narial opening, the conditions are more complex. The aperture of the nasal capsule (fenestra narina, Gaupp), which is destined for the *apertura nasalis externa,* looks ventrally and not forward or laterally as in many other mam-

mals. Ventrally, on each side, the septum is produced outward into a thick mass of cartilage, the *processus lateralis anterior* (Fischer), which forms the median border of the fenestra. Anterodorsally it thins out and, curving around the nasal opening, becomes continuous with the tectum. Posteriorly it goes over into the lamina transversalis anterior, from which there projects backward a short process, the *processus paraseptalis* (Pls. II and III, *proc. parasep.*). The precartilaginous tissue above mentioned, which lies in the fenestra basalis, is connected with the end of this process. Opposite

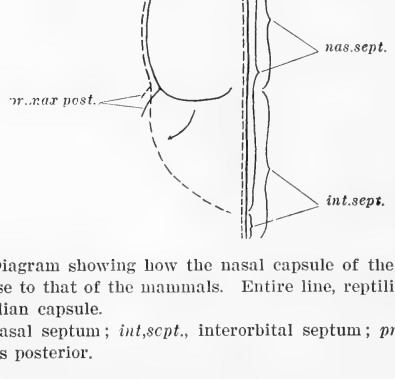

Fig. 11. Diagram showing how the nasal capsule of the reptiles has probably given rise to that of the mammals. Entire line, reptilian capsule; dotted line, mammalian capsule.

nas.sept., nasal septum; *int.sept.*, interorbital septum; *pr.max.post.*, processus maxillaris posterior.

to the point where the processus lateralis anterior becomes separated from the septum, the former is produced into a short ventrally-directed process which abuts against the anterior end of the premaxilla. The lamina transversalis anterior, the small band which bounds the fenestra narina behind, forms the only ventral connection between the paries nasi and the septum. Immediately in front of the lateral end of this band is a slender process, the *cartilago accessorius* (Fischer). It arises from the ventral border of the paries nasi, extends diagonally outward, downward and forward and ends in a bulbous expansion lateral to the outer angle of the processus

lateralis anterior. This process divides the fenestra narina into two parts, a larger anterior (destined for the external narial aperture of the adult) and a smaller posterior, through which the ductus naso-lacrymalis passes to open into the nasal chamber. The former opening is produced backwards by a slender notch in the side of the nasal capsule just in front of the origin of the cartilago lateralis.

The *fenestræ cribrosæ* are, as yet, simple triangular openings, one on each side of the posterior half of the nasal septum, which, dividing them, forms the crista galli. There is no indication of the cartilaginous bars which later divide the fenestræ and form the cribriform plate (lamina cribrosa). Each fenestra is in the form of an isosceles triangle, of which one side is only about half as long as the other two. The sharp corner is directed posteriorly, while the short side, which is bowed outward, is anterior and lateral. Each fenestra is nearly horizontal; the lateral angle is raised slightly above the level of the other two. Attached at this angle is the commissura spheno-ethmoidalis, which connects the capsule with the ala orbitalis.

The nasal cavity can be divided conveniently into a *pars posterior* or *subcerebralis,* lying ventrally to the fenestræ cribrosæ and a *pars anterior.* The maxilloturbinal, the homolog of the reptilian concha, is formed by the inrolled lower margin of the paries nasi. It lies in the floor of the recessus or pars anterior, and extends from the lamina transversalis anterior to the ductus nasopalatinus. Medianly it presents a flat surface, but as yet there is no splitting to form the double-rolled turbinal of the adult. The nasoturbinal is located internal to the pit mentioned above, (p. 202) which lies in front of the bulging recessus lateralis. Internally it presents a low vertical lamella and a forwardly projecting ridge, which together form a —(shaped turbinal. This seems to be the typical mammalian shape.

The pars posterior lies almost entirely ventral to the anterior part of the cerebrum and beneath the large fenestra cribrosa. Voit, in a forthcoming work on Lepus, divides its cavity into a *recessus lateralis* and a *recessus posterior* or *ethmoturbinalis,* the two being separated by a vertical plate (Pl. I, *ethmoturb.*) This is the first

of the ethmoturbinals to be developed and is the only one present at this embryonic stage. It springs from the lateral wall of the cavity a short distance behind the outer angle of the fenestra cribrosa and extends diagonally forward and inward halfway to the nasal septum. The recessus posterior thus forms a space shaped like the half of a cone with the base against the ethmoturbinal process, the flat side along the septum, and the apex in the posterior end of the nasal capsule.

The recessus laterale is divided by a horizontal shelf into a *recessus laterale superior* and a *recessus laterale inferior* (Voit). It is the large size of these that causes the prominent swelling on the side of the nasal capsule. The recessus laterale superior is oval in shape and lies in the highest part of the capsule. The recessus laterale inferior lies in the lower part of the capsule, posterior to the lower arm of the nasoturbinal. Posteriorly it is continued beneath the recessus posterior into a narrow cavity, the *sinus maxillaris* (Voit), which lies median to the processus maxillaris posterior. Of the finely developed ethmoturbinal system of the adult, there is as yet no indication.

But little has been said in this paper about the membrane bones. At this stage they are relatively unimportant. The larger ones, the premaxilla, maxilla, vomer, palatine, frontal, parietal and dentary, have already started to form, while the smaller ones, the nasal, lacrymal, jugal squamosal, tympanic and goniale, are as yet unossified.

Conclusions.

The principal results obtained from this study of the chondrocranium of the pig may be summarized briefly as follows:

The planum basale is broad and plate-like posteriorly, while in its anterior half it has been compressed from side to side by the large cochlear portions of the ear-capsules.

The occipital condyles are of the typical double mammalian type. The two synovial sacs surrounding the condyles are separate in most mammals, but in Sus they unite across the median line.

Instead of lying above the basal plate, as in Echidna, or passing continuously through this, as in most mammals, the notochord, near

the middle of its passage through the skull, dips beneath the plate and is connected with the dorsal wall of the pharynx in two places.

The cartilages, which will later form the ear-bones, are of the type common to the mammals at this stage of development. This region of the skull is very similar to the corresponding region in some of the streptostylic reptiles, or those with a movable quadrate.

A foramen nervus abducens is present. It is in the same position as the similarly named foramen in the reptiles, but the two are probably not homologous, that in Sus being formed by the secondary union of the processus clinoideus posterior with the ear-capsule.

The middle portion of the processus alaris is of less dense cartilage than the basal cartilage or the ascending part of the ala temporalis, indicating that the latter was probably formed independently in the pig, and had later united with the base of the skull.

Both roots of the ala orbitalis unite directly with the basal cartilage. They are well separated, leaving between them a large foramen through which passes only the optic nerve.

The evidence supports Gaupp's conclusion that the cranial cavities in the reptiles and mammals are not strictly homologous, but that the cavity in the mammals is larger morphologically than that of the reptiles; that it has been increased by the addition of the reptilian cavum epiptericum. Vestiges of the primitive side wall of the mammalian cranium are found in various forms. One vestige that Sus offers, which has not been described in any other form, is the rod of cartilage connecting the posterior clinoid process with the cochlear portion of the ear-capsule.

The commissura orbito-parietalis is very broad in Sus, resembling that in Echidna. This is probably a very primitive character of the mammals.

A true interorbital septum is present, supporting Gaupp's conclusion that the mammals belong to that group of vertebrates having a tropibasic or keeled type of skull. This allies them with the reptiles and separates them from the amphibians.

The nasal region is neither greatly lengthened nor shortened. The nasal cavities and the turbinals are of a very simple type which can be derived, without much difficulty, from that of the reptiles.

Taken as a whole, the chondrocranium of the pig is that of a generalized mammalian type. It shows certain specialized characters such as the narrowed anterior portion of the basal plate, the large size of the ear-capsules, and the secondary foramen nervus abducens, but these are less striking than the secondary characters of Echidna, Talpa, Lepus, or the primates.

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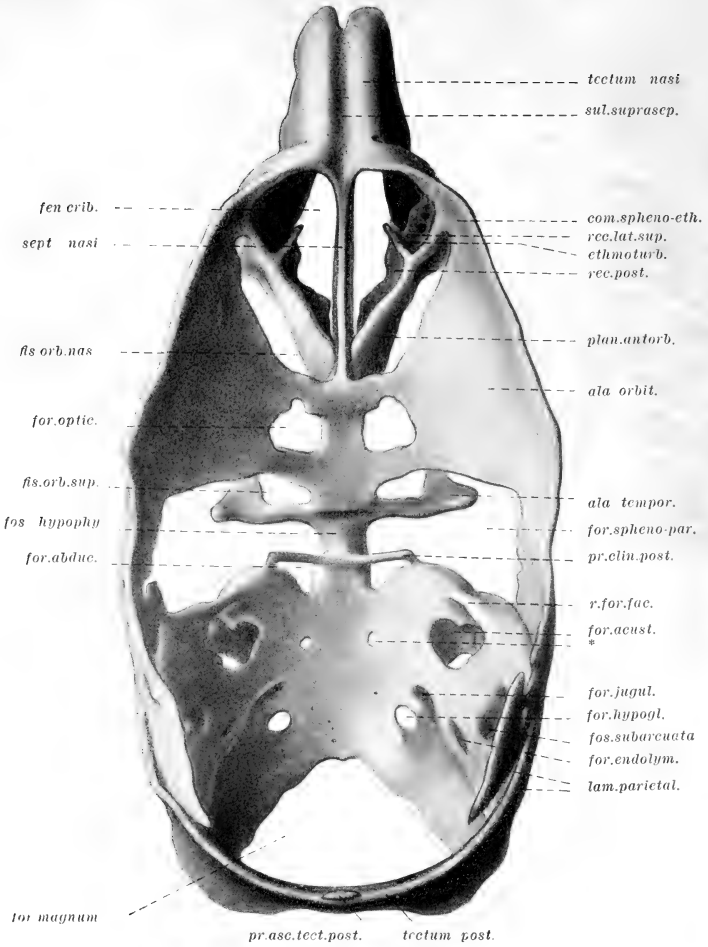
EXPLANATION OF PLATES.

Wax plate reconstruction of the chondrocranium of a pig, × 25. Length of head 12 mm., total length (head-rump measurement) 30 mm. All the plates are reduced to ½ the size of the reconstruction.

Plate I. Dorsal view of the chondrocranium. *sul.suprasep.*, sulcus supraseptalis *com.spheno-eth.*, commissura spheno-ethmoidalis; *rec.lat.sup.*, recessus lateralis superior; *rec.post.*, recessus posterior; *pr.clin.post.*, processus clinoideus posterior; *r.for.fac.*, roof over the facial nerve; *, opening in the thin lamella of cartilage between the basal plate and the auditory capsules; *fis.orb.sup.*, fissura orbitalis superior; *fis.orb.nas.*, fissura orbito-nasalis; *fen.crib.*, fenestra cribrosæ.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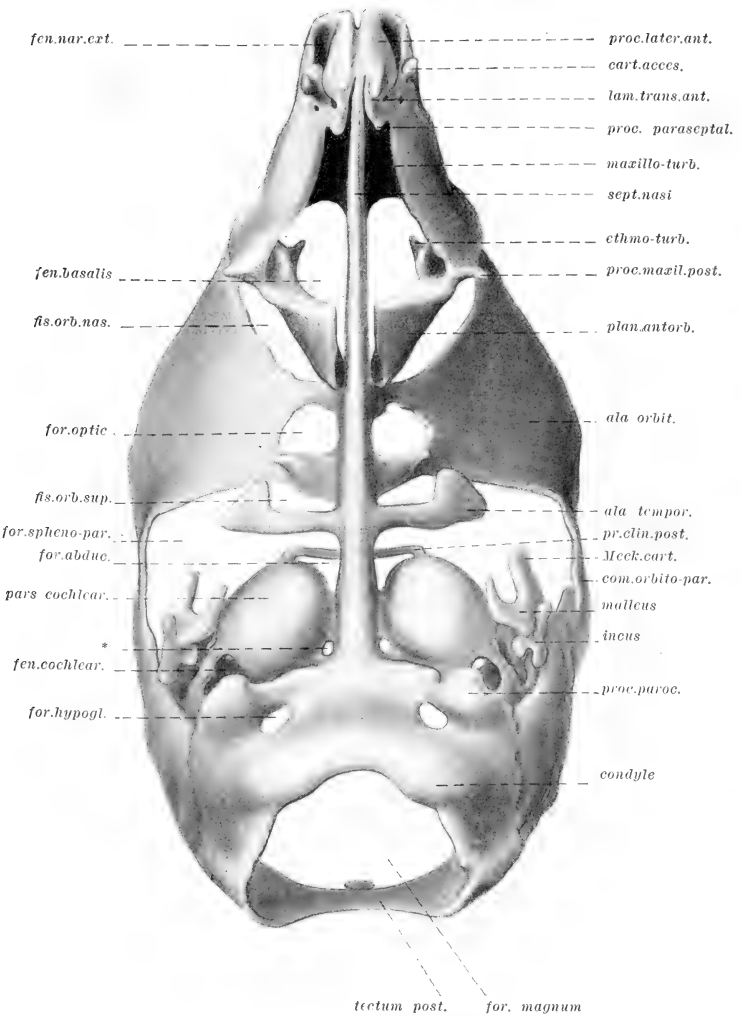
plan.antorb.
ala orbit.
for.optic.
fis.orb.sup.
ala tempor.
fos hypophy
for.spheno-par.
for.abduc.
pr.clin.post.
r.for.fac.
for.acust.
*
for.jugul.
for.hypogl.
fos.subarcuata
for.endolym.
lam.parietal.
for magnum
pr.asc.tect.post.
tectum post.

Plate II. Ventral view of the chondrocranium. The cornua hyalia and Meckel's cartilage have been removed. *proc.later.ant.*, processus lateralis anterior; *cart.acces.*, cartilago accessorius; *lam.trans.ant.*, lamina transversalis anterior; *pr.clin.post.*, processus clinoideus posterior; *proc.paroc.*, processus paroccipiltalis; *, opening in the thin lamella of cartilage between the basal plate and the auditory capsule; *fis.orb.nas.*, fissura orbito-nasalis.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proc.later.ant.
cart.acces.
lam.trans.ant.
proc. parasceptal.
maxillo-turb.
sept.nasi
ethmo-turb.
fen.basalis
proc.maxil.post.
fis.orb.nas.
plan.antorb.
for.optic
ala orbit.
fis.orb.sup.
ala tempor.
for.spheno-par.
pr.clin.post.
for.abduc.
Meck.cart.
com.orbito-par.
pars cochlear.
malleus
incus
*
fen.cochlear.
proc.paroc.
for.hypogl.
condyle
tectum post.
for. magnum

Plate III. Side view of the chondrocranium. *for. epiphan.*, foramen epiphaniale; *fis.lam.par.*, fissura laminæ parietalis; *par.asc.tect.post.*, processus ascendens, tectum posterius; *for.jug.spur.*, foramen jugulare spurium; *for.petro. occ.*, foramen petroso-occipitale; *proc.paroc.*, processus paroccipitalis; *man. malleus*, manubrium malleus; *proc.parasep.*, processus paraseptalis; *lam. trans.ant.*, lamina transversalis anterior; *cart.access.*, cartilago accessorius; *fen.nar.ext.*, fenestra narina externa.

CHARLES SEARING MEAD.

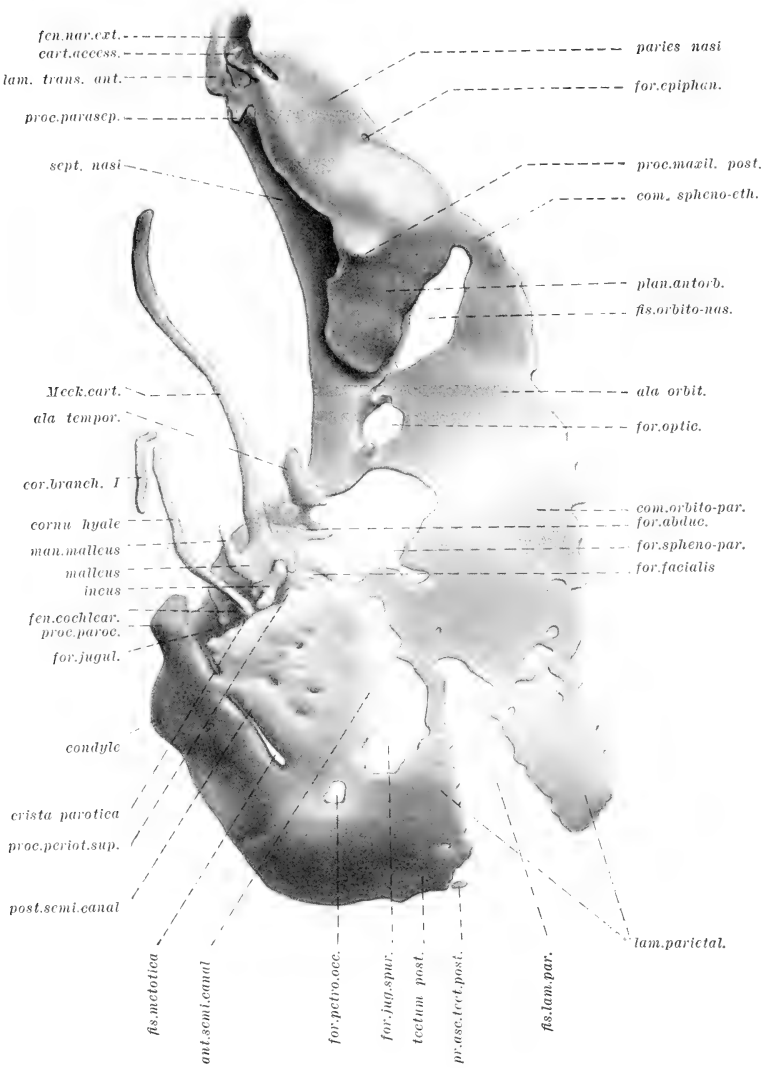
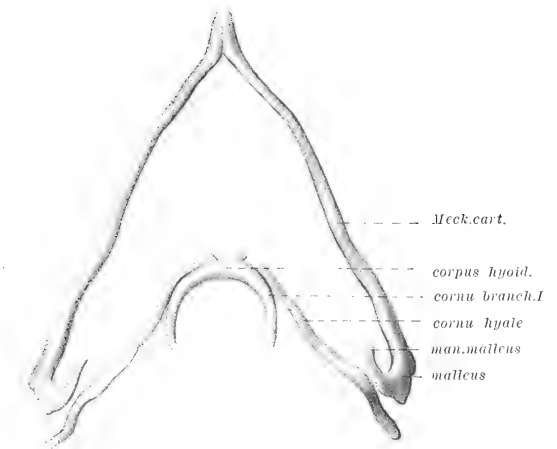


Plate IV. Ventral view of the lower jaw and the hyoid arches.


Meck.cart.
corpus hyoid.
cornu branch.I
cornu hyale
man.malleus
malleus

THE DEVELOPMENT OF THE HEART IN SHAD
(*Alosa Sapadissima, Wilson*).

With a Note on the Classification of Teleostean Embryos from a Morphological Standpoint.

BY

H. D. SENIOR.

(From The Wistar Institute of Anatomy and Biology, Philadelphia, and the Department of Anatomy in the College of Medicine, Syracuse University).

With 27 Figures.

CONTENTS.

Introduction.

A Brief Description of the Heart and Great Venous Channels of a Recently Hatched Shad.

In examining a recently hatched specimen of shad, it will be found that the heart (Fig. 1) is apparently widely open at the venous end. The atrium is separated from the ventricle in the usual way, but, on tracing the atrial wall back to where the sinus venosus should be, no sinus venosus, as such, is found to exist. The heart wall immediately succeeding the somewhat constricted venous end of the atrium represents the anterior wall of the future sinus venosus. This flares out abruptly, and its circumference, having reached the body-wall, is reflected forward upon the latter as the parietal pericardium. These relations are shown in Fig. 1; the general arrangement of the circulation, at this period, is diagrammatically indicated in Fig. 2.

The relations here are obviously peculiar; the peculiarity, however, does not consist in the continuity of the myo-epicardium with the parietal pericardium. Inasmuch as both the myo-epicardium and the parietal pericardium are developed from the mesothelium of the lateral plates in all vertebrates, this continuity is invariable. The peculiar feature in connection with the heart, in its present state, is that the anterior pole of the yolk in a sense replaces the posterior wall of the sinus venosus. Since the yolk is entirely naked, the vascular system, closed though it is, is not completely lined by vascular endothelium, as is very commonly the case in vertebrate embryos at a comparatively early stage of development.

The venous blood is returned to the heart through four veins, the jugulars and cardinals; also by means of a blood-sinus situate dorsal to the yolk, which may be called the supravitelline blood-sinus (or, for the sake of brevity, the supravitelline sinus). The supravitelline sinus is formed in the following way: The peritoneum has a ventral attachment on either side to the dorsal surface of the yolk; the peritoneal attachments involve almost the entire longitudinal extent of the yolk. Between the lines of attachment there is enclosed an arched tunnel, of which the floor is formed by the surface of the yolk,

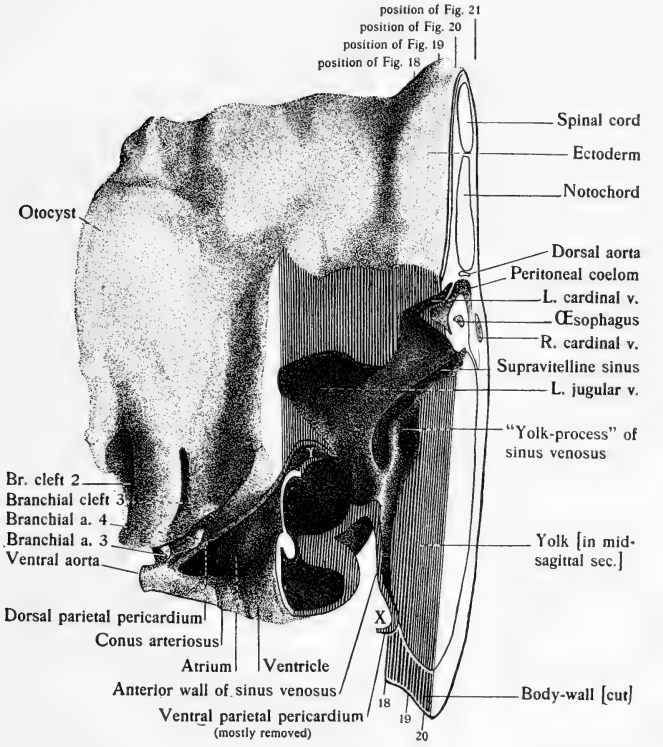


Fig. 1.—The left side of a reconstruction from the posterior gill region of a recently hatched shad, stage of 114 hours × 100 diams.

Sufficient body-wall has been removed to open the pericardial cœlom ventrally and laterally and to show the terminations of the left jugular and cardinal veins. The anterior pole of the yolk and the adjacent parts of the heart and pericardium are shown in mid-sagittal section.

and the roof by the ventral surface of the splanchnic mesoderm. The roof is entirely lined by vascular endothelium. The floor, having no endothelial lining, presents the naked periblast of the yolk to the blood-stream passing over it. Although the mid-sagittal plane on the embryo would fall (except near the anterior pole of the yolk, see Figs. 21 and 22) entirely within the tunnel, it would separate the latter into two unequal parts, of which the left would be greater than the right.

The supravitellins sinus, consisting of the tunnel described above, receives blood from the short *vena revehens* of the liver and discharges it into the, somewhat roomy, chamber embracing the anterior pole of the yolk. The liver, at this time, is situated dorsal to the posterior pole of the yolk (see Fig. 2). The chamber at the anterior

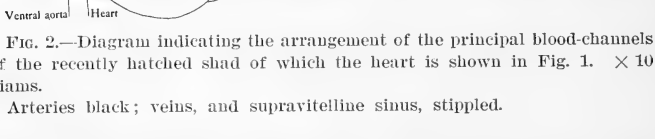


FIG. 2.—Diagram indicating the arrangement of the principal blood-channels of the recently hatched shad of which the heart is shown in Fig. 1. × 10 diams.

Arteries black; veins, and supravitelline sinus, stippled.

pole of the yolk, which corresponds in position with the future sinus venosus, is bounded as follows: Posteriorly by the naked anterior pole of the yolk; elsewhere by the portion of the heart-wall immediately succeeding the atrium, which is later to form the anterior wall of the sinus venosus. Venous blood enters the chamber from the jugular and cardinal veins and from the supravitelline sinus, and leaves it by passing through the orifice leading into the atrium.

The relation of the yolk to the vascular system is somewhat as follows: While it converts the partially formed sinus venosus and the (subintestinal) supravitelline sinus into a closed passage, capable of retaining and transmitting blood, it, at the same time, delays the formation of the posterior wall of the sinus venosus and of the vessel later to be formed by the vascular endothelium lining the supravitelline sinus.

In order to identify the parts briefly described above with the structures found in the adult, it may be said that during the rapid dwindling of the yolk the piers of the arched supravitelline sinus approach each other and that between them, by a process of rearrangement of vascular endothelium, there is formed a vein, the hepatic vein of the adult. The portion of the heart-wall connecting the venous end of the atrium with the parietal pericardium forms not only the anterior wall of the sinus venosus, but also the pericardial surface of the pericardio-peritoneal septum. The posterior wall of the sinus venosus, together with the peritoneal surface of the pericardio-peritoneal septum, is furnished by the anterior part of the splanchnic peritoneum.

No vitelline vessels, other than the hepatic vein (derived from the vascular endothelium lining the roof of the supravitelline sinus) are ever developed.

Contrast drawn between embryos of the shad type and those in which a vitelline vessel-network occurs. Suggested classification of teleostean embryos into morphological types depending on the relation of the ventral vessel system to the yolk.

The entire absence of a network of vessels on the ventral and lateral surfaces of the yolk imparts to the egg of shad (and to other eggs of the shad type) an appearance strikingly different from that of the teleostean eggs (*e. g.,* those of Salvelinus) which for some time before hatching display a vitelline vessel-network filled with corpuscles. The type of egg to which shad belongs may be called Type 1 in contradistinction to the type in which a vitelline vessel-network occurs, which may be called Type 2.

Type 1 appears to be almost universal in pelagic eggs. Uranoscopus scaber[1] is the only pelagic teleost of which I find it recorded

[1]The following are some examples of pelagic eggs in which a vitelline vessel-network is stated not to occur, or in which its absence has been inferred from figures depicting well-advanced stages: Elacate canada, Gadus morrhua (callarias), Chaetodipterus faber, Scomberomorus maculatus (Ryder '82, '84, and '87).

Hemipterus americanus, Temnodon saltator, Lophius piscatorius, Ctenolabrus (Tautogalabrus) adspersus, Tautoga onitis, Pseudorhombus oblongatus, Motella argentea (Agassiz and Whitman, '85).

Labrax lupus, Serranus cabrilla, S. scriba, Sargus Rondeletii, Box vulgaris,

that a vitelline network appears (Raffaele, '88). Type 1 is also commonly found in demersal eggs (among which the majority of the eggs belonging to Type 2 occur) including shad[2] itself; and, finally, although some of the viviparous eggs belong to Type 2 (*e. g.*, Zoarces and Gambusia) others[3] occur which conform to Type 1.

Since the difference between the two types referred to can scarcely be said to exist in the earlier stages of development, it is well to define, as exactly as may be, what is considered to be the essential difference between them. In both types the heart pulsates prior to the appearance of free blood corpuscles, and the space between the yolk and the extra-embryonic ectoderm is occupied by circulating blood-plasma. In the type in which a vitelline network occurs (Type 2), the blood, which acquires corpuscles comparatively early, is, sooner or later, confined upon the yolk, as elsewhere, in actual vessels.[4] In Type 1 no vessels are ever found upon the yolk, the

Scorpaena, Lepidotrigla aspera, Callionymus, Mugil (capito?), Gadus minutus, Coris (several species), Merlucius vulgaris, Motella vulgaris, Solea (several species), Rhombus laevis, Arnoglossus. Clupea, Engraulis encrasicholus, and several undetermined species (Raffaele, '88).

Hippoglossoides limatoides, Rhombus (Psetta) maximus, Pleuronectes platessa, P.cynoglossus, P.microcephalus, P.flesus, P. limanda, Solea vulgaris, Molva vulgaris, Centronotus (Pholis) gunellus, Motella mustela, Gadus morrhua, G. aeglefinus, G. luscus. G. merlangus, G. pollachus, Lophius piscatorius, Trachinus, Clupea sprattus, Trigla gurnardus, Callionymus lyra (McIntosh and Prince, '90).

Fierasfer dubius, Stelaphorus ringens (Eigenmann, '92).

[2]Also Pomolobus vernalis (pseudoharengus), Roccus americanus, Osmerus (Ryder, '84 and '87).

Typhlogobius californiensis (Eigenmann, '92).

Also Pseudopleuronectes americanus and, doubtless, many others.

[3]The examples found on record are Sebastodes auriculatus and Cymatogaster aggregatus (Eigenmann, '92 and '94). Probably a great many more of the viviparous perches also belong to Type 1. The absence of a vitelline vessel network in both the cases mentioned has been assumed from the figures alone. Through the courtesy of Dr. J. Percy Moore I have had an opportunity of verifying the type of Cymatogaster aggregatus.

[4]The arrangement of the vitelline vessels (which are invariably veins, Hochstetter, '87) varies considerably in different species, and these variations can be again classified into sub-types (see Ryder, '82, Wenckebach, '86, H. E. Ziegler, '87, Hochstetter, '87, Ziegenhagen, '94 and '96).

ventral surface of the splanchnic peritoneum is, however, lined by vascular endothelium which eventually forms the hepatic vein[5]

The hepatic vein then in Type 1, replaces the vitelline network of Type 2, and it might itself be considered a vitelline vessel, but for the fact that when it is fully formed the yolk is reduced to a very small size. The light in which the hepatic vein is regarded, however, does not affect the essential validity of the types: *Embryos of Type 2 differ from those of Type 1 in that they possess, at some period prior to the disappearance of the yolk, vitelline vessels lateral to the margin of the cœlon.*

In all cases in which the site of origin of the blood corpuscles has been investigated in embryos of Type 2,[6] it has been found to occur in the cardinal veins, which may be separate or partially conjoined (Stammvene). In embryos of Type 1[7] the cardinal veins have invariably been found, when they first appear, to contain no corpuscles.

At the present time, although the information at our command is rather suggestive, it appears neither safe to assume that the blood anlage is *always* developed within the cardinal veins in embryos of Type 2, nor that this *never* happens in embryos of Type 1. It would seem that more information is needed on the entire subject of blood-formation in teleosts, before a generalization of this kind can be made with safety. I refer particularly to the fact that Marcus ('05) has recorded for Gobius capito (Type 2) that corpuscles arise in the tail as well as in the cardinal vein region;

[5]It is neither assumed nor implied that the formation of the hepatic vein in other embryos belonging to Type 1 is similar in mechanism to that later to be described for shad; the serial sections of other Type 1 eggs, mostly pelagic, in the possession of the writer do not cover all the stages necessary for the determination of this point.

[6]Zeigler, '82 and '87, Salmo salar; Wenckelbach, '85, Perca fluviatilis, '86 Belone and Esox; Felix, '97, salmon and trout; Swaen and Brachet, '00, trout, '02, Leuciscus cephalus, and Exocoetus volitantes; Sobotta, '02, Trutta fario, T. iridea and Salmo salvelinus; Marcus, '05, Gobius capito.

[7]Wilson, '91, Serranus atrarius (Centropristis striatus, L); Sween and Brachet, '02, Clupea sprattus, Rhombus (?), Solea vulgaris, Pleuronectes microcephalus, Trachinus vipera, Caranx trachurus, and Callionymis lyra; Derjugin, '02, Lophius piscatorius.

this is probably a fact of great importance. The blood anlage of shad (Type 1) arises as a cord of cells in the tail, which forms a direct continuation backwards of the, then, partially developed caudal aorta and caudal vein; part of the blood anlage of Opsanus tau (Type 2) also arises in a similar manner (the remainder arising in the cardinal veins). It is possible that the tail is the site of blood formation common to all teleosts, and that the cardinal vein blood anlage occurs as a further source of corpuscles in the forms which acquire numerous corpuscles at a comparatively early stage of development. Without assuming this actually to be the case, I would venture to suggest that, in connection with the origin of the blood corpuscles, the tail deserves thorough examination in all teleosts, whether or not, in the species under examination, corpuscles are found to arise in the cardinal veins. Reference to investigations setting forth the surface of the yolk as the source of blood corpuscles has been purposely omitted.

It has been my experience that the yolk in eggs of Type 1 is in excellent condition for cutting after it has been fixed in formalin; whereas formalin-fixation produces in eggs of Type 2 a yolk difficult to cut and sometimes of almost stony hardness. This is not due merely to difference in size, but seems to point to a difference in chemical composition between the yolks of the two types of egg.

It is well known that the differences in structure, and in the general processes of development which occur among teleostean embryos of different species bear little or no relation to the structure and affinities of the corresponding adults; since, therefore, the type of embryo cannot be inferred from the systematic position of the adult, it would seem advantageous to classify the embryos themselves according to their own structural peculiarities.

The division of embryonic teleosts into the morphological types indicated above appears to be warranted by the present state of our knowledge and, since it is applicable alike to pelagic, demersal, and viviparous eggs it may prove of some service as a starting point for classification. The demand for some such division into types is, I think, indicated by the not infrequent use in the literature of the terms pelagic and demersal in a morphological connection. The

following objections to the use of the words pelagic and demersal in a morphological sense will sufficiently indicate their unsuitability.

(*a*) Their use, in this sense, is apt to entail the statement that a given egg is either demersal in habit and pelagic in structure, or *vice versa,* which is undesirable.

(*b*) Neither of these terms can be used to express the structure of a viviparous egg.

(*c*) These terms, as strictly applied, have no more morphological significance than has the term viviparous itself.

Statement of the object in investigating the development of the heart in shad, and of the scope of this investigation.

The development of the heart in embryos of Type 2 has received a large share of attention, particularly in Salmo and allied genera in which the process of heart-formation has been definitely made out.

The heart in embryos of Type 1 differs considerably from that in embryos of Type 2, particularly in its relation to the vascular system of the yolk. The development of the heart, however, appears to have received somewhat scant notice. Ryder has given a brief account of some of the changes undergone by the heart in Gadus morrhua (callarias), '82, and in Clupea (Alosa) sapadissima, '85; Boeke has described the early development of the heart in Muræna (endocardium particularly), '03. There are also some earlier investigations, dealing with the living embryo alone, which, necessarily, leave much to be desired.

It has been attempted here to give a consecutive account of the development of the heart in shad, as representing Type 1, from the earliest possible stage until the adult arrangement is recognizable.

Material and Methods.

The material investigated, the property of The Wistar Institute of Anatomy, Philadelphia, was collected during the seasons 1905, '06, and '07 at the hatchery of the Pennsylvania State Fish Commission, Torresdale, Pa. To the Commissioner, Mr. W. J. Meehan, I hereby tender my thanks for his many courtesies.

Prior to a period of development at which the embryo is capable of

self-extension after removal of the egg-membrane (so that it may be fixed in the extended position) the stages have been estimated by the number of somites. Stages have been designated in terms of length of embryo (in a straight line from end to end) from the time that the embryo is approximately straight until the, somewhat arbitrarily selected, period of hatching. After hatching the age is given as being the sole guide to the stage of development; the length of the embryo, unfortunately, does not convey the required information.

It is well known that development is relatively accelerated by a high water temperature. The period of development within the egg is actually shorter, however, in warm water, for the embryo is hatched in a progressively immature state in direct proportion as the water-temperature rises. To mention a few examples: My stage of 114 hours, 10.5 mm. (just hatched at a low-water temperature[8]) is much more advanced in development than the stage of 8.7 mm., 63 hours, (hatched about twenty hours in warmer[9] water); the latter is only slightly more advanced than a stage of 8.3 mm., 107 hours (still in the egg, water-temperature[8] low). Direct comparison, except in individual cases, is not easily made between the hatching stages in different water-temperatures because the embryos of any one batch do not hatch simultaneously but continue to hatch over a period lasting twenty-four hours or more; the hatching period must, therefore, be in any case somewhat arbitrarily determined. I have selected the stage of 114 hours as the just hatched stage (in preference to earlier "just-hatched" stages) because at all stages prior to this the length of the embryo, from the time it is capable of self-extension, accurately indicates the period of development.

Shad is anadromous; the egg, demersal and non-adhesive, is convenient for study on account of its transparency and because it is easily removed from its roomy capsule (diameter of egg proper is about 2 mm. capsule slightly under 4 mm.). Details of the spawning-habits and of the methods of rearing eggs and larvæ are given in "A Manual of Fish-Culture," published by the U. S. Fish Commission, revised edition, Washington, 1900.

[8]Average temperature 63° F.
[9]Average temperature 70° F.

All the methods of fixation in common use for teleosts were tried. That of Sumner (saturated corrosive sublimate containing 10 per cent glacial acetic; followed by 10 per cent. formalin; see Sumner, '00) proved the most satisfactory and was generally employed; all the figures were drawn from material fixed in this way except Fig. 8 (H. Virchow's method) and Figs. 10 and 11 (Pereny's fluid).[10]

The embryos were cut into serial paraffine sections ranging from 5 to 10 microns in thickness. Sections of the earlier stages were stained with iron hæmatoxylin, later stages were usually stained in toto with alcoholic carmine.

Eleven wax-plate reconstructions[11] were made, two hundred times larger than the originals after fixation (correction having been made for shrinkage in paraffine). In making the figures the reconstructions were photographed (natural size) and the outlines of the photograph traced. In finishing the drawings the irregularities due to the plates were omitted. All figures, representing reconstructions, have been reduced one-half in reproduction.

Below follows a list of the figures with data for identification of their sources. (*The numbers are from the catalogue of The Wistar Institute of Anatomy*):

Fig. 1.—Reconstruction 14504, from series 14275, sections 87 to 134.

Fig. 2.—Outline from embryo afterwards cut into series 14275.

Fig. 3.—From series 14556; 3A section 91, 3B 120, 3C 132, 3E 142, 3F 152, 3G 181.

Fig. 4.—Reconstruction 14534, from series 14533, sections 45 to 87; 4A section 53, 4B 57, 4C 62, 4E 71.

Fig. 5.—Reconstruction 14535, from series 14524, sections 91 to 151; 5B section 104, 5C 111, 5D 114, 5G 148.

Fig. 6. Reconstruction 14536, from series 14520, sections 63 to 110; 6B section 76, 6C 79, 6D 82, 6F 92.

Fig. 7.—Reconstruction 14537, from series 14532, sections 68 to 113; 7B section 82, 7C 86, 7D 89, 7G 113.

[10]Pereny's fluid, in which, unfortunately, all my material about the time of beginning heart-beat has been fixed, gives fair general results, but is extremely unfavorable for cytological study.

[11]Shown at the Chicago meeting of the Association of American Anatomists, Christmas, 1907.

Fig. 8.—Series 14567, section 39.

Fig. 9.—From the reconstruction used for Fig. 12.

Fig. 10.—From series 14667; 10B section 70, 10D 79.

Fig. 11.—Reconstruction 15011, from series 14670, sections 68 to 110; 11C section 79.

Fig. 12.—Reconstruction 15012, from series 14668, sections 39 to 69; 12C section 54.

Fig. 13.—Reconstruction 15013, from series 14671, sections 21 to 80.

Fig. 14.—Diagram from the reconstruction used for Fig. 13

Fig. 15.—Diagram from reconstruction 15014, from series 14672, sections 28 to 61.

Fig. 16.—Reconstruction 15015, from series 14568, sections 20 to 56.

Fig. 17.—Fig. 1 repeated.

Fig. 18.—Series 14275, section 126.

Fig. 19.—Series 14275, section 130.

Fig. 20.—Series 14275, section 133.

Fig. 21.—Series 14275, section 136.

Fig. 22.—Reconstruction 14507, from series 14538, sections 82 to 136.

Fig. 23.—Series 14538, section 128.

Fig. 24.—Series 14538, section 132.

Fig. 25.—Series 14538, section 140.

Fig. 26.—Series 15002, section 138.

Fig. 27.—Series 15002, section 148.

Development of the Heart.

The process of development of the heart in shad may conveniently be divided into four periods as follows:

1. Formation of the heart anlage.

2. Lasting until rhythmical contraction begins in the partially formed heart-tube.

3. In which the heart-tube is completed to form conus, ventricle and atrium, and assumes the adult position.

4. Formation of the sinus venosus and hepatic vein.

Period I. Formation of the heart anlage.

The myo-epicardium and the parietal pericardium are developed from the lateral plates of the mesoderm. That the endocardium is derived from mesoderm, and from mesoderm alone, has already been

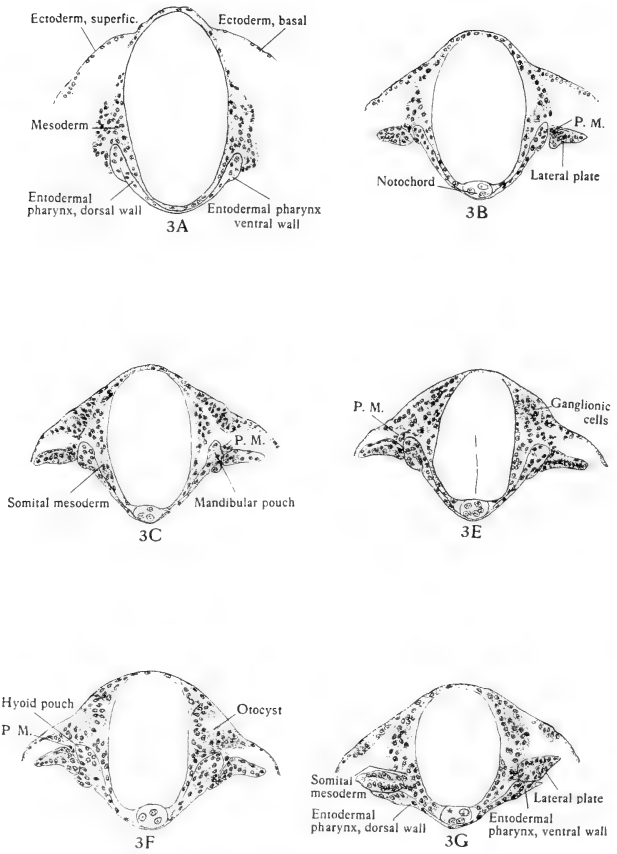


FIG. 3.—Transverse sections of shad, stage of 15 somites × 100 diams. For situation of sections see footnote on opposite page.

shown in other teleosts by Oellacher, '73, H. E. Ziegler, '87, Swaen and Brachet, '00, and by Sobotta, '02. Nothing has been found in shad supporting the view that the endocardium arises from entoderm alone or from entoderm and mesoderm together.

The particular part of the mesoderm from which the endocardium is derived is a, bilaterally symmetrical, cord of cells on either side immediately adjacent to the medial borders of the lateral plates; of this Swaen and Brachet have given a careful description (as found in Trutta fario) and to it they have applied the term "Portion moyenne du mésoblaste." In shad at a stage of 15 somites the portion moyenne is recognizable throughout most of its, eventual, longitudinal extent; although comparison with earlier stages shows that it has been recognizable for some time but in a less advanced stage of development.

In order to gain a clear conception of the portion moyenne, as it occurs in shad at the stage of 15 somites, it is necessary to enquire into the cause of its distinctness from the remainder of the head mesoderm. There are, as far as I can see, no features in the cells composing the portion moyenne which distinguish them at this stage from other mesodermal cells. The portion moyenne, as a whole, is distinguishable from the lateral plate by reason of the orientation of the cells of the latter (where this has occurred) around the, now virtual, cœlon; see Figs.[12] 3B, 3C, 3E and 3F. The lateral plate,

[12]To facilitate comparison between the sections shown in figures 3, 4, 5, 6, 7, 10, 11, and 12 the same letter has been used throughout to indicate a certain region. Seven regions have been selected as follows:

A is near the, eventual, anterior limit of the lateral plates.

B is between A and the mandibular entodermal pouch.

C is through the mandibular entodermal pouch.

D is midway between C and the gill anlage spoken of in the text as the hyo-branchial.

E is a short distance in front of the hyo-branchial anlage.

F is through the hyo-branchial anlage and the anterior part of the developing otocyst.

G is some distance posterior to the branchial region of the pharynx.

In figures of sections P.M. indicates portion moyenne.

The anterior aspect of the section is always represented; the structures on the right side of the embryo will, therefore, appear on the left side of the figure, and *vice versa*.

which is now in progress of differentiation from behind forwards, is not yet clearly distinguishable at the site of Fig. 3A (q.v.) and here the portion moyenne (although it is later well developed, see Fig. 4A) is not clearly defined.

The distinction of the portion moyenne from the somital portion of the head mesoderm depends on an actual separation of cells from the lateral region of the somite; or (perhaps more correctly) on an isolation of the cells situated between the somite and the lateral plate. The position of the portion moyenne, where portion moyenne occurs, bears a definite relation to the lateral margin of the entodermal pharynx. In the region of the entodermal pouches the pharynx is wide and the portion moyenne is always situated lateral to its lateral margin (see Figs. 3B, 3C, 3E and 3F). Posterior to the region of the entodermal pouches the entodermal pharynx is much reduced in width, and here the portion moyenne does not occur at all, for the lateral plate is in contact with the somite (see Figs. 3G and 5G). The transition between the wide and narrow portions of the entodermal pharynx occurs, somewhat abruptly, on a level with the middle of the otocyst. In the region of the entodermal pouches, the prominent lateral margin of the pharynx tends to insinuate itself between the somital mesoderm and the portion moyenne. Posterior to the entodermal-pouch region the margin of the narrow entodermal pharynx tends to remain altogether ventral to the somital mesoderm (see Figs. 3G and 5G).

Swaen and Brachet suggest that the portion moyenne is separated from the somital mesoderm under the combined influence of the prominent margin of the entodermal pharynx on the one hand, and of the ectoderm and the anterior part of the otocyst on the other; the conditions found in shad are quite in accordance with this view. The appearances in Fig. 3F clearly suggests the influence of the wide hyoid pouch and of the otocyst in causing separation of the portion moyenne. In Figs. 3B, 3C and 3E the ectoderm would appear to be of little assistance in causing separation of the portion moyenne, but it is probable that the ectoderm is normally in contact with the mesoderm and that the separation of ectoderm from mesoderm, seen in the sections, is artificial and mainly due to the action of the fixative.

The portion moyenne appears as a cord of mesodermal cells on either side, intervening between the somital and lateral mesoderm, which tends to remain in contact with the lateral plate. It extends from the middle of the otocyst forward to the anterior end of the lateral plate (see Fig. 4A). Small posteriorly, the portion moyenne becomes larger anterior to the mandibular pouch; here and there it comes into immediate contact with the somital mesoderm. Where contact occurs at this stage, the distinction between portion moyenne and somital portion of the head mesoderm cannot be made out because there is, as yet, no differentiation between the cells belonging to these two parts of the mesoderm. In spite of the fact that in occasional sections the portion moyenne is not quite clearly defined, it forms, as a whole, a perfectly definite structure.

That the portion moyenne, as found in shad, is directly comparable to that described with such admirable distinctness in trout by Swaen and Brachet cannot, I think, be doubted; for this reason I have ventured to adopt the term employed by them rather than run the risk of confusion by the unnecessary introduction of another name.

It has been said that the endocardium is derived from the portion moyenne of the mesoderm; before proceeding to trace the formation of the endocardium it may be stated that special care has been taken to determine whether the entire longitudinal extent of the portion moyenne is involved in the production of endocardium. In order to settle this question (among others) a series of four wax-plate reconstructions has been made from stages during which the cells of the portion moyenne are undergoing migration and differentiation to form the endocardium. *The result indicates that the endocardium, together with the central aorta, is derived exclusively from that part of the portion moyenne originally situated anterior to the transverse plane passing through a point midway between the mandibular and hyoid entodermal pouches.* That the posterior part of the portion moyenne takes absolutely no share in the formation of the endocardium is an important point which will be referred to later.

Fig. 4 is a diagrammatic representation of the ventral surface of a wax-plate reconstruction of the pericardial region of a shad's head. Stage of 18 somites (the embryo is one hour and a half older

than that used in the preceding figure). The parts shown[13] are (normally) in immediate contact with the yolk, and represent the region extending from the tip of the notochord back nearly to the anterior limit of the first body somite. Formation of the ventral wall of the pharynx, by the folding ventralwards of the (originally) lateral region of the gut-entoderm on either side, is complete in the anterior region and is rapidly extending backwards. The lateral plates are following the medial margins of the ventral pharyngeal wall in their progress toward the mid-line.

The outline of the entodermal pharynx presents, on each side, three prominences which require some explanation. Of these the posterior is the hyoid ectodermal pouch which has now reached, and blended with, the (very shallow) ectodermal pouch; this, for reasons stated below,[14] will be referred to as the hyo-branchial anlage. The middle prominence is the mandibular entodermal (solid) pouch; this blends with the ectoderm later but (as is well known) gives rise to no cleft; it undergoes disintegration soon after the perforation of

[13]Explanation of Figures 4, 5, 6, and 7.

Red, lateral plates, where these are covered ventrally by endocardium their outline is indicated by a red line.

Blue, endocardium.

Continuous black line indicates the position of the outline of the entodermal pharynx and adjacent part of the head-fold (see text); interruptions in this line indicate blending of pharyngeal entoderm, or of head-fold, with the basal layer of the surface ectoderm.

Broken black line, medial margin of (closing) ventral wall of pharynx.

Stipple, ventral wall of the entodermal pharynx and adjacent head-fold ectoderm.

Plain white, dorsal wall of (incomplete) pharynx.

Arrows on each side indicate the longitudinal limits of the "descent area" of portion moyenne.

[14]"Although the (solid) hyoid pouch is alone present at this stage the branchial entodermal pouches (also solid) are about to be laid on, very rapidly, from before backward. The hyoid and branchial entodermal pouches all reach a common ectodermal anlage and are separated from one another by an extremely delicate partition of mesoderm so that, at any given time, a very careful examination is necessary to determine the exact number of pouches actually present; in order to avoid a repeated analysis of the condition, which is unnecessary for this investigation, the entire series of compactly grouped pouches has been looked upon as a single structure.

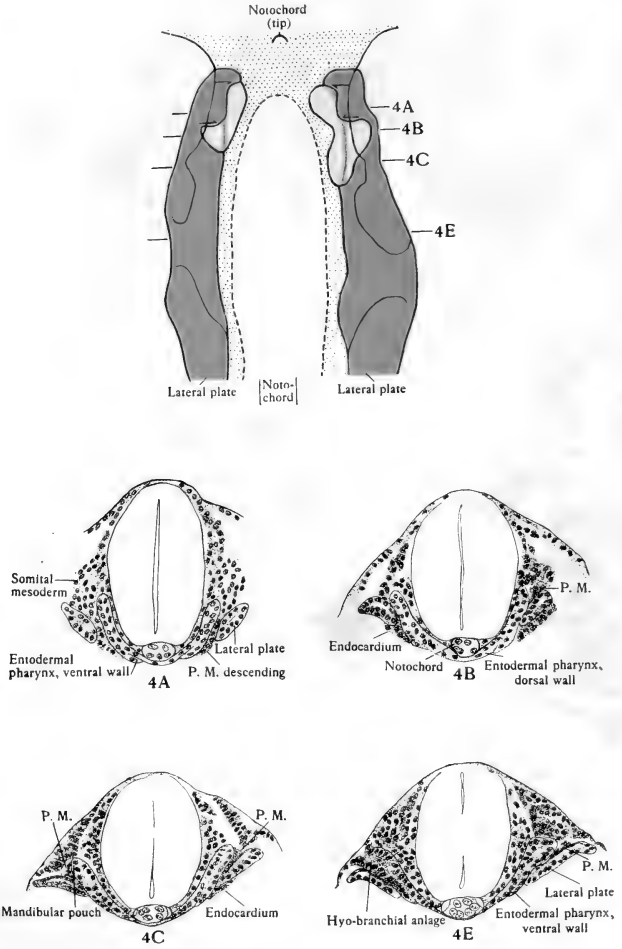


FIG. 4.—Diagram of the ventral surface of a reconstruction of the pericardial region of shad, stage of 18 somites × 100 diams. See explanation of figures, footnote on opposite page.

the hyoid cleft. The anterior prominence is not strictly pharyngeal; it represents the posterior limit of the head-fold ectoderm,[15] which, growing in from either side, blends with the anterior extremity of the closing entodermal pharynx. Inasmuch as it has been found difficult or impossible to distinguish the line of union between ectoderm and entoderm after blending has occurred, no attempt has been made to indicate this in Figs. 4, 5, 6 and 7; there is no doubt, however, that, although the lateral prominences consist of ectoderm, the axial region (as far as shown in the figures) is truly pharyngeal.

At 18 somites the anterior part of the portion moyenne is descending on either side, between the lateral plates and the, now closing, pharynx to gain a position ventral to these structures; the longitudinal area over which descent is occurring ("descent area") is indicated by the space between the arrows in the diagram. Fig. 4A is a section from this region. Sections from behind the descent area (Figs. 4B and 4C) show that the portion moyenne, which can be seen in them dorsal to the lateral plate, is not descending; descent is prevented, apparently, by the close contact between lateral plate and pharyngeal entoderm. It will be seen that the portion moyenne is

[15]The "optic region" of the head (anterior to the parts reconstructed) is separated from the yolk by a double layer of ectoderm which grows in, in this situation, from the basal ectoderm around the anterior periphery of the head. This ectoderm, which performs several functions, corresponds, in teleosts, to the head-fold of other vertebrates (see Froriep, '05). The formation of head-fold from this double layer of ectoderm occurs literally, in shad, only as far back as the hypophysial region, anterior to which no gut-entoderm occurs. There is a region, extending from some point (approximately) ventral to the hypophysis back to a point slightly posterior to the tip of the notochord, throughout which the ingrowing ectoderm encounters the anterior extremity of the pharyngeal entoderm; the ectoderm in this region, although its origin is intimately connected with that of the head-fold, has an entirely different subsequent history, briefly indicated as follows: Until the head of the embryo begins to arise from the yolk the double-layered ectoderm of the region in question forms a bond of union between the anterior end of the pharynx and the surface of the embryo. As the head rises from the yolk the layers of the head-fold ectoderm proper become separated to cover the contiguous portions of the head and yolk. Shortly before perforation in the oral plate occurs, the anterior cul-de-sac of the pharynx becomes widely dilated, and the ectoderm connecting the lateral margins of the anterior end of the entodermal pharynx with the basal ectoderm of the surface undergoes, very rapid, disintegration.

in process of an entire alteration in its distribution; it now consists, on either side, of three parts: One part still remains dorsal to the lateral plate, a second is on the ventral surface of this structure, and a third forms an isthmus between the other two and occupies the descent area. From now on it will be convenient to speak of the part ventral to the lateral plate as endocardium (for such it really is) and to retain the original term for the part which is still dorsal to the lateral plate and for the isthmus. The endocardium now appears as two patches (colored blue in Fig. 4) ventral to the entodermal pharynx and the lateral plates. These patches are not limited to the descent area, but are spreading backwards (more so on the left side of the embryo than on the right, see Fig. 4B and 4C). The backward (caudad) movement of the endocardium, which is now beginning, is soon to become very pronounced. The asymmetry seen in this reconstruction is thought to be due to unequal growth of the embryo, rather than to faulty building up of the plates, the entire right side of the head appears to be in a less advanced stage of development than is the left.

In an embryo of 22 somites (Fig. 5, one hour and a half older than the preceding stage) there has been considerable advance in development. Ventral closure of the pharynx has progressed rapidly from before backwards, and is also beginning in another place posterior to the gill-region. The medial margins of the lateral plates are approaching one another, and the notochord, slightly longer than before, is now fully formed as far as its anterior end is concerned. The endocardium has travelled back to a point posterior to the mandibular pouch (Fig. 5D), and the portions arising from each side have met across the mid-line. Fig. 5B (as compared with 4B) shows that the descent area has extended considerably backwards, but that descent is not yet occurring opposite the apex of the mandibular pouch is shown on the left side of Fig. 5C (right side of embryo).

At a stage of 26 somites (Fig. 6, one hour and a half later than the preceding stage) the ventral closure of the pharynx is approaching completion; rapidly, however, as closure of the pharynx is taking place, it has been overtaken by the backward growth of the endo-

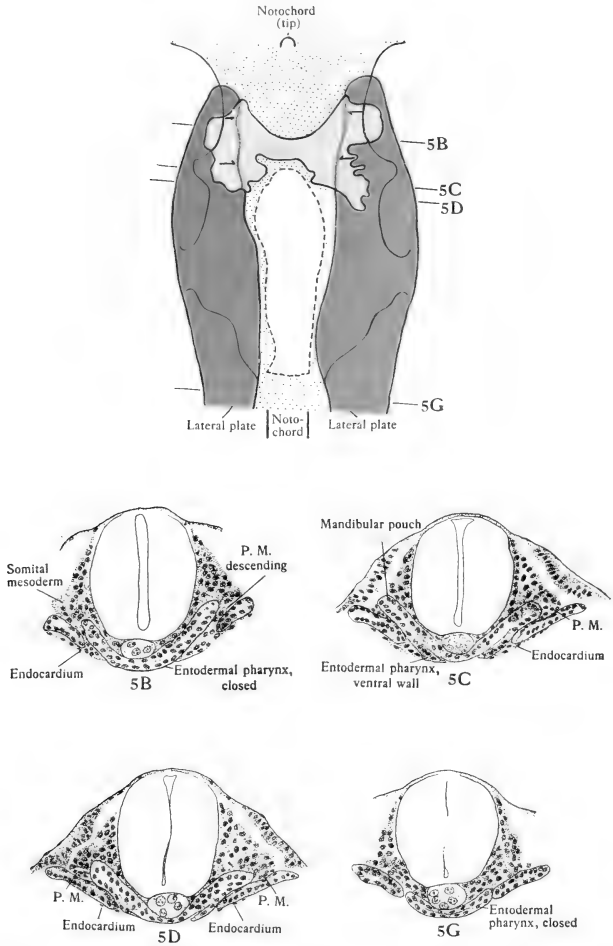


FIG. 5.—Diagram of the ventral surface of a reconstruction of the pericardial region of shad, stage of 22 somites 100 diams. See explanation of figures, footnote on page 226.

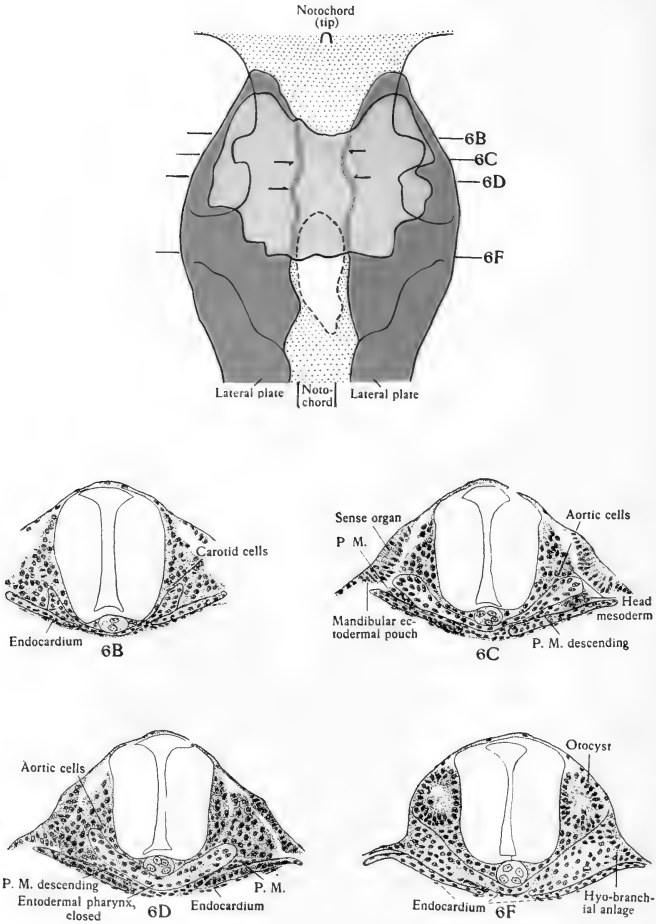


FIG. 6.—Diagram of the ventral surface of a reconstruction of the pericardial region of shad, stage of 26 somites × 100 diams. See explanation of figures, footnote on page 226.

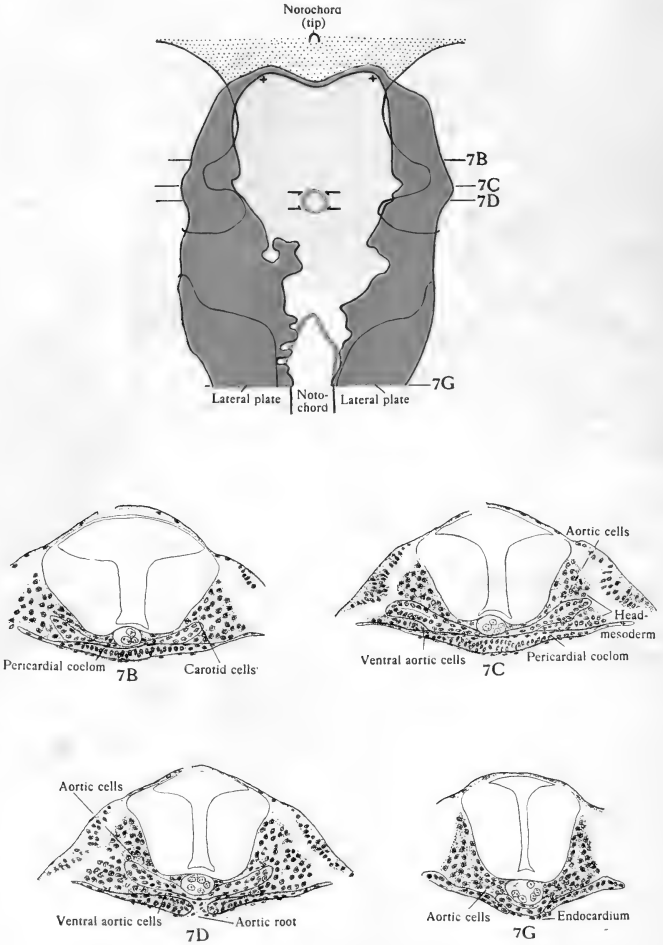


FIG. 7.—Diagram of the ventral surface of a reconstruction of the pericardial region of shad, stage of 32 somites × 100 diams. See explanation of figures, footnote on page 226.

cardium. The endocardium, which now covers a considerable area, not only lies over (ventral to) the closing edges of the entodermal pharynx, but partially covers the ventral surface of the future roof as well (see Fig. 6 and 6F). The portion moyenne is no longer descending in the region anterior to the mandibular pouch, for here it has, apparently, been exhausted (see Fig. 6B). The descent area on the left side (right in Fig. 6C) is situated toward the back of the mandibular pouch, and on the right (left in Fig. 6D) is altogether behind this structure; here again there is slight asymmetry. The lateral plates, which are now much increased in width, are approaching the mid-line; the notch in the medial border of each foreshadows the point at which the actual borders never meet, but at which they are about to embrace the root of the ventral aorta.

The lateral margins of the somital portion of the head mesoderm are, at this stage, spreading ventrally around the lateral margins of the entodermal pharynx in the region between the mandibular and hyo-branchial pouches (see Figs. 6C and 6D). The cells from the somital mesoderm, which now partially embrace the gut ventrally, will form the muscle and supporting framework of the hyoid arch. There is no difficulty *now* in distinguishing the head mesoderm proper from the undescended portion moyenne, with which it is, in many places, in close contact. The cells of the portion moyenne (like the endocardial cells ventral to the entodermal pharynx) are becoming plainly endothelial; they differ from the other cells of mesodermal origin in that the nuclei appear, in transverse sections of the embryo, to be small and rather flat and to be surrounded by a comparatively large amount of cytoplasm.

At a stage of 32 somites (Fig. 7, three hours later than the preceding stage[16]) the medial margins of the lateral plates have met and blended throughout the anterior three-fourths of the pericardial region except at one place foreshadowed in the preceding stage. The medial margins of the lateral plates, where they fail to

[16]In my stage of 30 somites (exactly intermediate in time between the stage of Fig. 6 and that of Fig. 7) the conditions in the heart-region are practically indistinguishable from those found at 32 somites (Fig. 7); the later stage has been used for reconstruction on account of its superior preservation.

blend, enclose a circular area which contains the cells about to form the root of the aorta (see Fig. 7D). Where blending has occurred (Figs. 7B and 7C) continuity is established between the somatic layers of the right and left lateral plates; the splanchnic mesoderm of the two sides becomes continuous across the mid-line in a similar manner. In the process of blending the cœlom becomes continuous across the mid-line by the apparent loss of the medial margin of each lateral plate; the medial margins together constitute the *dorsal mesocardium* which is, thus, early lost. The entire cœlom, paired or unpaired, occurring in Fig. 7 is pericardial. The site of discharge of the jugular veins (which later determines the points of separation, on either side of the embryo, between the pericardial and peritoneal regions of the original cœlom) will occur slightly behind the site of Fig. 7G.

The endocardium has undergone very rapid growth backwards (see Fig. 7G), and has now reached the anterior limit of the first body somite. The interval between the first body somite and the head is some little distance behind (caudad from) the posterior limit of the reconstruction and the future site of discharge of the jugular veins. The ventral surface of the entodermal pharynx and of the adjacent region of the (peritoneal) splanchnic mesoderm is, therefore, in the head region posterior to the reconstruction, covered ventrally by the endocardium. As far as has been ascertained, the endocardium does not encroach upon the region ventral to the first body somite itself; a re-investigation of this difficult point will form a part of a study of the origin of the body-vessels to be undertaken at a later date.

The descent area of the portion moyenne has moved slightly backwards since the preceding stage; it has narrowed considerably (in the antero-posterior dimension), and now consists of only a narrow cord of cells on each side. The term "descent area" which has hitherto been used to designate cellular connection between the endocardium ventral to the lateral plates and the portion moyenne dorsal to them is no longer applicable, for *descent has ceased;* the cells between the entodermal pharynx and lateral plate (seen on each side in Fig. 7D) represent the first (transverse) part of the ventral aorta, and the portion moyenne, as such, has ceased to exist.

The entodermal pharynx is now closed throughout, forming a flat tube with a horizontal (virtual) lumen; its ventral surface scarcely appears in Fig. 7 since this is almost entirely hidden by the peri- and endocardium.

The heart anlage is now complete, and, although it is quite flat, its component parts can be (by comparison with later stages) already recognized. If an isosceles triangle be described, the base of which corresponds to a straight line connecting the two crosses near the top of Fig. 7, and whose, truncated, apex skirts rather closely round the (red) circle which embraces the aortic root, the area of splanchnic

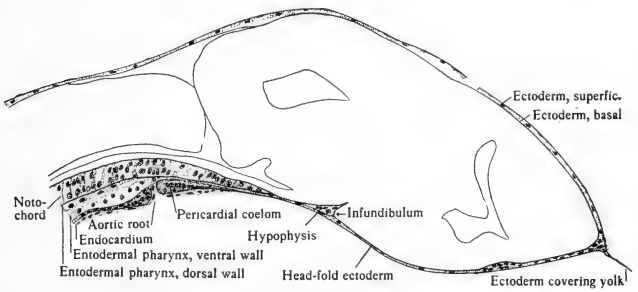


Fig. 8.—Mid-sagittal section through the head of shad, 30-31 somites × 100 diams.

Owing to slight obliquity the section passes through the brain mainly to the right of the ventricular cavity. Fixation by Virchow's method.

mesoderm contained within the triangle will represent conus, ventricle and atrium (in the order named, from behind forwards). The (pericardial) splanchnic mesoderm not included in the triangle will form the anterior wall of the sinus venosus and of the pericardio-peritoneal septum. All the somatic mesoderm anterior to the (future) site of discharge of the jugular veins will become parietal pericardium. The heart anlage in mid-sagital section is shown in Fig. 8. The embryo of Fig. 8 is slightly younger (30 to 31 somites) than that of Fig. 7 (32 somites).

Two points in regard to the descent of the endocardial cells have been brought out by the use of the plastic method of reconstruction

which, it appears from the literature, have previously escaped notice. Firstly: The endocardial cells do not descend in a hap-hazard fashion; descent proceeds, in a perfectly orderly manner, continuously from before backwards; the last cells to descend (*i. e.,* those in the region of the future aorta) are arrested, as it were, in the act of descent to form the first part of the central aorta. Secondly: In the region posterior to the aortic root descent of endocardial cells does not occur at all; the endocardium in this region is exclusively furnished by cells descending in, and derived from, the region anterior to the root of the aorta.

The part of the portion moyenne, which at 15 somites was easily recognizable in the region behind the (future) aortic root, extending back as far as the middle of the otocyst(see Figs. 3E and 3F), has disappeared long before the completion of the heart anlage, apparently by blending with the adjacent somital mesoderm. It is still recognizable at 18 somites (see right side of Fig. 4E), but posterior to this it has already disappeared or is very indefinite, see left side of Fig. 4E (through their hyo-branchial anlage). At 22 somites the portion moyenne, in this region, has entirely disappeared.

Review of the evidence bearing on the relation of the endocardium to the vascular endothelium of the head in general.

The development of the pericardial cœlom, including in this term the future myo-epicardium, may be looked upon as a subject practically complete in itself which can be considered independently of that of other structures. The case of the endocardium is entirely different; the fact alone that part (at least) of the ventral aorta arises, in common with the endocardium, from the portion moyennne is quite sufficient to indicate that the endocardium cannot be considered as an independent structure. It is only reasonable to suppose that the origin of the aorta is essentially similar throughout the head, so that separation of the aorta from the endocardium, in this connection, would be artificial and, therefore, not conductive to a clear conception of the origin and relations of the latter. During the study of the process of formation of the heart anlage several facts bearing on the origin of the vascular endothelium became

evident; mention of these, since they had no direct bearing on the subject in hand, has, hitherto, been omitted. In the following notes the facts alluded to are briefly reviewed, and an attempt made to estimate their significance.

The portion of the head shown in Fig. 7 may be divided transversely into three regions: anterior, middle and posterior; of these the middle region extends from the back of the red circle, indicating the aortic root, back to the place at which the entodermal pharynx becomes narrow behind the hyo-branchial anlage; the anterior region corresponds to the pericardial area in front of the middle region, and the posterior to the corresponding area behind it.

In the embryo of 15 somites, from which sections are shown in Fig. 3, the cells immediately adjacent to the medial borders of the lateral plates are isolated from the remainder of the mesoderm to form the portion moyenne of Swaen and Brachet; this isolation of the portion moyenne occurs only in the anterior and middle regions (comparing the stages of 15 and 32 somites approximately) and not in the posterior. The endocardium, as seen in Fig. 7, is derived exclusively from the portion moyenne of the anterior region. Having recalled these points regarding the portion moyenne and endocardium, the occurrences bearing on the vascular endothelium belonging to the three regions mentioned will, as far as possible, be considered separately.

The anterior region may be examined first. In Fig. 7D the lateral, now pericardial, plate is not in contact with the ventral surface of the entodermal pharynx, as is the case elsewhere (save in the two neighboring sections), contact being prevented by a cord of cells on either side. The cells of these cords were derived from the portion moyenne, and, like other cells known to come from the same source, they are at this stage easily distinguishable from the ordinary mesoderm.

Fig. 9 is from a reconstruction of the aorta at the earliest stage in which it appears as a well defined vessel throughout the head (42 somites; the ventral surface of this reconstruction is shown as Fig. 12). The cells between the entodermal pharynx and pericardial plate in Fig. 7D correspond exactly in position with the first (or transverse) part of the ventral aorta.

In Fig. 7C the entodermal pharynx and pericardium are in contact, and here cells descending from the portion moyenne have been arrested *lateral to the line of contact* between the two; reference to Fig. 9 will show that these cells correspond in position to the second (or oblique) part of the ventral aorta. This is not all; in Fig. 7C (also 7B and 7D) just dorsal to the entodermal pharynx there are one or two cells on either side which differ markedly from the surrounding mesodermal cells; the cells in question (which can be dis-

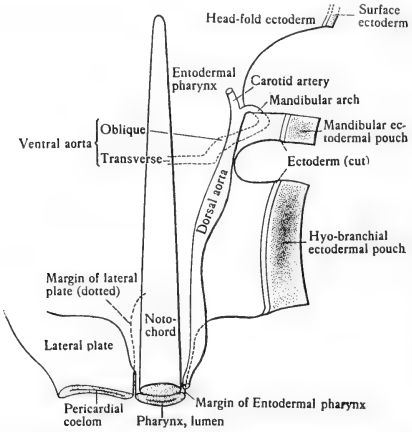


Fig. 9.—Diagram showing the course taken by the right aorta throughout the pericardial region of shad, stage 42 somites × 100 diams.

The dorsal surface of the entodermal pharynx is shown on the right side, and the dorsal surface of part of the lateral plate on the left.

tinguished in practically every section used in the reconstruction) are found (see Fig. 9) to invariably occupy the line of the dorsal aorta and are, without doubt, aortic endothelium. It is hoped that the position of the aortic cells has been sufficiently indicated in the figures referred to; there are rarely more than two dorsal aortic cells on each side of a section, the differentiation of these will be illustrated in a future study of the aorta itself.

The aortic cells do not appear suddenly. At 26 somites (Figs. 6B, 6D, etc.) a break is apparent, in most sections, in the line of the future dorsal aorta, (the breaks together forming a tunnel) the cells about the break have small nuclei and a large amount of cytoplasm; at 32 somites the aortic cells are not only clearly endothelial but tend to line the tunnel. In the light of later stages the break referred to can be distinguished in many sections at 22 somites (Fig. 5), but it is questionable whether the cells about it have undergone much differentiation. The differentiation of the cells of the dorsal aorta thus goes hand-in-hand with that of the cells known to be derived from the portion moyenne, and the dorsal aorta is recognizable before the ventral aortic cells have taken up their definitive position. Finally, the history of the carotid artery is similar to that of the dorsal aorta, and, at 32 somites, the cells of the ventral and dorsal aorta are continuous around the front of the mandibular pouch through the mandibular aortic arch.

This entire chain of circumstances suggests very strongly that the dorsal aorta and carotid of the anterior region, like the ventral aorta and endocardium, arise from the portion moyenne.

In the middle region (of the three before mentioned) there is also isolation of the cells adjacent to the medial borders of the lateral plates to form the portion moyenne. Soon after the portion moyenne (which is here less bulky than in the anterior region) ceases to be recognizable the differentiation of the cells about to form the dorsal aorta can be followed in exactly the same manner as in the anterior region. Here are two sets of facts, the disappearance of the portion moyenne, and the gradual differentiation of the dorsal aorta nearer to the mid-line; they are, of course, not necessarily dependent on one another, but considered in connection with the circumstances in the anterior region they may be looked upon as suggestive.

By reference to Fig. 9 it will be seen that the dorsal aorta in the anterior and middle regions is always situate *medial* to the line of the lateral margin of the entodermal pharynx (usually very much so). Figs. 3B, 3C, 3E and 3F show that the portion moyenne is always in a position lateral to the above mentioned line. From these two facts it follows that although the conditions in the anterior

and middle regions suggest the origin of the dorsal aorta from the portion moyenne, any attempt to prove this supposition is met with the difficulty that the aortic cells are known to differentiate *in situ*. To trace a few undifferentiated aortic cells in their migration through mesodermal cells which they closely resemble would scarcely be possible by ordinary embryological methods.

In the posterior region the cells adjacent to the medial borders of the lateral plates are not isolated, as in the anterior and middle regions, to form portion moyenne. Fig. 9 shows that the aorta in the posterior region is situate immediately dorsal to the lateral margin of the entodermal pharynx, and, in the posterior part of this region, just dorsal to the medial margin of the lateral plate as well (sections from this part of the posterior region are shown in Figs. 3G and 5G; 7G is slightly anterior to it). The aorta is thus placed exactly in the line of the mesodermal cells adjacent to the medial borders of the lateral plates *apparently arises directly from them.*

In the foregoing notes an attempt has been made to bring together some evidence bearing on the development of the vascular endothelium of the head in order to arrive at a conception of the nature and relations of the endocardium. The evidence in question appears to justify the statement that the endocardium in shad arises from mesodermal cells which are found, after differentiation of the lateral plates, to be placed in the region bordering on the medial margins of the latter; further, that the mesodermal cells in this situation appear to be given up exclusively to the formation of vascular endothelial cells of which the endocardium only forms a part.

A few words may be added by way of re-examination of the "Portion moyenne du mésoblaste" of Swaen and Brachet. In the anterior pericardial region the cells which later form the endocardium must necessarily be separated from the somital portion of the mesoderm, for the former eventually take their place as endocardium ventral to the lateral plates, while the latter retains its position dorsal to them. In the middle and posterior pericardial regions, separation of the cells bordering on the lateral plates is not a necessity, for neither region produces endocardium. Nevertheless, separation occurs in the middle region but not in the posterior; the dif-

ference in behaviour of the cells in question does not necessarily depend on the nature of the cells themselves, but appears to be due to conditions occurring in the middle region which are not found in the posterior. It may be said that the portion moyenne appears to consist in the main of cells which will later form vascular endothelium, but it is difficult or impossible to show that in the anterior region it includes all of these, or that in the middle region it does not include more. In other words, although the portion moyenne forms a well defined group of cells which is of great assistance in following the movements of the endocardium, it does not appear, in itself, to be a structure of real morphological importance.

Greil, in his recent paper on the origin of the blood and blood-vessels, '08, traces the origin of the endocardium in Ceratodus, and in some amphibia and selachii, from two sources which he distinguishes as the Angioscleroblast and Angiohæmoblast. Greil does not refer to teleosts in this connection, but his statement gives additional interest to the description by Boeke, '03, of a two-fold origin of the endocardium in Muræna. Boeke describes the major part of the endocardium as arising from cells developed in the head-region, but traces the origin of some of the cells lining the venous end of the heart from the region of the closing blastopore (the latter cells would seem to correspond to those described by Greil as emanating from the hæmangioblast). I have looked carefully for cells corresponding to those described by Boeke as arising from the region of the blastopore without result, and believe that any cells which may migrate forward from this region in shad must be arrested posterior to the junction of the first body somite with the head.

It is intended to re-investigate the origin of the aorta in the head, and to look for additional evidence regarding the origin of the jugular veins, and afterward to study the origin of the vascular endothelium of the body vessels.

PERIOD 2. LASTING UNTIL RHYTHMICAL CONTRACTION BEGINS IN THE PARTIALLY FORMED HEART-TUBE.

Between the stages of 30 and 32 somites, the heart anlage has undergone little change; at 33 somites the beginning of progress

becomes apparent. In mapping out the heart anlage (see page 231) it has been shown that the splanchnic mesoderm contained within a triangle drawn with its apex at the aortic region and its base near the anterior end of the pericardial plate would correspond to the future conus ventricle and atrium. By referring to Figs. 7B, 7C and 7D it will be seen that the splanchnic mesothelial cells included in this triangle are columnar in shape, while those lateral to the triangle are much flatter. Passing forward from the region 7B, the cells within the triangular area diminish in height until they become cubical, and finally, near the anterior limit of the pericardial plate, quite flat.

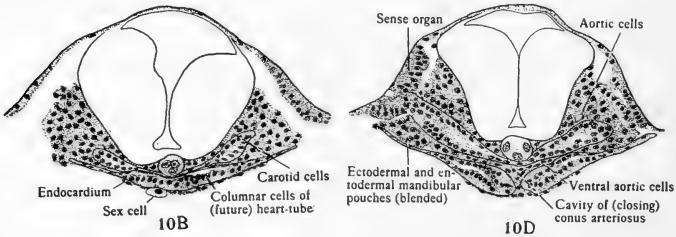


Fig. 10.—Two transverse sections of shad, stage of 34-35 somites × 100 diams. 10B passes through the mid-sagittal plane of the embyro anterior to the mandibular pouch, 10D midway between the mandibular and hyoid pouches. The left side of the figures represents a region anterior to that seen on the right. Fixation by Perenyi's method.

The splanchnic mesoderm included within the triangular area is soon to form a tube, which may provisionally be called the heart-tube; the columnar mesothelial cells will form the myo-epicardium.

Before formation of the heart-tube begins, the triangular area spoken of undergoes a migration toward the left, carrying with it the underlying endocardium; this is the movement which begins at 33 somites. The sinistral movement of the "columnar area" of the splanchnic mesoderm and of the endocardium, which begins at 33 somites, is very pronounced an hour later at the stage of 34-35 somites from which Fig. 10B is taken. Reference to Fig. 10D shows that in the aortic region there has been no sinistral movement; this

movement takes place around the aorta as a center, and becomes progressively greater as the base of the triangle referred to is reached. In the region of Fig. 10B (anterior to the mandibular pouch) the columnar area of the splanchnic mesoderm, being about two-thirds to the left of the mid-line, has accomplished its migration. The movement of the columnar area of the splanchnic mesoderm is not quite simple; it is accompanied, and partly brought about, by a slighter movement to the left of the entire pericardial plate (see Fig. 10B). The slighter sinistral movement of the entire pericardial plate began as early as the stage of 32 somites (see Fig. 7B).

In Fig. 10D the initiation of heart-tube formation can be recognized. Union is about to take place between the splanchnic mesoderm in the region of the original medial margins of the right and left lateral plates. This union takes place around the lateral and posterior circumference of the aortic root. The splanchnic mesoderm to the right and left of the aortic root is seen in Fig. 10 D to be somewhat prominent ventrally, so that, by uniting in the mid-line (which it is just about to do), it will enclose a small chamber, the conus arteriosus; the latter contains a small amount of endocardium directly continuous with the endothelium of the aorta.

At a stage of 36 somites (see Fig. 11, one hour older than the preceding stage) the formation of the heart-tube has made considerable progress. The arterial extremity of the heart-tube has been formed by the blending of the splanchnic mesoderm about the circumference of the aortic root symmetrically in the mid-line. The remainder of the heart-tube is also undergoing formation by the blending of splanchnic mesoderm on either side. The axis about which the further blending of splanchnic mesoderm occurs corresponds to a straight line connecting the middle of the base of the triangle spoken of with its apex. The apex of the triangle corresponds in position with the aortic root; the middle of the base is now placed (owing to rotation of the triangle to the left around its apex) quite near to the left side of the anterior margin of the pericardial plate. The axis, then, along which union of the splanchnic mesoderm is proceeding, extends from the (medially placed) aortic region forward; as it passes forward it diverges to the left so as to form an acute angle with the mid-sagittal plane of the embryo.

In the formation of the heart-tube anterior to the aortic region, the splanchnic mesoderm of the right side undergoes active movement, while that of the left remains comparatively passive. The splanchnic mesoderm to the right of the heart-tube axis arises abruptly from the somatic layer to form a crest which moves over to the left; this crest becomes imminent and falls to the left somewhat in the manner of a wave breaking upon the shore (see Fig. 11C). The splanchnic mesoderm to the left of the axis rises slightly to meet the splanchnic mesoderm from the other side as the latter falls; between the two a tube of splanchnic mesoderm is formed of which the ventral wall is derived mainly from the right side, and the dorsal wall mainly from the left.

Fig. 11 shows the heart-tube in process of formation, as indicated by a reconstruction of the stage of 36 somites; contact of the two sides has occurred at the posterior (arterial) end. The splanchnic mesoderm on each side (right particularly), for some little distance anterior to the contact area, shows evidence of preparation for bending in the manner described above (Fig. 11C). Heart-tube formation is now in rapid progress; the posterior (arterial) end now being complete, the venous end will be progressively formed, from behind forward, along an axis deviating to the left.

The irregular black line in Fig. 11 indicates the outline of the endocardium; a small quantity of the latter has been included in the heart-tube.

The heart shown in Fig. 11 (such as it is) is contracting rhythmically, and has been doing so for some 10 or 15 minutes. The heart which was quiescent at the stage of 35 somites began beating (after very little preliminary oscillation) at a rate of 52 beats per minute, about 15 minutes before the thirty-sixth somite was completely marked off.[17]

It may be questioned whether the rhythmical contraction of the

[17]Water temperature 62° F. (July 11, 1907). Some evidence has been obtained which suggests that in higher water temperatures the heart begins to beat at an earlier stage of development (as estimated by the number of somites). In order to exclude the possibility of a miscount of somites the data on which this evidence rests require to be controlled by comparison with the results of further observations, preferably made on eggs of another species.

heart anlage is of assistance in the folding over of the splanchnic mesoderm to form a tube; that it is not essential to this process is

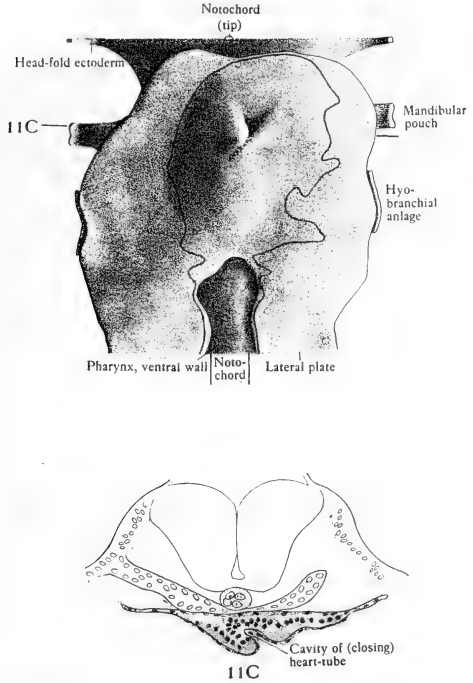


Fig. 11.—Ventral surface of a reconstruction of the pericardial region of shad, stage of 36 somites × 100 diams.

The heart-tube is in process of formation, contact of the two sides has occurred along the broken line. The continuous black line encloses the area covered by endocardium. Fixation by Perenyi's method.

indicated by the fact that a stage of 35 somites (half an hour earlier) the folding over has already commenced, although it is not so advanced as at 36 somites.

PERIOD 3. IN WHICH THE HEART-TUBE IS COMPLETED TO FORM CONUS, VENTRICLE AND ATRIUM, AND ASSUMES THE ADULT POSITION.

Stage of 42 somites.

Fig. 12 is from a reconstruction of the ventral surface of the pericardial region of an embryo of 42 somites. The ventral wall of the pericardial cœlom is very thin and to some extent moulded upon the heart-tube, so that the outlines of the latter are clearly indicated. A portion of the splanchnic mesoderm forming the ventral wall of the pericardial cœlom has been removed over an area mainly to the right of the mid-line. In the area referred to a portion of the heart wall and of the somatic mesoderm are seen near the mid-line. Toward the lateral region of this area the somatic mesoderm and some of the mesoderm of the pharyngeal floor are represented as having been removed in order to uncover the ventral aspect of the entodermal pharynx and of the ventral aorta.

The heart-tube, which has only just been completed, is cone-shaped; the (venous) base of the cone is directed anteriorly and to the left. There is, as yet no external indication of separation into conus, ventricle and atrium, but the wall becomes progressively thinner in passing from the arterial to the venous end.

The splanchnic mesoderm, not included in the heart-tube, forms the ventral wall of the pericardial cœlom; it becomes continuous with the wall of the hart-tube at the venous end of the latter. In closing, the two sides of the heart-tube do not appear to blend where the splanchnic mesoderm first meets; some further adjustment occurs in order to bring the columnar area of the latter (and the endocardium in contact with this) within the limits of the tube. Completion of the heart-tube is effected by the blending of splanchnic mesoderm of the right and left sides, and continuity of the ventral wall of the pericardial cœlom is maintained by a similar process; the two processes together entail loss of the ventral mesocardium.

Comparison of Figs. 11 and 12 shows there is a large amount of endocardium not included in the heart-tube; also that, after closure of the latter, the endocardium tends to move over to the left side.

Before passing from the stage of 42 somites to that of 6.2 mm. (the next one to be examined) it will be necessary to make a digres-

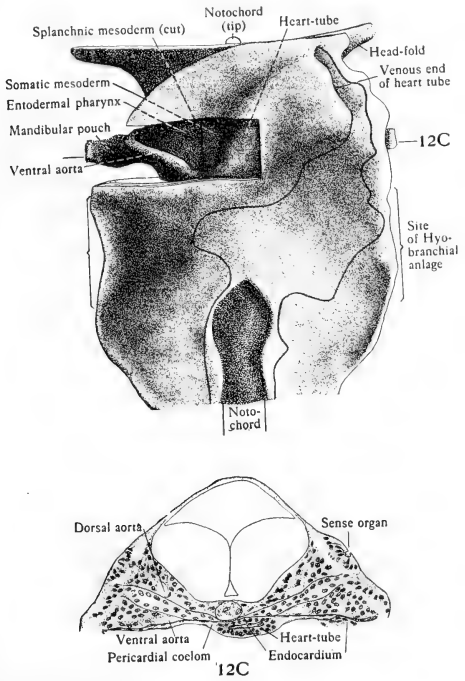


Fig. 12.—Ventral surface of a reconstruction of the pericardial region of shad, stage of 42 somites × 100 diams.

Over an area mainly to the right of the mid-line the ventral wall of the pericardial cœlom has been removed to show heart-tube and somatopleure; also, laterally (where somatopleure has been removed), the pharynx and ventral aorta. The continuous black line encloses the area covered by endocardium.

sion; firstly, to correlate the stages as estimated by the number of somites with those designated by the length in millimeters; secondly, to mention some points in connection with the general circulation.

Correlation of stages.

The embryo becomes sufficiently straight to yield a satisfactory end-to-end measurement when it has about 51 somites; its length is then 3.6 mm. From this time on the stages are designated by the length in millimeters; it may be mentioned that the last somite (59th or 60th) is formed at the stage of 5.2 mm.

Mechanism of the circulation at different stages of development.

Prior to the stage of 42 somites there has been no true circulation, because the aorta opposite the first three somites, late in being formed, is still wanting. *At 42 somites* the aorta is practically complete as far back as the anus; posteriorly it bifurcates, and the two vessels form a loop encircling the gut and join the subintestinal vein. The blood plasma (for there are no corpuscles) flows from the heart into the aorta, turns forward near the anus, into the subintestinal vein, which, in turn, discharges it on to the posterior pole of the yolk. The plasma flows ventral and lateral to the yolk in a wide channel between the ectoderm and the yolk-periblast and, following the contour of the yolk, enters the venous end of the heart-tube. The plasma is not in contact with the periblast ventral to the peritoneum, for it is excluded from this situation by the attachment of the lateral margins of the latter to the yolk; as far as can be determined the periblast elsewhere is bathed in plasma. The caudal aorta and vein are as yet unformed, but a cord of cells ventral to the notochord in the tail represents these vessels together with the blood-anlage. The jugular and cardinal veins are not yet developed; there is no liver.

The jugular veins have reached completion at a stage of 4.4 mm., and discharge their blood upon the yolk immediately posterior to the vagus ganglia. It may be mentioned that the endothelial cells of the jugular veins have been recognizable for some time prior to the complete formation of the veins themselves, certainly as early as the stage of 42 somites. The place of termination of the jugular veins is a point of great interest, for here the cœlom is separated into its pericardial and its peritoneal portions. The orifices of discharge of the jugular veins are placed laterally to the lateral

plates in a situation approximately corresponding to the lower end of Fig. 12. The cœlom anterior to the orifices become pericardial; posterior to them it becomes peritoneal; the cœlom between the two orifices ceases to exist.[18] Since separation of the pericardial from the peritoneal portion of the cœlom occurs at a situation in which the lateral plates are still separate, the posterior end of the pericardium extends farther backward on each side than in the mid-line.

At the *stage of 6.2 mm.* the caudal aorta and caudal vein replace the anterior end of the cord of cells found in the tail at 42 somites, and the blood contains a very few corpuscles. Blood passes from the dorsal aorta through the short caudal aorta and then forward through the caudal vein. The caudal vein meets the cardinals[19] near the anus, and from the point of junction two veins pass ventrad (embracing the gut) to join the subintestinal vein; through these two veins most (or all) of the blood from the caudal vein enters the subintestinal.

The subintestinal vein is now involved in the rapidly growing liver; its extreme anterior end (*vena revehens* of liver) is free, and discharges its blood ventral to the peritoneum. Blood is now retained in the space ventral to the peritoneum (supravitelline sinus) by the very agency which formerly prevented its flowing there, *i. e.,* by the lateral attachment of the peritoneum to yolk. At the site of discharge of the jugular veins the blood from these meets that flowing from the supravitelline sinus; and the blood from these two sources enters the venous end of the heart. There appears to be no special mechanism for retaining the blood in the space between the ventral wall of the pericardial cœlom and the yolk, for the pericardial plate is attached peripherally to the ectoderm and not to yolk.

The condition of *the circulation in the stage described in the introduction* differs from that in the stage just described in that the cardinal veins are fully formed. The blood from the, now practi-

[18]Conditions at the site of discharge of the jugular veins are much complicated by the fact that the mesenchyme of the pectoral fins is arising from the somatic mesoderm in this region. A more thorough study will be undertaken later in connection with the veins themselves.

[19]The cardinal veins, at this stage extend only from the anus as far as the anterior end of the liver. I am unable to determine their functions in connection with the general circulation.

cally complete, caudal vein is received entirely by the cardinals. The subintestinal vein has lost all connection with the caudal and the cardinals, and is now the portal vein.

Stage of 6.2 mm.

Fig. 13 represents the ventral surface of a reconstruction of the pericardial region of an embryo of 6.2 mm.; the reconstruction extends further forward than those shown in the previous figures; it, in fact, includes the whole of the anterior part of the head which remains at this time in contact with the yolk. The ventral wall of the pericardial cœlom has been partially removed to show the condition of the heart-tube. The conus, ventricle and atrium are now quite distinct, but there is no prominence on the ventricle corresponding to its future apex. There is evidence, at this stage, that the ventral wall of the pericardial cœlom is attached, rather extensively, to the yolk just to the right of the venous orifice of the atrium. Over the area of attachment no endocardium is present; the exact distribution of endocardium over the remainder of the ventral wall of the pericardial cœlom is difficult to make out owing to the extreme tenuity of the latter.

The venous end of the atrium has moved forward and is now placed ventral to the posterior half of the left eye (it was altogether posterior to the eye in the preceding stage); the anterior end of the pericardial plate has moved forward even more than the atrium and now *appears* to have reached the anterior limit of the head-fold. There are several changes going on, however, which tend to complicate matters by shifting former landmarks; these changes can be partially appreciated by reference to Figs. 13 and 14. In the first place the head is rising from the yolk: this is accomplished by forward growth of the head, by shrinking of the yolk, and by a horizontal separation of the head-fold into its original two layers. The dorsal and ventral layers of the head-fold are now being added to the surface ectoderm (basal layer) of the continuous regions of head and yolk respectively. In the second place the, now separating, head-fold is moving bodily backwards so that it approaches the mandibular pouch; the latter shows evidence of antero-posterior com-

pression and is, in its turn, crowded back so as to approach the hyomandibular anlage. The backward movement of the above mentioned structures produces an effect of advance in the tip of the notochord, this is relative only and not actual.

Having found that the heart-tube represents conus, ventricle and atrium, it remains to be seen how the different parts of the wall of the pericardial cœlom attain their definitive positions; this can be most conveniently studied from the left side.

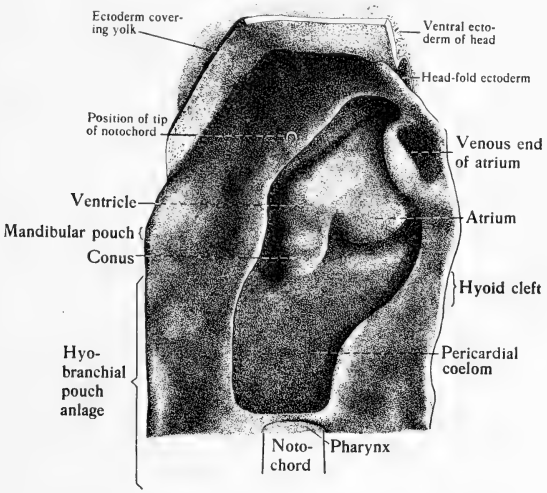


Fig. 13.—Ventral surface of a reconstruction of the pericardial region of shad, stage of 6.2 mm. × 100 diams.

Part of the ventral wall of the pericardial cœlom has been removed to uncover the conus, ventricle, and atrium.

To make a satisfactory drawing of the heart at this, and the following, stage, from the left side would be very difficult; a complete view of the organ is obstructed partly by the dorsal and partly by the ventral wall of the pericardial cœlom. The parts considered essential to the elucidation of the changes about to occur are, therefore, shown diagrammatically and the less important parts eliminated altogether.

In Figs. 14 and 15 the heart has been almost entirely omitted, the position of the venous orifice of the atrium (as it lies near the surface of the yolk) is indicated by a broken line; the other structures shown are indicated as they would appear in a mid-sagittal section of the embryo.

Fig. 14 is a diagram made from the reconstruction used for Fig. 13, the left side is represented. The relations of the head-fold ectoderm to the surface have been approximately determined by comparison with earlier stages, and are indicated in the diagram. The line of separation of head from yolk is indicated externally, in the

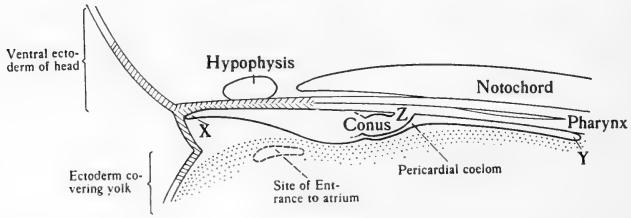


Fig. 14.—Diagram made from the reconstruction shown in Fig. 13 as seen from the left side, × 100 diams.

The venous end of the atrium is indicated by a broken line; other structures are represented as cut in the mid-sagittal plane. Ectoderm belonging to the original head fold is shaded. The yolk is stippled.

embryo, by a U-shaped groove; the anterior part of this groove (corresponding to the cross-piece of the U) appears in section in Fig. 14. The anterior margin of the pericardial plate is attached to the head-fold ectoderm and is, at present, dorsal to the groove referred to (see Fig. 14); the lateral margins of the pericardial plate are attached to the surface ectoderm just ventral to the groove. The pericardial cœlom is, as in previous stages, dorso-ventrally compressed; three points in its wall will require to be noticed in the following stages. The points referred to are: the anterior end, the posterior end and the aortic root (apex of conus arteriosus); these are indicated, respectively, by the letters X, Y and Z.

Stage of 7.3 mm.

Fig. 15 is a diagram, made in the same way as Fig. 14, from a reconstruction of the pericardial region of an embryo of 7.3 mm.

The head fold proper no longer exists, having been absorbed into the basal ectoderm of the surface. The pharynx is dilated, and the oral plate is soon to be perforated. Antero-posterior compression of the head is still more marked than in the preceding stage, and is accompanied by the formation of a head-bend of the mid-brain

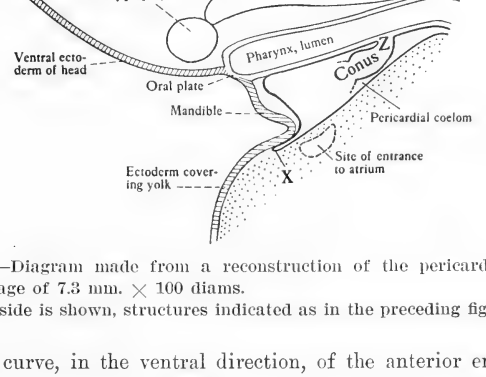


Fig. 15.—Diagram made from a reconstruction of the pericardial region of shad, stage of 7.3 mm. × 100 diams.

The left side is shown, structures indicated as in the preceding figure.

and of a curve, in the ventral direction, of the anterior end of the notochord. There is an ectodermal band connecting the lateral margin of the anterior end of the entodermal pharynx, on either side, with the basal ectoderm of the surface. The ectodermal bands referred to represent the posterior margin of the original head-fold ectoderm, and have a developmental history similar to that of the head-fold proper (see footnote on page 227.) These ectodermal bands and the mandibular anlage (combined entodermal and ectodermal mandibular pouches) are now undergoing disintegration.

The heart differs from that of the preceding stage in that the apex of the ventricle is well-marked.

Fig. 15 shows (compare with Fig. 14) that the ventral layer of the head-fold, in completely separating from the dorsal layer, has

carried with it the (attached) anterior periphery of the pericardial cœlom. The point X has, therefore, moved in a ventral direction, and is now placed between ectoderm and yolk some distance below the head of the embryo. (The point Y, which has not altered its position, is not shown.) The groove between the head and yolk is now deep and, anteriorly, very narrow. The ectoderm bounding the groove impinges upon the dorsal wall of the pericardial cœlom and constricts it. Accompanying this constriction there is a diminution in the area of the ventral wall of the pericardial cœlom, and the venous orifice of the atrium is brought nearer to the mid-line. The, somewhat dome-shaped, part of the pericardial cœlom dorsal to the constriction (and, therefore, on the side of the groove towards the head) contains the conus and ventricle. The lateral periphery of the pericardial cœlom, together with the venous orifice of the atrium, is ventral to the constriction (and, therefore, on the side of the groove towards the yolk).

Stage of 8.75 mm., and a comparison of the heart with that of the stage described in the introduction.

Fig. 16 represents a reconstruction from an embryo of 8.75 mm. The reconstruction, which is shown from the left side, was made from sagittal sections. The last section (on the left side) passes through the yolk on a level with the left margin of the pericardial cœlom, and fails to complete the left wall of the pharynx.

In the preceding stage the groove between the head and yolk was narrow and nearly horizontal; the periphery only of the pericardial cœlom was ventral to it. The groove in question at this stage has become, by reason of the recession of yolk from head, oblique and much wider. Practically the entire pericardial cœlom is now on the yolk side of the main axis of the groove, and radical changes have taken place in the arrangement of its walls. The point X has moved so far back that the somatic mesoderm between X and Z has been stripped from the ventral wall of the pharynx and will form the ventral parietal pericardium (compare Figs. 16 and 17). The somatic mesoderm between the points Z and Y has remained stationary and will form the dorsal parietal pericardium. The

splanchnic mesoderm between the points X and Y, which has hitherto formed the ventral wall of the pericardial cœlom, has undergone considerable contraction and is stouter than before; it will form the anterior wall of the sinus venosus (compare Figs. 16 and 17).

Between the stage of 8.75 mm. and that of 114 hours, noticed in the introduction (see Fig. 17), the heart is brought into the adult position; the arterial end is fixed (by the ventral aorta) to the floor of the pharynx, while the venous end follows the retreating yolk.

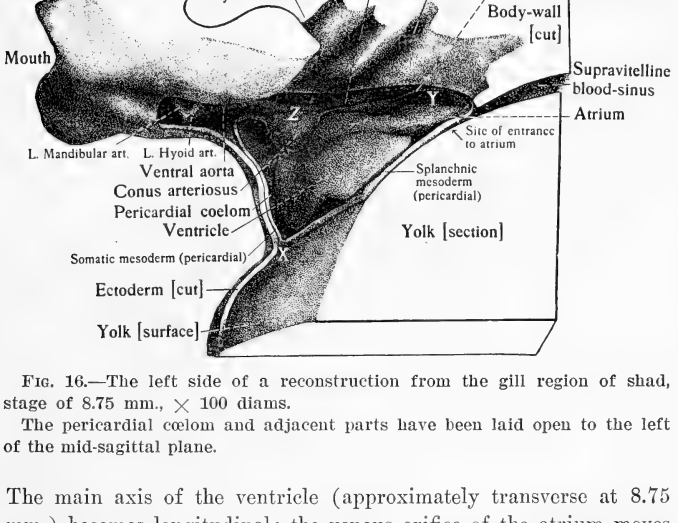


Fig. 16.—The left side of a reconstruction from the gill region of shad, stage of 8.75 mm., × 100 diams.

The pericardial cœlom and adjacent parts have been laid open to the left of the mid-sagittal plane.

The main axis of the ventricle (approximately transverse at 8.75 mm.) becomes longitudinal; the venous orifice of the atrium moves from left to right until it reaches the mid-sagittal plane of the embryo.

On comparing the parts of the pericardium and heart, as described in the introduction (Fig. 17), with those of the original heart anlage (Fig. 7), it will be seen that the entire parietal pericardium has been derived from somatic, and the myo-epicardium from splanchnic

mesoderm. Part of the original splanchnic mesoderm was employed in the formation of the tube which has become the conus, ventricle and atrium; the remainder (hitherto referred to as the ventral wall of the pericardial cœlom) is now about to take part in the formation of the sinus venosus.

PERIOD 4. FORMATION OF SINUS VENOSUS AND HEPATIC VEIN.

Fig. 17 is from an embryo 10.67 mm. in length, designated (for reasons already stated) the stage of 114 hours. The structures shown have been briefly mentioned in the introduction; it is now necessary to describe them more fully.

The portion of the reconstruction posterior to the venous orifice of the atrium may be divided into two regions by a vertical transverse plane passing through the orifices of discharge of the cardinal veins. The region anterior to the plane mentioned is the site of the future sinus venosus and may be called the sinus-venosus region; the region posterior to it corresponds to the anterior end of the supravitelline sinus and may be called the hepatic-vein region.

In the sinus-venosus region the peritoneal cœlom has extended forwards dorsal to the pericardial cœlom. The peritoneal cœlom is here small (and remains so) being practically confined to the region dorsal to the gut. The splanchnic mesoderm extending between the points X and Y is to form the anterior wall of the sinus venosus. The part of this immediately to the right of the venous orifice of the atrium is known to have been attached to the yolk since the stage of 6.2 mm. The anterior wall of the sinus venosus is now, at the site of attachment, drawn out into a long process referred to below as the "yolk-process" of the sinus venosus. The posterior surface of the anterior wall of the sinus venosus is in contact with (and possibly attached to) the yolk around its ventral and lateral periphery. Endocardium lines the posterior surface of the anterior wall of the sinus venosus where the latter is not in contact with yolk, and, at this stage, is beginning to migrate from the yolk process on to the yolk itself (see Fig. 18). Elsewhere the yolk is entirely destitute of endocardium (see Figs. 19, 20, 21 and 22). The yolk process, extending obliquely up-

ward from the anterior wall of the sinus venosus to a projection on the yolk (dorsal to the anterior pole of the latter) forms an

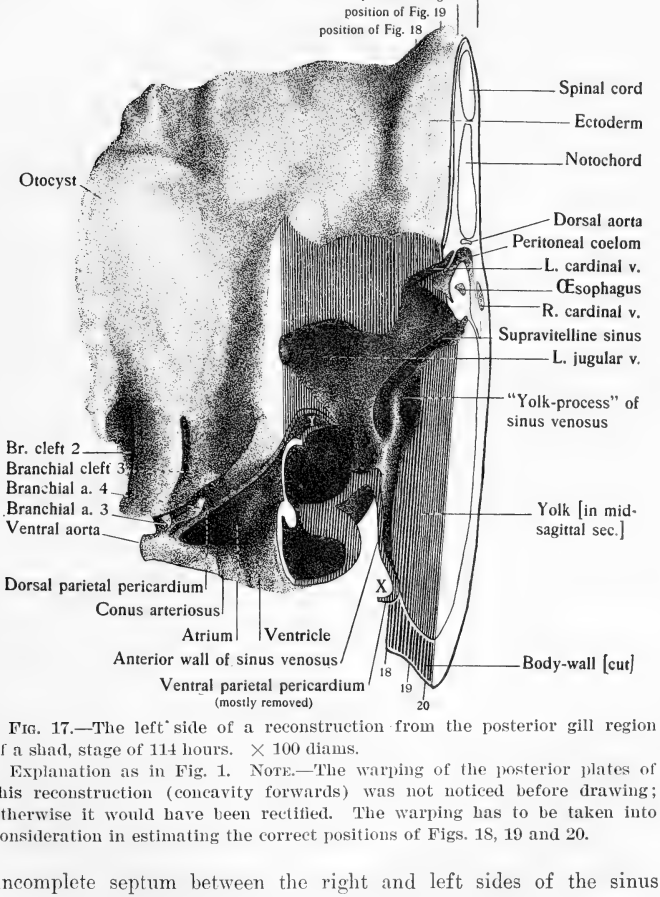


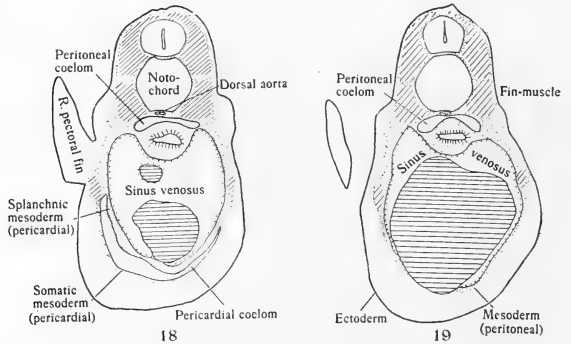
Fig. 17.—The left side of a reconstruction from the posterior gill region of a shad, stage of 114 hours. × 100 diams.

Explanation as in Fig. 1. Note.—The warping of the posterior plates of this reconstruction (concavity forwards) was not noticed before drawing; otherwise it would have been rectified. The warping has to be taken into consideration in estimating the correct positions of Figs. 18, 19 and 20.

incomplete septum between the right and left sides of the sinus venosus. The two sides of the sinus venosus are in communica-

tion both anterior (dorsally) and posterior (ventrally) to the yolk process.

Conditions in the hepatic-vein region become more readily intelligible after preliminary examination of a section from 0.02 mm. posterior to the reconstructed part of the embryo. Here (Fig. 21) the peritoneal mesoderm (in which the cœlom is to a large extent virtual) covers the yolk laterally and dorsally and is generally in contact with it. Contact between the yolk and peritoneum is interrupted on the left side by the interposition of



Figs. 18 and 19.—Two transverse sections of which the positions are marked on Fig. 17. × 50 diams.

Yolk horizontally shaded, voluntary muscle obliquely shaded. The distribution of vascular endothelium, including endocardium, is indicated by large dots.

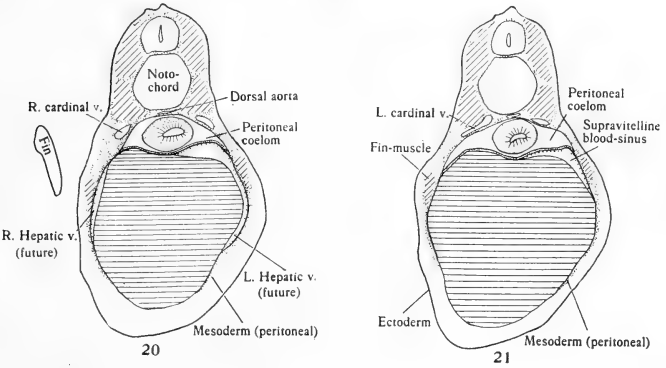
the supravitelline blood-sinus (see introduction). In passing tailward in the series from the position of Fig. 21, the yolk increases in size and the supravitelline sinus becomes more dorsally placed with regard to the yolk, but is always mainly to the left of the mid-sagittal plane.

In Fig. 20, from the hepatic-vein region of the reconstruction, contact between the peritoneum and yolk is interrupted on both right and left sides. The interruption on the right of the figure (left of embryo) corresponds to the future left hepatic vein and

is a direct continuation forward of the main supra-vitelline sinus. The interruption on the left side of the figure (right of embryo) corresponds to the future (smaller) right hepatic vein, which is not at present in direct communication with the supravitelline sinus. The future right hepatic vein communicates anteriorly with the future sinus and posteriorly ends blindly.

The dorsal mesentery in this stage, as in the adult, is absent in the region anterior to the stomach.

Fig. 22 is a left lateral view of a reconstruction of the posterior



Figs. 20 and 21.—Two transverse sections of which the positions are marked on Fig. 17. × 50 diams.

Yolk and voluntary muscle shaded and vascular endothelium dotted as in the preceding figure.

gill region of a shad of 166 hours (9.46 mm. in length). At this stage the peritoneal cœlom in the hepatic-vein region is so large that it can be opened by merely removing the body-wall; the anterior pole of the yolk is shown *in situ*. The plane of separation between sinus-venosus and hepatic-vein regions is indicated, as before, by the point of discharge of the left cardinal vein.

It will be convenient to consider the hepatic-vein region first. In this region the peritoneal cœlom has undergone great expansion by reason of the extensive separation of splanchnic from somatic meso-

derm (compare Figs. 20 and 24). The shrinkage of the yolk has had an effect on the shape of the future hepatic veins; the latter are now semilunar rather than crescentic in section. The ventral

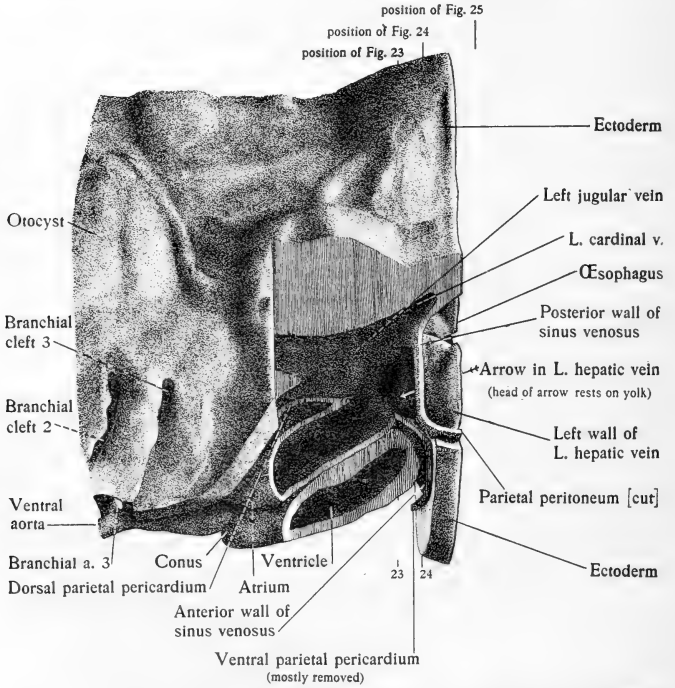


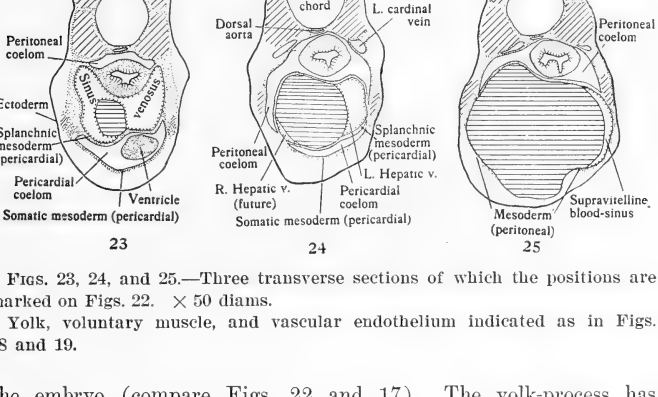
Fig. 22.—The left side of a reconstruction from the posterior gill region of shad, stage of 166 hours. × 100 diams.

Sufficient body-wall has been removed to open the pericardial cœlom laterally and ventrally and the peritoneal cœlom laterally. The anterior pole of the yolk is intact, the ventricle and atrium have been opened. The vein immediately ventral to the left jugular is the left inferior jugular.

part of the pericardial cœlom has extended backward, so that the endocardium lining the anterior wall of the sinus venosus assists in the formation of the hepatic veins (see Fig. 24). The expansion

of the peritoneal cœlom affects only the hepatic-vein region (Fig. 22). It ends so abruptly that the splanchnic peritoneum, passing peripherally from the anterior ends of the hepatic veins to become somatic, forms a partition between sinus-venosus and hepatic-vein regions. This partition of splanchnic peritoneum represents the posterior wall of the sinus venosus, and has been labelled accordingly in Fig. 22.

In the sinus-venosus region shrinkage of the yolk has brought about marked diminution in the vertical dimension of this part of



Figs. 23, 24, and 25.—Three transverse sections of which the positions are marked on Figs. 22. × 50 diams.

Yolk, voluntary muscle, and vascular endothelium indicated as in Figs. 18 and 19.

the embryo (compare Figs. 22 and 17). The yolk-process has become thicker and much shorter; the space between its posterior surface and the yolk (which constituted the posterior communication between the right and left sides of the sinus venosus) is now obliterated.

The most striking change taking place at this stage occurs, alike, in the sinus-venosus and hepatic-vein regions; it is a process of rearrangement of the vascular endothelium. The vascular endothelium is rapidly spreading from the splanchnic mesoderm, both pericardial and peritoneal, on to the yolk so as to exclude the latter from the

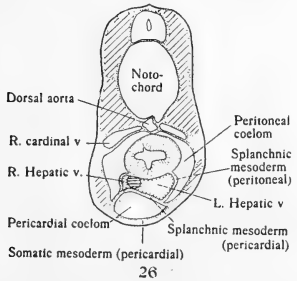
vascular system (see Figs. 23 and 24). This process has already progressed so far that the sinus venosus has a complete lining of vascular endothelium, which is true also of the adjacent part of the left hepatic vein. The yolk is still uncovered in the region of Fig. 25.

The sinus venosus, since the yolk has been consigned to a position definitely external to it, may now be looked upon as a complete structure in which the achievement of the adult condition is merely a matter of detail. The layers of splanchnic mesoderm, pericardial and peritoneal, which have been called the anterior and posterior walls of the sinus venosus are, to some extent, in mutual contact just behind and above the apex of the ventricle (see Fig. 22). Where these two layers are in contact the terms anterior and posterior wall of sinus venosus are not strictly applicable, for they together form the anlage of the pericardio-peritoneal septum. During the process of disappearance of the yolk, the pericardio-peritoneal septum undergoes further increase, and later gowth produces the extensive structure of the adult.

A few words may be added with regard to the completion of the hepatic vein. The conditions indicated in Figs. 24 and 25 are tending in a direction which ends in the complete formation of the hepatic vein, as shown in Figs. 26 and 27. The latter figures come from sections of an embryo eight days old, and correspond in position to Figs. 24 and 25. In order to understand the condition of the hepatic vein at eight days it is necessary to appreciate the following facts: The yolk is not only smaller at its equator but the distance between the poles is also much diminished. The liver, which has grown forward so as to keep pace with the posterior pole of the yolk, is now very much nearer the heart than before. Redistribution of vascular endothelium, excluding the yolk from the vascular system (see Figs. 26 and 27), has now occurred throughout the entire extent of the yolk (*i. e.*, from heart to liver).

The hepatic vein is, at this time, rather a long vessel and consists of a main stem bifurcating anteriorly into short right and left branches; both stem and branches replace the original supravitelline blood sinus. The left branch formerly transmitted all the blood

passing from the liver to the sinus venosus; this function is now shared by the right branch. At eleven days the yolk has entirely disappeared; the final disappearance of the (latterly torpedo-shaped) yolk leaves the anterior pole of the liver still some distance posterior to the pericardio-peritoneal septum. The liver now grows forward (somewhat slowly) into the space formerly occupied by the yolk and, therefore, receives its peritoneal covering from the splanchnic mesoderm formerly enveloping the yolk and supravitelline sinus. The advancing liver receives into itself the entire main hepatic stem and the adjacent parts of the right and left branches. Finally, the anterior pole of the liver reaches the posterior surface of the peri-



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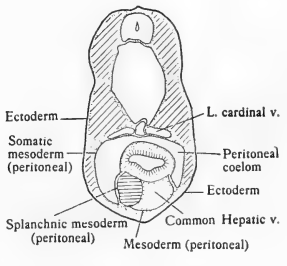

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Figs. 26 and 27.—Transverse sections of shad, stage of 8 days. × 50 diams. The transverse diameter of the sections is diminished from a tendency of the notochord to collapse. Yolk, voluntary muscle, and vascular endothelium as in Figs. 18 and 19.

cardio-peritoneal septum and protruding from its anterior end are the right and left hepatic veins of the adult which transmit blood into the sinus venosus.

The vascular endothelium lining the heart and the hepatic veins has been derived from two sources: Firstly, from the vascular endothelium (of which the origin has been described in the section dealing with the formation of the heart anlage) which has been referred to as endocardium. Secondly, from the vascular endothelium originally lining the roof of the supravitelline sinus, of which the origin has not been studied.

The endocardium originally lined the ventral surface of the splanchnic mesoderm anterior to the junction of the head with the first body somite. Of the splanchnic mesoderm lined ventrally by endocardium the (larger) part anterior to the site of discharge of the jugular veins became pericardial and the (smaller) part between the site of discharge of the jugulars and the first body somite became peritoneal. The disturbance of the original relations between the structures in the posterior head region which occurred between the stage of 7.2 mm. and that of 166 hours has been so extensive that it is very difficult to determine the exact amount of splanchnic peritoneum which was originally situated anterior to the line of junction of the first body somite with the head; nevertheless the account given below of the eventual distribution of the endocardium is probably not very far from the truth. The endocardium lining the ventral surface of the pericardial splanchnic mesoderm was partially included in the conus, ventricle and atrium to line these cavities; the remainder formed the lining of the anterior wall of the sinus venosus. The anterior wall of the sinus venosus assists in the formation of the anterior ends of the right and left hepatic veins (see Fig. 24). The vascular endothelial cells, migrating from the anterior wall of the sinus venosus on to the yolk to furnish the ventral lining of the hepatic veins, are, thus, derived from endocardium.

The splanchnic peritoneum forming the posterior wall of the sinus venosus is so close to the site of discharge of the jugular veins that the vascular endothelial cells lining the posterior wall of the sinus venosus are undoubtedly (like those lining the remainder of the heart) endocardial in origin. Since the ends of the hepatic veins immediately adjoining the sinus venosus are lined ventrally by endocardial cells migrating from the anterior wall of the sinus venosus, there is a great probability that the cells migrating from the splanchnic peritoneum to provide their dorsal lining (see Fig. 24), are also endocardial in origin.

The main stem of the hepatic vein is composed of vascular endothelium which originally lined the roof of the supravitelline sinus, and it is not at all unlikely that the adjoining roots of the right and left hepatic veins (eventually contained within the liver) are of similar origin.

There seems to be nothing unusual in the method of development of the heart valves. The sinu-atrial valves (two, right and left) are developed very late. I have serial sections of an embryo of 114 hours (No. 14,994) in which the heart has apparently ceased to beat during atrial systole. In this specimen the (valveless) venous orifice of the atrium is tightly contracted as by a sphincter. There is little doubt that this specimen indicates the normal mechanism of atrial systole prior to the formation of the sinu-atrial valves.

The true circulation does not begin with the initiation of rhythmical contraction in the, partially formed, heart-tube (36 somites), but with the completion of the aorta (42 somites), which occurs at a time when the heart-tube has been completely formed. Prior to the division of the primitive heart-tube into conus ventricle and atrium, the mechanism of systole of the entire tube is, in all probability, similar to that described for the atrium.

Syracuse, N. Y., November 2, 1908.

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HISTOGENESIS AND HISTOLYSIS OF THE INTESTINAL EPITHELIUM OF BUFO LENTIGINOSUS.

BY

MARY A. BOWERS.

With 4 Plates and 1 Text Figure.

The various phenomena associated with metamorphosis present many problems which are of great biological interest. They have attracted a large number of investigators, and the literature, including all phases of the subject, the macroscopic and microscopic changes which occur at the time of transformation and the experimental and theoretical work on the causes of metamorphosis, has become most voluminous. However, previous to 1906 but two papers had been published on the modification of the tadpole intestine, Ratner, '91, and Reuter, 1900. Ratner describes the changes which occur in the subepithelial layers; Reuter, using Alytes obstetricans, gives a very full account (Part I) of the macroscopic changes of the alimentary tract; (Part II) the degenerative and regenerative modifications of the epithelium. I should mention that Kingsbury, '99, published a brief abstract of work which he had begun on Bufo. Since my work was begun another paper has appeared, by Duesberg, '96, but as his observations and interpretations are not in accord with those of Reuter and as his material, Rana fusca, is different from mine, it seems worth while to state briefly my results.

This investigation was undertaken at the suggestion of Professor E. G. Conklin, and has been carried on in the laboratories of the University of Pennsylvania. I wish to express my sincere appreciation of Professor Conklin's valuable assistance and his kindness to me throughout my work. I am also greatly indebted to Mr. Herbert G. Kribs, who most carefully prepared the photomicrographs and kindly aided me in all my photographic work.

Material and Methods.

The material used for this investigation has been our common toad, Bufo lentiginosus, supplemented by the examination of many series of the bullfrog, Rana catesbiana, and of the green frog, Rana clamata.

The most satisfactory fixing fluids were Flemming's and Zenker's. Paraffin embedding was employed and sections cut 6 2/3 microns thick. Considerable difficulty was experienced in cutting the first series, especially the young stages, owing to the diatoms and sand in the intestine. This trouble was avoided by feeding the tadpoles for a day before killing, on fine yellow corn meal, which replaced the gritty contents. A long series of substances, flour paste, egg (yolk and white), beef juice gelatine, and meat were tried, but were not satisfactory.

The Flemming material was stained with Heidenhain's iron hæmatoxylin and orange G. Delafield's hæmatoxylin (in toto) and eosin (on the slide) were used for the larvæ preserved in Zenker. All figures were drawn with the aid of an Abbé camera lucida. The photomicrographs were taken with violet light.

Living tadpoles were used for all X-ray photographs except Figs. 3-10; these were taken from preserved specimens whose alimentary tracts contained only the normal food. Larvæ were prepared for all other photographs in the following way: subnitrate of bismuth, a non-irritating powder and insoluble in water, was placed in a flat-bottomed glass dish and the tadpoles allowed to feed on it for four or five hours. Larvæ which are not transforming feed almost continuously, by night as well as by day, so the whole canal is kept well filled. They were then placed in a 5 per cent solution of ether and when quiet (usually in about 15 minutes) they were taken out and arranged on a thin piece of paraffin paper, to protect the X-ray plate, and exposed to the rays for thirty seconds. The animals were immediately replaced in pure water where they revived in from 5 to 10 minutes.

Macroscopic Changes.

Reuter, in the first part of his work, describes the gross changes which occur during metamorphosis, in the alimentary tract of Alytes

obstetricans, and Duesberg reports that Rana fusca shows complete agreement with these conditions. The phenomena are essentially the same in Bufo lentiginosus. I shall refer only briefly to a few points brought out by the method used in studying Bufo. The employment of subnitrate of bismuth and the X-rays was suggested to me by Cannon's paper ('02) in which he describes its use in his physiological work upon the movements of the intestines of the cat. By employing this method with Bufo it has been possible to figure successive changes in the same individual from day to day, and the danger of displacement by dissection is done away with. Also the exact time at which the larvæ begin to take food is easily determined. With animals reared under normal conditions (20° C.), this occurred on the sixth day after hatching (Fig. 1), which corresponds with the time of rupture of the stomodeal septum.

This series, exposed first upon the sixth day, was subjected to the X-ray eight times. These rays had no effect upon development or metamorphosis, as could be determined by comparison with control larvæ.

The coil of the small intestine increases in length until a maximum is reached at a time when the hind legs are well developed, but not yet drawn up on the body (earlier than Fig. 3). The short œsophagus is followed by the stomach and duodenum, which lie on the extreme right side and dorsal to the large coil. The rectum lies on the left, also dorsal in position.

As Yung and Babak have shown experimentally, the extreme length of intestine in larval Anura is an adaptation to the plant-eating habit—a difference of 58.15 per cent was obtained by Babak ('06) in the length of intestine of two sets of larvæ, one fed upon plants, the other upon meat. As Ratner points out, the Urodeles are meat eaters throughout life, and in Anura this plant-eating habit is secondary. The change in adult Anura to meat eating and the short intestine is a return to the normal. In the early differentiation of the alimentary tract, two or three days after hatching, the anterior part which is to form the stomach, lies in the normal position for this organ in Vertebrates, on the left. There appears to be a

passive crowding of this anterior portion to the right side by the rapid differentiation of the large coil from the posterior yolk mass. This conclusion seems justified from experimental work, which will be described later.

When the coil has reached its maximum length, the liver, lying anteriorly on the right, is very small, but it increases rapidly in size. This growth of the liver on the right, plus a decrease in size of the intestinal coil on the left, and a slight growth in length of the stomach, probably combine to effect the interchange in position of stomach and coil. This is clearly represented in the X-ray series, Figs. 3 to 10. In the twenty-four hours immediately following the breaking through of the fore legs, the shortening of the coil is usually completed (Figs. 7-9) and stomach and coil pass each other in the median line (Fig. 9). At the time the tail begins to shorten (usually on the second day), the change has as a rule been made (there is some individual variation), and the stomach has taken the adult positon on the left side, the reduced coil lies on the right (Fig. 10). This transformation of the intestine is described by Reuter and Duesberg as occurring in Alytes obstetricans and Rana fusca *before* the breaking through of the fore legs. Reuter says the rectum changes position slightly or not at all in Alytes. In Bufo it follows the coil from the left to the right side, or, in some cases, only to the median line. It becomes shortened and widened. Besides the shortening of the small intestine, there is also a marked diminution in the diameter,—compare Fig. 18 (before reduction of coil), and Fig. 20 (after reduction), noting the increase in thickness of the circular and longitudinal muscle layers.

In striving to get a full bismuth-X-ray series, extending through this critical change of shortening and narrowing, it was incidentally determined at exactly what time the larvæ stop feeding in preparation for the renewal of the intestinal epithelium. Figs. 11 to 13 indicate that feeding continues to the day before the appearance of the fore legs. At the time when the fore legs break through, X-ray plates of many different series show that the bismuth has been eliminated from the rectum (except in a few cases, as Fig. 14). Although the normal food seems to be not so freely eliminated as the bismuth,

the examination of a large number of alcoholic specimens was required before the tadpoles in Figs. 7-10 were found.

After the appearance of the fore legs and the coincident shortening and narrowing of the intestine, there remains one more marked change before metamorphosis is completed, the absorption of the tail. This shortening usually begins about 48 hours after the fore legs have come through and is completed in from 2 to 4 days. Feeding is resumed on the third or fourth day after metamorphosis is completed.

Microscopic Changes.

I. *Historical.* As stated in the introduction, there have been but three papers on the modification of the intestine of Anura during metamorphosis,—those of Ratner, '91, Reuter, '00, and Duesberg, '06.

Ratner's work does not consider the *epithelial* changes; it is concerned with the subepithelial layers. Reuter, using Alytes obstetricans, and Duesberg, working with Rana fusca, disagree on the main points of the process.

Reuter recognizes that the epithelium of young larvæ is composed of two forms of cells, first, cylindrical, and second, basal round cells, the latter relatively few in number. These "round cells" show fine granular contents and might be mistaken for leucocytes. During degeneration of the epithelium they become more numerous, large and may be multinucleate. They contain great masses, food substance in process of absorption, which are colored brown with Flemming. He considers them special absorbing cells. Somewhat later "giant cells" appear, also basal in position and multinucleate, and differing from the "round cells" only in not having the brown masses and in their fate. Reuter claims that both round and giant cells originate by amitosis from the cylindrical epithelium.

At the beginning of metamorphosis the larvæ stop feeding, the round cells receive from the cylindrical cells the last absorbed food, but they do not pass it on into the lymph channels,—they cease secreting, become overloaded, die, and with the degenerating cylindrical cells are cast off into the lumen when shortening of the intestine occurs. The giant cells remain, gain the ability to divide *mitotically*, and form the definitive epithelium.

Duesberg describes the epithelium of the coil at a time before the appearance of the hind legs, as composed of principal cells and, between their proximal ends, scattered basal cells. Later, as the hind legs appear, other basal elements are noticed, which clearly correspond to Reuter's round cell. Vacuoles and granules appear in the protoplasm, neighboring cells become confluent and form a single cell, the brown masses become more numerous, the nuclei go through chromatolysis, and the final stage in this process is the typical "round cell." These cells present none of the characteristics of active absorbing cells, on the contrary they show marked degenerative phenomena. They are cast off as described by Reuter.

Duesberg figures the definitive epithelium as forming from the basal cells of the larval epithelium, which are at first not distinguished by any peculiar structure, but later show the characteristics of Reuter's "giant cells." He has not seen in any of his preparations the formation of round or of giant cells by amitotic division of the principal cells.

My conclusions, which agree in the main points with those of Duesberg, were formed independently before the reading of his paper.

II. *Description.* The histological differentiation of the small intestine of Bufo, *i. e.,* the degeneration and regeneration of the epithelium, takes place progressively from the anterior to the posterior end. Therefore, for clearness of comparison in successive stages, one particular region has been selected for description, the duodenum near the entrance of the bile duct, and all figures except 16 and 44 are from this region of the intestine.

At the time of hatching the stomodeum and proctodeum are already formed and the rest of the alimentary canal is a mass of yolk, through the dorsal portion of which runs an irregular lumen. Anteriorly, extending back to the level of the pronephros, the lumen is definite, and in the dorsal and lateral walls may be distinguished scattered nuclei and very faint irregular cell walls.

Fig. 22, five days after hatching, shows the earliest differentiation of the duodenal epithelium into a definite layer of columnar cells. The cell walls are very faint, the ciliated border is not formed, the cells are solidly packed with yolk spherules and often contain two nuclei.

The stomodeal septum is usually broken through on the sixth day (larvæ 11 mm. long). On the seventh day the coil is completely formed and fills the whole ventral part of the abdominal cavity. Figs. 23 to 25 show this stage in cross section. There is a low, ciliated, columnar epithelium, having large nuclei at the proximal end. The yolk is mostly absorbed, only an occasional cell being well packed with small spherules. Rarely one finds a basal cell (Figs. 23 and 24), and in the distal border a mitotically dividing cell (Fig. 25). The sub-epithelial tissue forms a very delicate layer. Figs. 26 to 31 (two weeks after hatching) show the ciliated columnar epithelium thrown into folds. Many mitotic figures appear at the bases of the folds. These cells as a rule are clearer than those that form the folds,—their appearance is suggestive of special activity, perhaps of a grandular character. Bataillon ('91) noticed in the epithelium of Alytes obstetricans, "a curious localization of karyokinetic figures" at the bases of the folds. He suggests that perhaps the irritation of compression causes activity at these points.

Figs. 26, 27 and 28 show resting, spireme and anaphase stages of basal cells, the first differentiation of the giant cells of Reuter and Duesberg. This stage is characterized by many mitotically dividing cells in the distal zone of the epithelium (Figs. 29 to 31). A few round cells appear (Fig. 31).

Fig. 32 (three weeks after hatching) again shows the activity and relative clearness of the protoplasm of cells at the base of a fold. The other cells are well filled with fat, stained black with Flemming. Figs. 32 and 33 were made from larvæ which correspond approximately to individuals I and II of Fig. 2, the hind legs having appeared.

Histolysis. For the sake of clearness the histolytic phenomena which now begin to appear in a marked degree, will be followed through succeeding stages; histogenesis will be considered later, although the two processes go on side by side. Figs. 18 and 35 to 37 are cross sections of No. III, Fig. 2. The cytoplasm still shows the fine mottled appearance of the preceding stages, only rarely (Fig. 34) showing small vacuoles and products of degeneration, brown and yellow granules (Delafield and eosin stain). Fig 34 shows also one of the many "round cells" which have now made their appearance.

It is filled with degenerating material. Its nucleus, like those of the columnar cells, shows an early stage of chromatolysis. There is a pale nuclear groundwork, the chromatin is in deeply stained clumps, the membrane is slightly thickened and is beginning to be irregular in outline. The structure marked *ch.* appears to be a mass of chromatin in an early stage of degeneration (compare Figs. 38 to 41, showing later stages of chromatolysis). The chromatin must undergo chemical change, for increasingly large areas fail to take the hæmatoxylin. These unstained areas, in a later stage, are filled with small yellow granules, and in a final stage the whole mass has a clear straw color (Delafield and eosin stain).

Figs. 35 to 37 show the characteristic appearance of the cells at this stage (No. III, Fig. 2). There appears to be fragmentation of the nuclei, also a breaking down of cell walls and a clumping together of nuclei (Fig. 37).

No. V (Fig. 2) has pushed one fore leg through. Feeding has ceased, the muscular contraction is pronounced (see Figs. 19 and 42 for cross sections). The cytoplasm has now become vacuolar, and throughout the cells are scattered globules and granules of brown, yellow and black degenerating substance. The nuclei have become more irregular.

In No. VI, Fig. 2 (both fore legs through, tail not absorbed), contraction has been completed and the organs of the alimentary tract are in their adult position (Figs. 20 and 43). Degeneration of the old cells is nearly completed, the cell walls have become indistinct, the cilia have disappeared.

Marcelin ('03) in his tabulation of the histogenetic changes of the intestinal epithelium of Rana esculenta, shows that the disappearance of the cilia occurs at the time when the intestine is at its maximum length, that is, relatively much earlier than in Bufo. He believes that the reason for this disappearance of the cilia is to be found in the fact that their function, the propelling of the food, is now usurped by peristaltic contraction of the muscles, which have grown stronger. In Bufo the cilia remain throughout the larval life, although there is a strong peristaltic movement, even in very young larvæ.

Figs. 16 and 44 (from the posterior end of the small intestine of

No. VII, Fig. 2) show the appearance of the final stage of degeneration of the columnar and round cells and the manner in which they are pinched off into the lumen when the final muscular contraction occurs. The debris is given off through the anus. Duesberg suggests that some of it may be absorbed as nourishment.

Histogenesis. At the stage represented by No. III (Fig. 2) many giant cells can be distinguished in the duodenum (Figs. 18 and 35 to 37). Owing to the fact that a giant cell, as it now becomes active, takes a diffuse deep blue stain, with hæmatoxylin, it can be easily distinguished, even when it has but one nucleus, and more readily when there have been repeated mitotic divisions and a syncytium or a cyst has been formed (Fig. 18, g. c.). Figs. 35, 36 and 42 show stages in the formation by mitosis of the syncytium and the characteristic appearance of the nuclei, large and plump, with fine chromatin network and large nucleolus. These nuclei and the small amount of deeply staining cytoplasm in which they are irregularly massed (Fig. 42) soon form a cyst, a hollow sphere, which breaks open on the side toward the lumen of the alimentary tract (Fig. 43). This condition corresponds to that found in Alytes obstetricans. In Rana fusca the developing epithelium does not go through a cyst stage,—the syncitia appear as scattered patches of new epithelium, which meet and fuse when contraction occurs. This broken cyst opens more widely (Fig. 44), and at the same time the nuclei become oriented, with their long axes parallel to one another. Cell walls appear and a cuticular border is formed. Eventually these isolated crypts of new epithelium become joined edge to edge (Fig. 16); thus the continuous, definitive epithelium is formed (Figs. 17 and 45). As the process of histogenesis is progressive from stomach to rectum, it was possible to find these two last stages (Figs. 44 and 45) in the same individual (No. VII, Fig. 2).

The new epithelium forms much earlier, in comparison with the progress of degeneration, in Bufo than in Alytes (compare Fig. 43 with Reuter's Fig. 38).

This account of the histological changes which occur in Bufo agrees, I believe, in the main with that given by Duesberg for Rana fusca. It has been limited chiefly to a statement of the facts, because

the arguments have already been fully set forth by him, also because it is believed that the figures given in this paper offer their own argument.

It is probable that the significance of the histolytic and regenerative phenomena which have been described in the three Anurans, Alytes obstetricans, Rana fusca, and Bufo lentiginosus, will be more clearly seen when more extended work has been done upon other forms, upon amphibia in general.

It appears from the comparative work which has already been done that in the tadpole of Anura we have merely a temporary adaptation, and, as in the case of the larvæ of those insects which undergo complete metamorphosis, that these conditions have no phylogenetic significance.

Experimental Work.

I. *Mechanics of the early differentiation of the alimentary canal.*

Text figure A shows the successive changes which had occurred in one individual of Rana palustris by the second, third, fourth and sixth days after hatching. Rana palustris was used because the lack of pigmentation at this stage allows one to see clearly, without dissection, the changes in the alimentary tract. Dissection of different individuals of Bufo on successive days indicates that the changes are the same as in Rana palustris.

Text figure A, I, shows the œsophagus, stomach, anterior part of the duodenum and the rectum formed; the rest of the tract is an undifferentiated mass of yolk from which the coil will be formed. The stomach lies on the left side, in the normal adult position. Further growth, *i. e.,* differentiation of the coil, takes place in the region marked x, and because of this vigorous growth, the stomach and duodenum are crowded into the temporary larval) position on the right. Theoretically this transfer from the left to the right side would appear to be due to a passive crowding, not to an active movement of the anterior part of the digestive tract, for the cellular structure of this region is already laid down in the stage shown in I.

To test experimentally this question of crowding, that is, whether the stomach would remain on the left if the coil did not usurp its

place, a small cut was made through the abdominal wall of several Bufo larvæ, one day after hatching. In some cases these healed so rapidly that development went on normally, but in several indi-

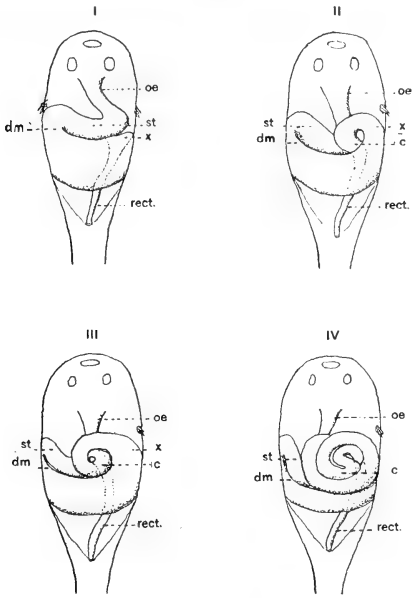

Fig. A. Rana palustris. Ventral. × 10.
I. Two days after hatching.
II. Three days after hatching.
III. Four days after hatching.
IV. Six days after hatching.
c., coil; dm., duodenum; oe., oesophagus; rect., rectum; st., stomach; x., point at which the differentiation of coil from yolk mass takes place.

viduals as the coil developed it pushed through the opening in the body wall, forming a hernia outside the body. In these cases the stomach, which was not crowded by the coil, remained on the left. Of these individuals none lived over four days.

II. *Carmine injection test for leucocytes.*

Reuter speaks of the superficial resemblance of the round cells of the epithelium to the round cells of the blood, viz., the leucocytes. Bizzozero ('02) and others have considered them to be leucocytes, but Reuter strongly maintains that the two should not be confused, —those of the epithelium arise as epithelial cells and remain epithelial cells throughout life. He says that they may migrate to the outer zone of the epithelium and undergo division.

The round cells of Bufo do not have the appearance of leucocytes, and as they are degenerating cells, it is improbable that they migrate to the distal zone and divide. It was not possible to identify as leucocytes the numerous mitotically dividing cells of the distal zone (Figs. 29-31), in fact none were distinguished in the epithelium.

To test their presence, and to determine, if possible, whether phagocytosis plays any part in the histolysis of the epithelium, the method employed by Mercier ('06) in his study of phagocytosis in the tail of Rana temporaria, was tried. Mercier injected powdered sterilized carmine into the dorsal lymph sacs of tadpoles, and the animals were killed 24 hours later. He found that the leucocytes had taken up the carmine granules and wandered with them into the muscular tissue of the tail. It was hoped that leucocytes might be traced by this method into the degenerating epithelial tissue of the intestine.

Tadpoles just ready for metamorphosis were etherized, and sterilized powdered carmine was injected under the skin of the back. On the first, second and third days after the injection tadpoles were killed in corrosive-acetic, stained with Delafield's hæmatoxylin, and imbedded in paraffin. Several series have been carefully examined, with but negative results thus far, no carmine having been found in any of the layers of the intestine.

III. *Contraction of the Intestine.*

Reuter and Duesberg refer to the contraction which results in the shortening and narrowing of the intestine as a peristalsis, moving in a cranio-caudal direction. Duesberg states that this peristaltic contraction is like true peristalsis with the exception that it is permanent. The reduction in size of the intestine, he says, always occurs

during the period between the appearance of the posterior and the anterior limbs and takes place in a relatively short time. According to Reuter 48 hours is the maximum time for contraction in Alytes obstetricans.

Bufo, at a stage corresponding to No. I (Fig. 2), often has a mid-ventral longitudinal strip of the body wall which is free from pigment. Beneath this unpigmented area the normal peristaltic movement of the intestine can be clearly seen. The food is pushed toward the anus by the typical slow, rhythmic, wavelike contraction. Older larvæ, in which the whole ventral surface was deeply pigmented, were etherized, a longitudinal cut was made in the ventral body-wall, and they were then placed in normal salt solution. The normal successive contraction and expansion was observed as before. Many series of tadpoles, corresponding to the different stages represented in Fig. 2, were examined by means of this method.

Among one lot of especially large strong tadpoles, two individuals were found whose whole bodies remained abnormally free from pigment throughout their development. The intestinal movement could thus be observed without opening the body cavity. These two tadpoles were etherized and observed daily until the end of metamorphosis, one individual for a week, the other for twelve days.

In no case was anything like a "permanent peristalsis" observed. The records obtained from the unpigmented individuals show that the contraction of the coil is a slow process, extending over the week or ten days previous to the appearance of the fore legs. I should say that X-ray photographs of the same individual on successive days also prove this to be true.

From observation of gross conditions, the shortening and narrowing of the intestine in Bufo would appear to be accomplished by a very gradual, even contraction of all the muscles, longitudinal and circular, of the intestinal wall.

Bataillon ('91) ascertained by the measurement of dissociated muscle fibres, that their contraction corresponds exactly to the total shortening of the intestine.

Although individual variation occurs, this slow contraction of the coil has, as a rule, been completed at the end of the first day after

the breaking through of the fore legs. In two individuals, opened at this stage, the reduced intestine was observed to move slowly and steadily, but without visible action of the muscles of the alimentary tube, from its larval position on the left to its adult position on the right. The loop, which had traveled across the body, then made a double coil.

It is not possible to draw conclusions with certainty from the observation of two cases, but this whole movement *appeared* to be due to contraction of the mesentery; the intestine seemed to be passive. The mesentery usually contains a large amount of pigment. The fact that aggregations of pigment are often found on the alimentary tube after it has passed to the right side of the body, and not before, would seem to add evidence to the view that the contraction of the mesentery is the agent in this movement.

Bataillon has tested experimentally the question whether the modifications of the coil are localized. He curarized tadpoles several days before the appearance of the anterior legs, opened the abdominal cavity and marked equal distances on the small intestine by means of fine pieces of silk thread, which were held in place by the mesentery. The cavity was then closed and the animals soon recovered. At the end of metamorphosis examination showed that the modifications are not localized, except that the shortening is slightly more marked at the summit of the coil.

The cause of the contraction of the intestine may be involved in the puzzling question of metamorphosis, which has never been satisfactorily answered, although many interesting theories have been advanced.

Reuter suggests that the contraction of the intestinal muscles is perhaps in response to a stimulus derived from the degenerating epithelial cells. In Bufo the first appearance of epithelial degeneration and the beginning of the slow muscular contraction are coincident, and they may be causally related. Bataillon holds that the changing conditions of respiration and circulation which result in partial asphyxiation, are the causes of metamorphosis. This could hardly be the cause of the contraction, since epithelial degeneration and muscular contraction begin several days before these changes

in respiration and circulation take place. Likewise they are initiated before there is any change in the feeding habits. It appears probable that the muscular contraction is in some way dependent upon the epithelial condition, perhaps both chemical and mechanical changes, which accompany degeneration.

Why the degeneration of all but the giant cells of the larval epithelium should take place seems to me to be a question of life cycles, which cannot be answered until we know more of the laws of growth and senescence. My observations of the histological conditions of the Bufo larva lead me to believe that there is an early differentiation of the two sets of epithelial cells, one set, the principal and round cells, destined to function through the larval existence, to run its whole life cycle within six or eight weeks, the other, the giant cells, becoming active only after a latent period of four or five weeks, and functional as absorbing and secreting cells only after metamorphosis is completed.

Summary.

I. Between the time of hatching and the end of metamorphosis the alimentary tract of Bufo undergoes striking changes, both macroscopic and microscopic.

II. The macroscopic changes consist of:

1. The differentiation, from the posterior part of the yolk mass, of a huge intestinal coil, which crowds the stomach and duodenum to the right side of the body.

2. This intestinal coil reaches its maximum size at a time when the hind legs are well developed, but not yet drawn up on the body.

3. A gradual shortening and narrowing of the intestine then takes place, by means of a slow, even contraction of the longitudinal and circular muscles. This contraction occurs during the week or ten days previous to the breaking through of the fore legs and is usually completed in the following 24 hours.

4. With the completion of the intestinal reduction the stomach resumes its original position on the left, and the intestine moves, apparently by means of contraction of the mesentery, to its adult position on the right.

III. Miscroscopic changes:

1. From earliest differentiation of the larval epithelium two kinds of cells may be distinguished, the principal cells and the basal giant cells.

2. The principal cells form the temporary, larval epithelium. Degenerative phenomena begin to appear in these cells about two weeks before the breaking through of the fore legs; they are coincident with the shortening and narrowing of the intestine.

3. As degeneration begins in the principal cells, the giant cells become active, increase in size, divide mitotically, and form syncitia.

4. These syncitia form cysts, hollow spheres. The nuclei then become oriented, their long axes radiating from the center, and cell walls form, making a single layer of columnar epithelium.

5. The cysts break open on the side toward the degenerating epithelium, unite with each other and form the definitive epithelium.

6. With the final muscular contraction, the degenerating epithelium is pinched off into the lumen of the alimentary tract and eliminated through the anus.

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LIST OF ABBREVIATIONS.

b. c., Basal cell.
c., Coil.
ch., Chromatin.
d. e., Degenerating epithelium.
e., Epithelium.
g. c., Giant cell.
m., Mitosis.
ms., Muscles.
n., Nucleus.
n. e., New epithelium.
p. c., Principal cell.
r., Rectum.
r. c., Round cell.
st., Stomach.
vac., Vacuole.
y. g., Yellow granules.
y. s., Yolk spherules.
z., Detail shown in Fig. 22.

PLATE I.

FIG. 1. X-ray photographs. Four tadpoles, sixth day after hatching. The intestinal coil filled with subnitrate of bismuth. Dorsal view.

FIG. 2.—Photograph. Seven stages in the development of the tadpole, from 3 weeks after hatching to 3 days after the appearance of the fore legs. Ventral view.

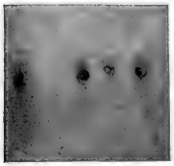
FIGS. 3-10.—X-ray photographs. Stages in the development of the tadpole alimentary tract, from about 10 days before to 2 days after the appearance of the fore legs. Alcoholic material. Normal food in the alimentary tract. Ventral view.

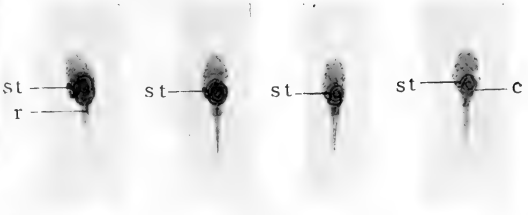
FIGS. 11-15.—X-ray photographs. Living material. Subnitrate of bismuth in the alimentary tract. The same individual photographed on June 10th (Fig. 11), June 14th (Fig. 12), June 15th (Fig. 13), June 16th (Fig. 14), just after the appearance of the fore legs, June 16th (Fig. 15), a few hours later than Fig. 14. Ventral view.

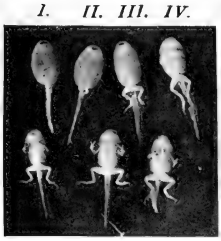
FIG. 16. Photomicrograph. Cross-section of the posterior part of the intestine of No. VII, Fig. 2. Detail (ne) shown in Fig. 44. × 165.

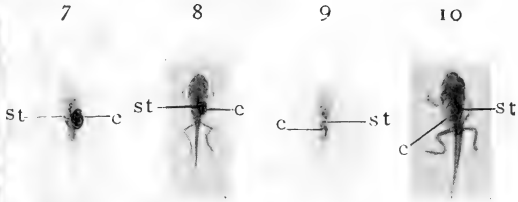
FIG. 17. Photomicrograph. Cross-section of the duodenum of No. VII, Fig. 2. Detail shown in Fig. 45. × 165.

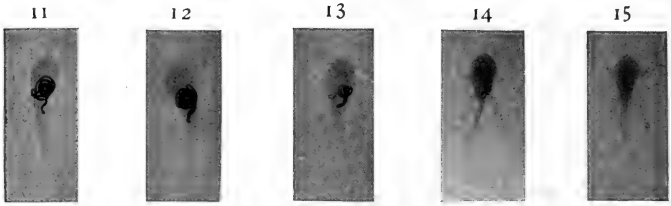
MARY A. BOWERS.











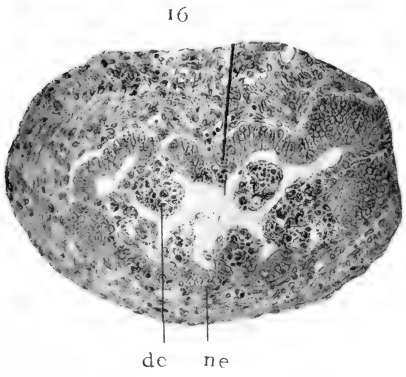


PLATE II.

FIG. 18. Photomicrograph. Cross-section of the duodenum of No. III, Fig. 2. × 165.

FIG. 19. Photomicrograph. Cross-section of the duodenum of No. V, Fig. 2. × 165.

FIG. 20. Photomicrograph. Cross-section of the duodenum of No. VI, Fig. 2. × 165.

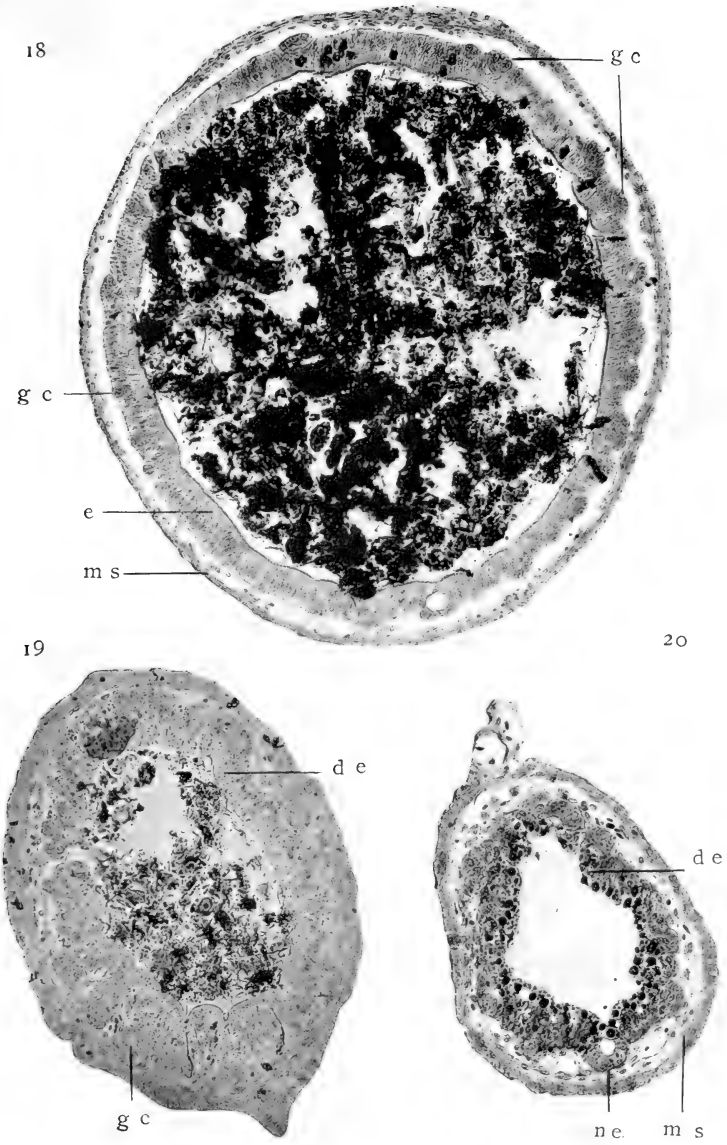

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Plate III.

Fig. 21. Cross-section of Bufo tadpole, at the level of the pronephros, 5 days after hatching. Detail (z) shown in Fig. 22. × 80.

Fig. 22. Duodenum, 5 days after hatching. Cross-section. × 990.

Figs. 23-25. Duodenum, 1 week after hatching. Cross-section. × 990.

Figs. 26-31. Duodenum, 2 weeks after hatching. Cross-section. × 990.

Fig. 32. Duodenum, 3 weeks after hatching (like No. I, Fig. 2). Cross-section. × 990.

Fig. 33. Duodenum, from a tadpole like No. II, Fig. 2. Cross-section. × 990.

Fig. 34. Duodenal epithelium from tadpole No. III, Fig. 2. Cross-section. Micr. Leitz. oc. 4, obj. im. $^1/_{16}$.

Figs. 35-37. Duodenum. Tadpole No. III, Fig. 2. Cross-section. × 990.

Figs. 38-41. Stages of chromatolysis. Nuclei from duodenal epithelium of tadpole No. IV, Fig. 2. Oc. 4, obj. im. $^1/_{12}$.

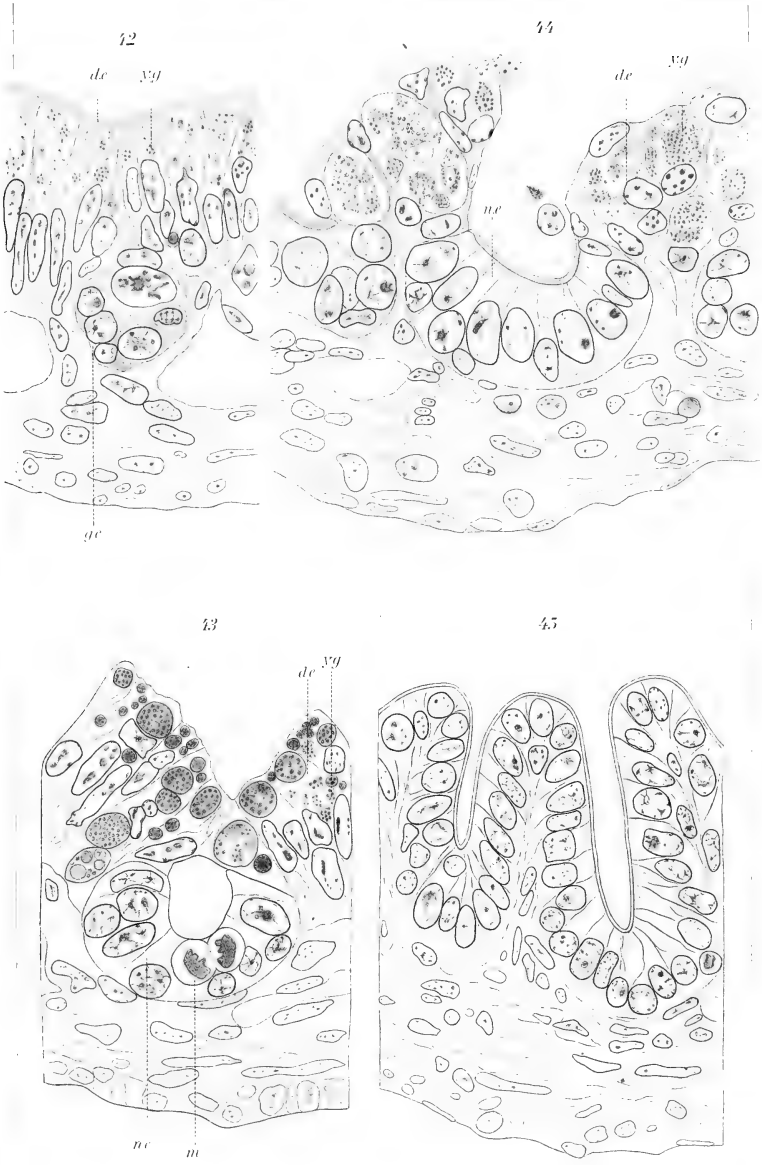
PLATE IV.

FIG. 42. Duodenum. Tadpole No. V, Fig. 2. Cross-section. × 750.

FIG. 43. Duodenum. Tadpole No. VI, Fig. 2. Detail of Fig. 20. Cross-section. × 750.

FIG. 44. Cross-section of the posterior part of the small intestine of tadpole, No. VII, Fig. 2. Detail of Fig. 16. × 750.

FIG. 45. Duodenum. Tadpole No. VII, Fig. 2. Detail of Fig. 17. Cross-section. × 612.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ON THE EARLIEST BLOOD-VESSELS IN THE ANTERIOR LIMB BUDS OF BIRDS AND THEIR RELATION TO THE PRIMARY SUBCLAVIAN ARTERY.*

BY

HERBERT M. EVANS.

From the Anatomical Laboratory of the Johns Hopkins University.

WITH 20 FIGURES.

CONTENTS.

I. INTRODUCTORY.

Our knowledge of the origin and method of formation of the vascular system in vertebrate embryos is still far from satisfactory, and, indeed, in many instances surprisingly inadequate. This is true, too, in spite of a long series of observations and a number of important contributions in this field. We owe much, for instance, to Rathke, whose classical paper on the aortic arches and the vessels derived from them, dates from 1857, and to Hochstetter, whose persistent labors have yielded such a wealth of facts on the development of various vessels.

But the knowledge which these and other researches have given us consists chiefly in an idea of the position and connections of the

*A portion of the observations recorded in this paper were made while occupying one of the research rooms endowed by The Wistar Institute of Anatomy at the Marine Biological Laboratory, Woods Hole, Mass. I would acknowledge here my obligations and thanks to The Wistar Institute for this privilege, and also to Professor Frank R. Lillie, Director of the Marine Biological Laboratory.

main blood-vessels present in successive stages. They tell us little of the beginnings or of the method of development of any of these vessels.

Significant advances in anatomy have owed much to improvements in the method of investigation. Thus von Lenhossek has declared it probable that a complete nerve cell, with all its processes had never been seen before the introduction of Golgi's method of staining.

In almost all instances, the study of the development of the vascular system has been done merely by the usual methods of histological investigation, and, as a consequence, has suffered keenly the limitations this method entails. The shrinkage which dehydration always produces in tissues prepared for serial sectioning is unavoidable. In perfect technique, this shrinkage is never great and is so uniform that great distortion cannot result; but it is present, nevertheless, and on no system or tissues of the embryo does it impress itself, even though slightly, as it does on the embryonic vessels. These delicate endothelial tubes easily collapse and the finest of them almost invariably do so. Thus the observer is fortunate to trace clearly the chief vessels and their tributaries. If new vessels, in the embryo as in the adult, arise by simply capillary sprouts and plexuses, these must imperfectly be seen, and so in the development of most of the vessels we have doubtless missed these earliest and important stages.

But more serious errors than this have arisen, for the employment of tissue prepared in the usual way has often led the observer to consider the larger uncollapsed vessels as constituting the entire system present, and thus led him to the conception of the outgrowth of vascular trunks *as such*. This, however, is never the case.

To recognize the origin and the method of growth of the early blood-vessels, we must have a complete picture of the vascular tree, of all its capillaries and even of their endothelial sprouts. Can any method accomplish this much?

Fortunately the experience of this laboratory during the last ten years has shown the existence of such a method. I refer to the method of injection.

The first publication from this laboratory, referring to the injection of embryos, was made in 1900, in the research by Flint[1] on the blood-vessels of the adrenal. Injections were used extensively by Sabin[2] in the study of the development of the lymphatic system, and in her first publication on this subject (1902), mention is made of the injection of the blood vascular, as well as lymphatic systems, in young pig embryos.

In 1904, Professor Mall's "Development of the Blood-Vessels of the Brain in the Human Embryo"[3] appeared, and in it he reported briefly some of the methods and results of the injection of embryos, which he had been conducting for some time. His paper outlined a method of obtaining splendid double injections. India ink was injected into the liver and being taken to the pulsating heart, driven by the latter through the arterial tree. The embryo was then cooled and the beat of the heart arrested, whereupon a second injection by way of the liver now filled the veins. At other times single injections of the arteries, or of the veins, were made by the same method, *i. e.*, through the liver, and these were, in many cases, remarkably complete. Thus the feasibility of demonstrating all the chief vessels of the embryonic venous or arterial system became an established fact.

But until recently, though injections of most of the vascular tree had been secured, it was necessary to perfect the method so that an approximately complete filling of all the body's capillaries could be secured, and so that even the very youngest embryos could be successfully treated in this way.

Many hundred trial injections, with living embryos under the best conditions obtainable, have evolved methods delicate, and yet effective enough, to successfully fulfil these two conditions. I

[1]Flint J. M. "The Bloodvessels, Angiogenesis, Organogenesis, Reticulum, and Histology of the Adrenal." Contributions to the Science of Medicine, dedicated by his pupils to William Henry Welch. Baltimore, 1900. Pp. 153-228.

[2]Sabin, F. R. "On the Origin of the Lymphatic System from the Veins and the Development of the Lymph Hearts and Thoracic Duct in the Pig." Amer. Jour. of Anat., Vol. I, 1902, pp. 367-391.

[3]Mall, F. P. "On the Development of the Blood-Vessels of the Brain in the Human Embryo." Amer. Jour. of Anat., Vol. IV, 1904.

need not refer here to the details of such methods, since they will be described adequately elsewhere. Recently Knower[4] has described carefully a method of using glass bulbs in this connection, which is very useful.

Whatever the details of the procedure are, fine glass canulæ, the helpful binocular microscope, and living embryos comprise the essentials. I used every possible channel as a starting place from which to reach the general circulation. The veins, the liver, and the heart itself were successivly chosen only to be abandoned. The larger arteries, however, permitted the employment of great pressure without danger of rupture. Thus the entire vascular system could be filled with the minute carbon particles of the injection mass (India ink), which, passing through arteries and capillaries, and again streaming into the heart by the veins, are pumped out again into the circulation. The more perfect of these beautiful specimens were subjected to detailed study and at length convinced us that in some instances we had attained a complete injection. The endothelial sprouts themselves were everywhere filled to their tips. We beheld the growing vascular system!

The revelations which such injections have made are in no place more striking than in the case of the origin of the chief vessels to the limbs; the femoral and the subclavian arteries. The results here, too, are of peculiar interest, inasmuch as the latter vessels have been studied with most painstaking care and, in the recent work of Müller and of Rabl, have already been traced to a very primitive condition.

The present communication deals chiefly with the first blood-vessels which grow into the anterior-limb buds, and is almost entirely based on a long series of chick and duck embryos, though I have also studied the early mammalian limb and the arm bud in man in this connection.

Before presenting these observations, however, some account of the literature on the origin of the avian-subclavian artery will not be out of place.

[4]Knower, H. McE. "A New and Sensitive Method of Injecting the Vessels of Small Embryos, etc." Anat. Record, Vol. II, No. 5, August, 1908.

II. Historical.

Five important investigations have been made on this subject—the researches of Mackay, Hochstetter, C. G. Sabin, Rabl, and of Müller; each paper containing a significant contribution and, with the exception of Müller's account, which does not include very young embryos, each successfully carrying the subject to still earlier stages of development.

Twenty years ago Mackay[5] published the first valuable account of the origin of the carotid and subclavian arteries in birds, and pointed out that the latter vessel in this class was entirely different from the subclavian trunk in most mammals.

It had been known for a long time that this vessel lay dorsal to the superior caval vein and the vagus nerve in the mammals but always ventral to these structures in the birds, and if the subclavians of these two classes were regarded as identical this difference in relations was quite unexplained. Moreover, the opinions of embryologists here were not helpful, for they did not describe a ventral but a dorsal origin for the bird's subclavian, Rathke figuring it as a derivative of the dorsal end of the fourth aortic arch, and Sabatier, following the earlier account by von Baer, as a dorsal derivative of the third aortic arch. Embryology should have thrown some light on the subject and Mackay set about to rework the embryology. It is his chief contribution to have shown clearly that the bird's subclavian had ample reason to be different in position and in all its relations from the mammalian vessel, for it arose at an entirely different point embryologically and in its growth, must come into entirely different relations with the thoracic structures. Mackay deserves the credit of being the first to recognize that the definitive avian subclavian grows down from the ventral portion of the third aortic arch and is thus from the beginning, and during its entire development, a ventral vessel, in no sense homologous with the dorsal outgrowth of the aorta which becomes the subclavian artery of mammals.

[5]Mackay, J. Y. "The Development of the Branchial Arches in Birds, with Special Reference to the Origin of the Subclavians and Carotids." Phil. Trans. Roy. Soc., London. Vol. 179, 1888.

Mackay went further and declared that in the Amniota generally there occur these two distinct subclavians—the dorsal vessel, possessed by man and by most of the mammals, and by the Lacertilia, the ventral vessel occurring in birds, Chelonia, Crocidilia, and the Cetacea. Moreover in one form—in Chamæleo vulgaris—both kinds of subclavians occurred, the ventral vessel, however, supplying chiefly the shoulder muscles.

The next advance was made by Hochstetter[6] who, in 1890, published his paper on the "Origin of the Subclavian Artery in Birds." Independently of Mackay, he also had reached the conclusion that the dorsal subclavian of mammals was not represented in the adult bird, but that the definitive wing vessels in the latter class arose from the ventral segment of the carotid arch. In addition, Hochstetter announced the discovery that in still earlier embryos the birds possessed a subclavian which corresponded to that vessel in the mammals. The secondary subclavian, the adult vessel of the birds and the trunk which Mackay had discovered, did not arise, said Hochstetter, till the beginning of the sixth day in the chick. Preceding it, on the fifth day, the primary subclavian vessel had arisen and extending into the early limb buds, had grown to trunks of considerable size, furnishing a splendid arterial supply to the growing extremities. This primary aortic subclavian was a branch of the fifteenth dorsal segmental artery on each side and Hochstetter was thus inclined to regard the subclavians as a modification of the pair of segmental vessels. On the sixth day, the final vessel began its downgrowth from the ventral portion of the third aortic arch and could be seen anastomosing with the primary vessel derived from the aorta. Thus, during the sixth and seventh days, the chick's wing buds were supplied by a double source, a condition corresponding to that seen by Mackay in the adult Chamæleo.

Hochstetter's account established the fact that even in those amniotes where the definitive vessel is the ventral subclavian, there arise in development, nevertheless, typical dorsal subclavians which correspond to those in the mammals, and in fact to the subclavian

[6]Hochstetter, F. "Ueber den Ursprung der Arteria Subclavia der Vögel." Morph. Jahrb. 1890.

of the Anamniota, since this is also the vessel which occurs in both the fish and amphibia. Thus this important paper emphasized the fact that in all vertebrates possessing fore limbs, the primary subclavian artery arises from the dorsal aorta.

In 1905, C. G. Sabin,[7] working in Locy's laboratory, reworked the subject and furnished us for the first time with good illustrations of the origin and course of both the primary and secondary subclavians in the chick. Sabin not only pushed the time of origin of the primary vessel to a stage much earlier than Hochstetter had suspected, *i. e.,* to the third, instead of the fifth, day of incubation, but he also made another observation which must be considered an important contribution to the subject. Sabin was the first to show that the primary subclavian was not at first a branch of the dorsal segmental artery but instead was primarily an independent outgrowth of the aorta and only secondarily came to be included as a branch of the dorsal vessel.

The admirable paper published in the summer of 1907, by Rabl,[8] was by far the most complete account we possessed of the subclavian arteries in birds and it marked the discovery also of a new fact of great importance, namely that the single primary subclavian is itself preceded by a row of some three segmental subclavians. These early segmental subclavians had been entirely overlooked.

Rabl's research was based on a careful study of sections of duck embryos. The account which he has given us is of the greatest interest, for it indicates that the metamerism of the limb is as distinctly expressed in its primitive vessels as it is in the nerves and myotomes that enter into its structure, a point which was first discovered by Erik Müller[9] in selachians and in reptiles, and which has been emphasized by the latter observer in several articles on the arm vessels.

[7]Sabin, C. G. "The Origin of the Subclavian Artery in the Chick." Anat. Anz., Bd. XXVI, 1905, p. 317.

[8]Rabl, Hans. "Die Erste Anlage der Arterien der vorderen Extremitäten bei den Vögeln." Arch. f. mik. Anat., Bd. 69, pp. 340-387.

[9]Müller, Erik. "Beiträge zur Morphologie des Gefässsystems." Anat. Hefte, June, 1903; Dec., 1904; and Feb., 1908.

It was from this standpoint, among others, that Professor Mall suggested that we study carefully the earliest arm vessels and it occurred to me that in the embryos of the bird we had a splendid opportunity for delicate injections. The greatly expanded extra-embryonic vessels furnished a good channel of entrance into the circulation of the embryo proper.

A segmental blood supply to the early limbs must now be regarded as an established fact. Keibel and Elze[10] and the writer[11] have shown it to be a normal stage in man, and observations which I have recently been able to make on mammalian embryos show its occurrence there also.

However, even the segmental subclavians are not the earliest plan of blood supply to the limb buds, as my injections plainly show; for in the birds, at any rate, the capillaries which first nourish the young limb bud, course at first entirely regardless of any segmental alignment and in a profusion hitherto unsuspected.

III. Observations on the Origin and Character of the First Blood-vessels in the Anterior Limb Buds of Chick Embryos.

Both chick and duck embryos were employed in the present study and it is for this reason highly probable that the results are generally applicable to the class Aves. Most of my description, however, will be confined to the condition in chicks, inasmuch as it was easier to secure here a larger series of the embryos for study. Nevertheless, in view of Rabl's study of the duck, it was important to see if the conclusions reached in the chick could apply here also, and I consequently incubated a considerable number of duck eggs and, at length, obtained successful injections of these embryos.

The drawings illustrating my findings have been done with the greatest care and fidelity possible.

I shall report first the conditions observed in the chick.

[10]Keibel u. Elze. "Normentafeln zur Entwick. des Menschen." Jena, 1908.

[11]Evans, Herbert M. "On an Instance of two Subclavian Arteries to the Early Arm Bud of Man and its Fundamental Significance." Anat. Record, II, 9, Dec., 1908.

The injections gave apparently complete pictures of the entire capillary system throughout the body and showed a wealth of these delicate vessels often where they had previously been poorly revealed. It consequently became of great interest to know if the early body wall—the somatopleure—were supplied by capillaries before any portion of it was elevated to form the limb buds, and such was, indeed, found to be the case.

In embryos of some twenty somites, the capillaries which lie in the upper somatopleure, and which form a small plexus in the angle between the duct of Cuvier and the posterior cardinal vein, now begin to grow downward. Behaving like typical capillaries, these vessels frequently anastomose and so begin to form a narrow plexus filling most of the somatopleure. Often their endothelium meets and coalesces with that of the posterior cardinal vein. By the time this simple plexus has reached the position of the earliest wing bud, the latter structure begins to form and perhaps through some influence exerted by the mitoses of the limb cells, endothelial outgrowths from the aortic wall are now stimulated. These outgrowths are again typical capillaries in size and character, sometimes anastomosing almost immediately after their origin from the lateral wall of the aorta (producing the appearance called "insel-bildung"), but more commonly growing rapidly toward the limb cells where they meet the chain of capillaries previously mentioned. Thus there is established in the newly-formed limb its first circulation, a circulation merely of capillary character through the simple mesh work of these anastomosing vessels. Coincident with the establishment of a circulation, however, transformations occur in the capillary mesh, for there come into force now hydrodynamic laws which are at work everywhere in the circulation. The path from arm bud to Cuvier's duct now receiving a good current of blood becomes an important drainage channel and this rôle almost immediately fashions from the capillary mesh a fairly direct and constantly enlarging path—the umbilical vein. No more striking instance of the applicability of those mechanical laws which Thoma discovered could be found, for it is of the greatest significance that the mesh of capillaries in the somatopleure remains of this primitive character

until the appearance of a good circulation. Not until then is a vein formed.

Portions of this system of capillaries constituting the later umbilical vein—the primary body-wall plexus, I have called them—have been seen before. Brouha mentioned them and Rabl has described them in some detail. However, no figures of them have ever been published, nor has the whole story of their origin and downgrowth ever been adequately described.

In order to facilitate a description of the various embryos studied, the subjoined table can present a summary of the chief facts relating to the limb vessels.

TABLE SHOWING THE NUMBER OF SUBCLAVIAN ARTERIES PRESENT IN CHICK EMBRYOS OF FROM 24 TO 48 SOMITES.

Periods in the Development of the Subclavian Artery.	Number of the Embryo.	Hours of Incubation	Somites Present.	Extent of Downgrowth of Primary Body-wall Plexus.	Number of Aortic Capillary Outgrowths. (Subclavian Arteries.)		General Region of Origin of Subclavian Arteries.
					Left.	Right.	
	1	..	24	Opposite 9th	0	10	.....
	2	..	30	14th	0	1	13th intersomitic space
Period of primary subclavian capillary plexus.	3	60	32	20th	4	10	14th–17th "
	4	60	..	"	5	11	14th–18th "
	5	65	31	21st	5	6	15th–19th "
	6	60	..	"	4	6	15th–19th "
	7	66	33	"	3	4	15th–18th "
Period of multiple segmental subclavians.	8	72	..		4	4	" " "
	9	72	..	Completely	3	4	16th–18th "
	10	72	34	"	2	4	18th–19th "
	11	78	36	"	2	2	18th–19th "
Period of primary subclavian artery.	12	80	45	"	1	2	18th–19th
	13	84	..	"	1	1	18th " "
	14	96	48	"	1	1	18th " "
	15	96	..	"	1	1	18th " "
	16	116	..	"	1	1	18th " "
	17	70	38	21st	5	6	16th–20th "

It will be seen by referring to the table that the definitive primary subclavian artery is at the level of the eighteenth inter-somitic space. It is a branch of the dorsal segmental vessels of that interspace. My injections, controlled by careful study of serial sections, show that in the embryos embraced by the table, *i. e.*, in chicks possessing from twenty-four to forty-eight somites, the first interspace (*i. e.*,

that between the first and second myotomes) is not occupied by a dorsal segmental artery. *The series of dorsal segmental arteries in these embryos begins with the vessel present in the interspace between the second and third somites.* Consequently the vessels present in the eighteenth interspace are really the seventeenth pair of the series actually present, and I have so labelled them in all the drawings. It is to be borne in mind, then, that the seventeenth segmental vessels of the figures are in the eighteenth inter-somitic septum. Inasmuch as the first four somites of the chick are to be considered cephalic, rather than cervical, in their ultimate fate, and as the third actual segmental artery later courses near the first cervical nerve, and is hence the first cervical artery, the primary subclavian artery arises from the fifteenth cervical segmental artery, a vessel occurring in the eighteenth interspace and the seventeenth of the series actually present.

There are five periods in the history of the bird's subclavian and not four as Rabl maintained. These may be briefly enumerated as follows:

I. Period of capillary outgrowth from the aorta forming a primary-limb plexus, not influenced in its arrangement by metamerism.

II. Period of multiple segmental subclavians, a condition resulting from the atrophy of all capillaries in the preëxisting plexus not at segmental points.

III. Period of the establishment of the primary subclavian artery from the persistence of one of the pairs of segmental subclavians—*i. e.,* that of the eighteenth segment.

IV. Period of double arterial supply through contemporary existence of dorsal and newly arisen ventral subclavians.

V. Period of enlargement of the permanent channel, the secondary subclavian, and coincident atrophy and disappearance of the primary vessel.

The last three periods or phases were described by Hochstetter and Sabin, the second period, in which segmental subclavians exist, by Rabl and Müller, *the earliest or first period for the first time in the present study.*

It will be seen that the embryos included in the table presented comprise only those in the first three periods or stages of the development of the subclavian artery

Embryos 1 and 2 show some early steps in the downgrowth of the primary body-wall plexus, the system forming the latter umbilical vein.

Embryo 1, with twenty-four somites, shows this mesh of capillaries extended caudally in the somatopleure to the level of the ninth inter-somitic space.

In embryo 2, with thirty somites, these capillaries have reached a point opposite the fourteenth interspace. In addition, from the aorta itself several capillaries have now grown out, one of which has joined the main plexus at a point near the thirteenth interspace and its dorsal segmental vessels. These apparently unimportant endothelial sprouts are but the first of a considerable series to grow from the lateral aortic wall, and though there is as yet no external indication of a limb bud, they are probably to be regarded as the first limb capillaries.

In the next succeeding stage, embryo 3, of thirty-two somites, a slight swelling of the somatopleure constitutes an infinite, yet undoubted, limb bud, and we find a considerable row of these aortic or rather subclavian capillaries. A glance shows that these vessels are not segmentally arranged (Figs. 2a and 2b).

In such injected specimens one may look down on the preparation as a whole and examine carefully any area. No reconstruction is necessary; no doubt about relations exists, for the entire picture is spread out before one. The dorsal segmental vessels stand out sharply and it is easy to determine the relation of these to the new subclavian capillaries. It is impossible to say that the latter vessels are determined in position by the former *for the whole appearance given is of a profuse irregular outgrowth of capillaries which form a simple plexus.* On the right side, as the figure shows,

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Fig. 1.—Chick embryo of 30 somites (embryo 2 of table). Showing downgrowth of primary wall plexus, × 53½. Ant. Card. V., anterior cardinal vein. Ext. Jug. V., external or inferior jugular vein (linguo-facial vein). Post Card. V., posterior cardinal vein. P. b. w. p., primary body wall plexus.

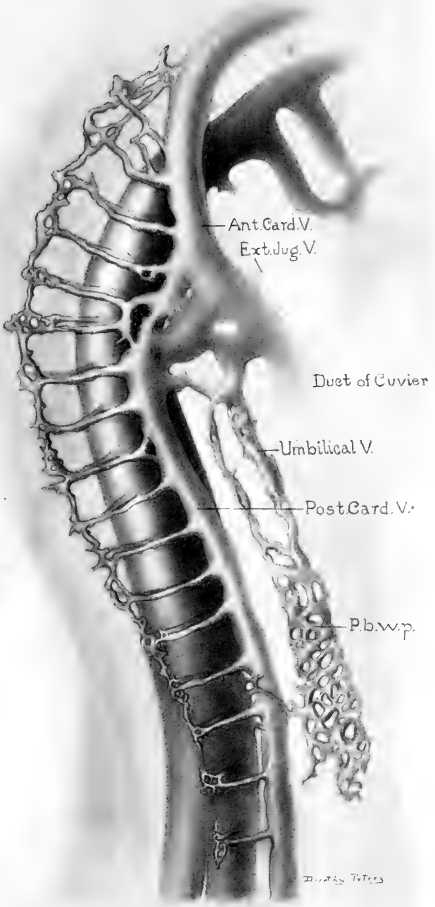
Ant. Card. V.
Ext. Jug. V.
Duct of Cuvier
Umbilical V.
Post. Card. V.
P.b.w.p.

FIG. 1.

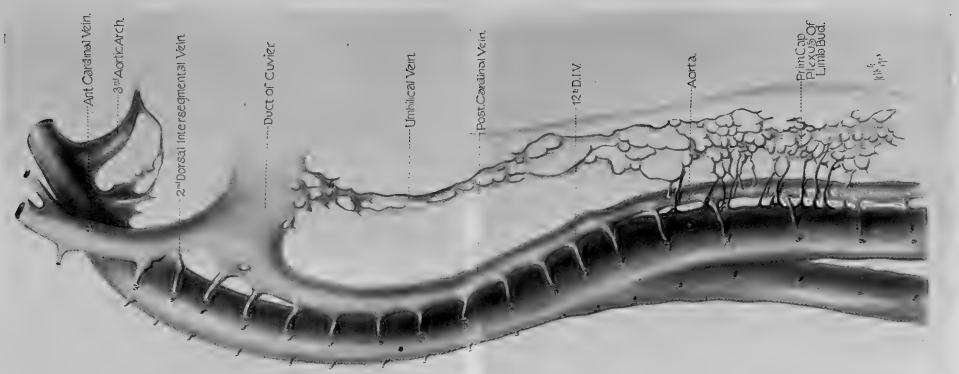
Ant. Cardinal Vein
3rd Aortic Arch
2nd Dorsal Intersegmental Vein
Duct of Cuvier
Umbilical Vein
Post. Cavalinal Vein
12th D.I.V.
Aorta
Prim. Cap. Plexus of Limb Bud
10th D.I.V.

FIG. 2a.

there are as many as ten of these vessels and on the left side, four. They are all remarkably delicate, being smaller, on the whole, than

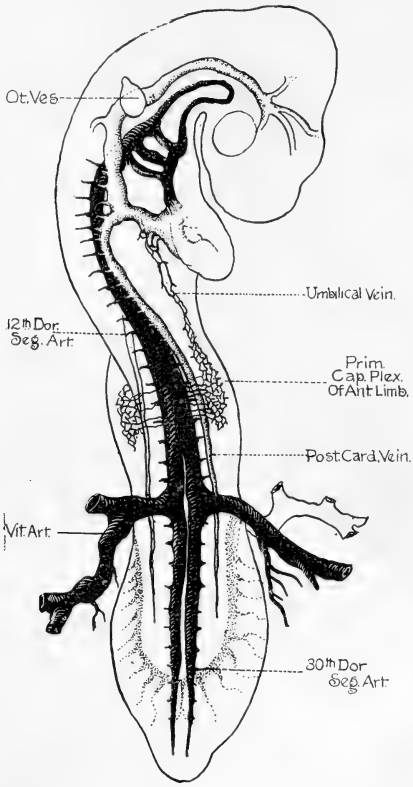

FIG. 2b—Chick embryo of 32 somites (embryo 3 of table) showing establishment of primary capillary plexus of limb bud by union of subclavian capillaries with primary body wall plexus, × 53½.

most of the capillaries in the somatopleure. One can see that under ordinary conditions such structures might be difficult to trace, and

especially so if empty and collapsed, as is most often the case with the capillaries generally.

The early subclavian capillaries are true lateral derivatives of the aorta, and at the present stage arise at considerable intervals laterally from the points of origin of the dorsal segmental vessels. In the figure (Fig. 2b), the latter vessels are represented for convenience as if cut off, but they may be seen forming a typical plexus on the sides of the spinal cord. For a clearer picture of the behavior of these dorsal segmental vessels, I have drawn with care their entire course and capillary bed in a slightly older embryo. (Embryo 17; Fig. 3.) The cross section, shown in Fig. 4, is also helpful here. With the aid of such preparations, one can determine accurately the extent of capillary growth in this region of the body, for the only vessels present at this stage, besides those in the early limb, are these capillaries surrounding the spinal cord and a few to the Wolffian body. The injections show that the dorsal segmental vessels are concerned as yet solely in the supply of the lateral aspect of the cord. The segmental artery reaches the cord near its ventrolateral angle, and from this point there radiates the capillary plexus confined as yet entirely to the lateral aspect of the cord. On the dorsal surface of the cord no vessels are yet present, the upper limit of extension of the lateral vessels is well marked by the level of emergence of the segmental veins. A few sprouts are pushing ventrally, but as a whole, that surface of the cord, like the dorsal, is as yet non-vascular. Thus it appears that the capillaries supply first those areas of the spinal cord where the greatest development or cell activity occurs, for it is well known that the lateral region of the cord is at this stage concerned in the formation of the spinal nerves, their ganglia and their roots. It is not improbable that the same correspondence is found in other tissues with an early blood supply, and that at the stage we are discussing, the appearance of the subclavian and nephric capillaries is similarly related to marked cell activity in these areas, activity which is responsible in the one region for the outgrowth of the limb bud and in the other, in the formation of the mesonephros.

The drainage of the early limb bud is interesting. When one

FIG. 3.—Chick embyro of 38 somites (embryo 17 of table) showing multiple subclavians and their relation to the dorsal segmental vessels, × 53½.

5th Dor. Seg. Ves., fifth dorsal segmental vessels, occupying the sixth intersomitic septum.

Ot. Ves., otic vesicle.

4th Subcl. Art., fourth subclavian artery of right side, opposite the seventeenth dorsal segmental vessels (those of the eighteenth septum) and hence likely to become the chief primary subclavian artery of later stages.

U. V., umbilical vein, here subserving no function other than draining the limb bud.

The figure shows clearly the dorsal segmental vessels and their distribution as a simple capillary plexus investing the lateral walls of the spinal cord.

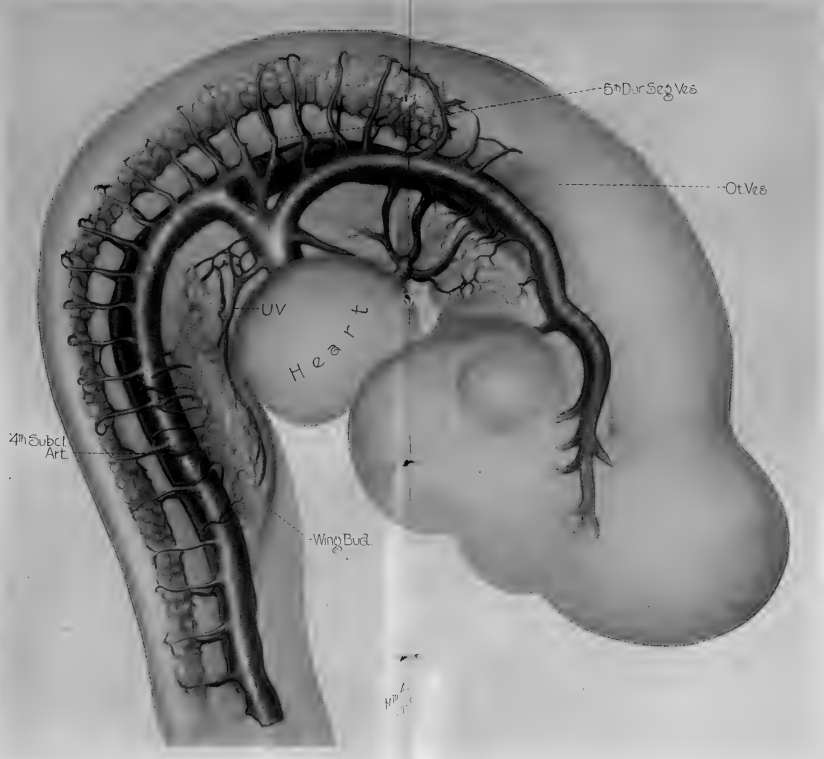

FIG. 3.

considers the story told in the earliest stages (embryos 1 and 2), it is evident that we must consider the umbilical vein as its earliest drainage channel. This vessel grows rapidly in size and receives a constantly increasing number and caliber of venules from the limb. But a process of coalescence of the limb's capillaries with the posterior cardinal vein opens up other avenues of drainage, so that there comes to be a row of venules opening from the capillary plexus of the limb bud into the posterior cardinal vein. In embryo 3, there are four of these venules on the right side but many others are soon to be formed.

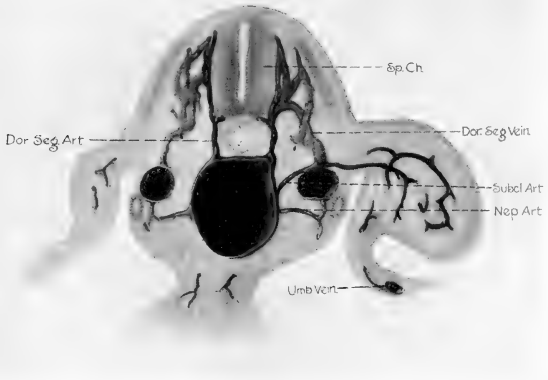

FIG. 4.—Cross section of chick embryo of 33 somites (embryo 7 of table), showing the first left subclavian artery, × 53½.

Sp. Ch., spinal cord.

Dor. Seg. Vein, dorsal segmental vein.

Dor. Seg. Art., dorsal segmental artery.

Subcl. Art., subclavian artery. One notes its origin from the mid-lateral aortic wall.

Nep. Art., nephric artery.

The next embryo, No. 4, is remarkable in possessing the greatest number of subclavian capillaries found in the series (Fig. 5).

Eleven of these vessels spring from the lateral aortic wall and anastomosing, form a simple plexus in the limb. Six venules may be seen entering the posterior cardinal trunk. The umbilical vein, seen faintly in the somatopleure, is of considerable size and receives many tributaries from the limb (Fig. 5).

It is well to call attention here to a constant phenomenon observed in the spread of the capillaries through the limb bud. They extend in every direction through the limb tissue, and fill it with a uniform mesh of vessels, save in a definite border zone. This border zone or marginal non-vascular[12] area is never invaded by capillary sprouts and remains uninvaded till the time of establishment of the border vein. The latter structure, constructed out of the most peripheral portion of the limb's plexus, thus marks the old boundary between the primary vascular and non-vascular zones.

Embryos 5 and 6 (Figs. 6 and 7) furnish other instances of the variations in the exact pattern of these capillaries forming the primary limb plexus. Both the anastomosis of the subclavian capillaries soon after their emergence from the aortic wall, and the occasional division of these vessels soon after their origin, are to be expected from the usual behavior of capillaries. Instances of this are seen in both figures. Some writers have seen fit to especially remark

[12]The significance of non-vascular areas is as yet unsolved. However, careful studies on a series of complete injections show them to be a definite feature in the circulation of every region of the early embryo. We have to do here perhaps with a matter of cell chemistry and tropisms, for endothelium apparently avoids certain areas in the embryo—the non-vascular areas. In the case of the arm buds, the early non-vascular area consists of a narrow strip of denser mesenchyme adjoining the ectoderm.

Fig. 5.—Chick embryo of sixty hours incubation (embryo 4 of table), showing profuse outgrowth of primary subclavian capillaries into early limb bud, × 53½.

14th D. I. V., fourteenth dorsal intersegmental vein, that of the fifteenth inter-somitic space.

Prim. Cap. Plexus, primary capillary plexus of the limb bud.

Post. Card. Vein, posterior cardinal vein.

Prim. Subcl. Art., primary subclavian artery of left side, the lowest one of four subclavians here, but opposite the seventeenth dorsal inter-segmental vessels and already the largest of the series.

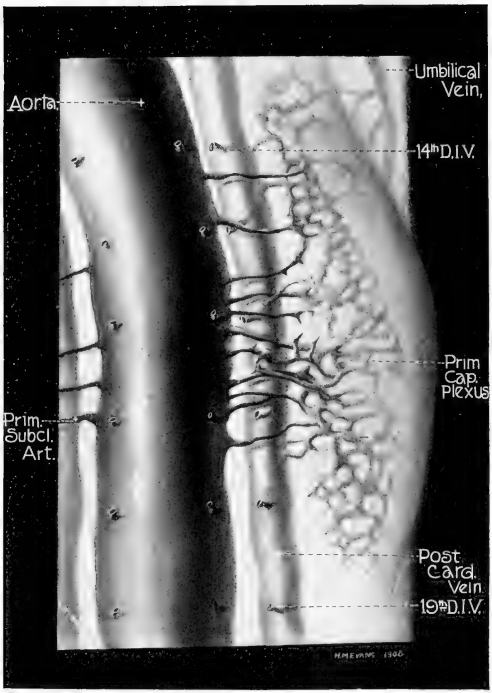
Umbilical Vein.
Aorta
14th D.I.V.
Prim. Cap. Plexus
Prim. Subcl. Art.
Post. Card. Vein.
19th D.I.V.

FIG. 5.

on these occurrences, to call them "unusual divisions" and "island formation," etc., yet this is all to be expected of capillaries tending to plexify.[13] It is, indeed, more remarkable that the subclavian capillaries usually cross the posterior cardinal vein before anastomosing to any great extent. The general appearances given by the limb vessels in these two embryos are quite significant. They must

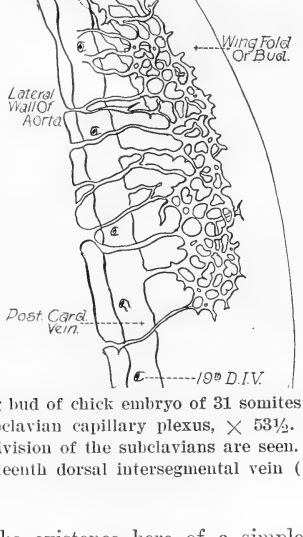

Fig. 6.—Right wing bud of chick embryo of 31 somites (embryo 5 of table), showing primary subclavian capillary plexus, × 53½. Instances of "inselbildung" and early division of the subclavians are seen.

19th D. I. V., nineteenth dorsal intersegmental vein (that of the twentieth interspace).

convince one of the existence here of a simple plexus—a plexus the first meshes of which are elongated, as a rule, but a true plexus, nevertheless, of true capillary vessels. Nor would any of the appearances lead one to believe that this primitive plexus in the limb is in any way arranged according to a segmental plan.

I have designated Embryo 7 as the last of those having the sub-

[13]Thus Rabl seems surprised at these appearances. "Die mittlere der drei Subclavien zeigt eine besondere Eigentümlichkeit, indem sie sich unmittelbar nach ihrem Ursprung teilt." P. 355, loc. cit. See also his text figures.

clavian vessels still in the first phase or period of development. It is, in every respect, slightly older than the embryos we have just been considering. Some of the primary subclavian capillaries have undoubtedly disappeared and there now remain but a total of seven of these vessels on both sides, four on the right and three on the left. I have considered it as belonging to the first general period

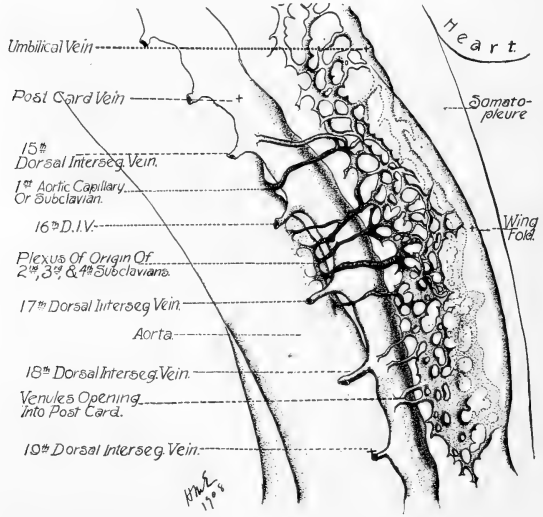

Fig. 7.—Right wing bud of chick of sixty hours incubation (embryo 6 of table), showing primary subclavian capillary plexus, × 53½.

in the development of the subclavian, however, for the majority of the vessels are at unsegmental points. With the existence of so large a proportion of the vessels out of harmony with the segmental plan, I think we can hardly classify this embryo as in the period of segmental subclavians. Of the four subclavians present on the right side, the first arises opposite the sixteenth dorsal segmental vessels, the second midway between the sixteenth and seventeenth segmentals, the third exactly opposite the seventeenth, and the fourth

about half-way between the seventeenth and eighteenth dorsal segmentals. Thus two of the subclavian arteries on the right side are true segmental vessels—those opposite the sixteenth and seventeenth dorsal segmental arteries—but an equal number, two, are completely out of harmony with the segmental plan. On the left side, the first subclavian is opposite the seventeenth dorsal segmental vessels, the second about midway between the seventeenth and eighteenth, and

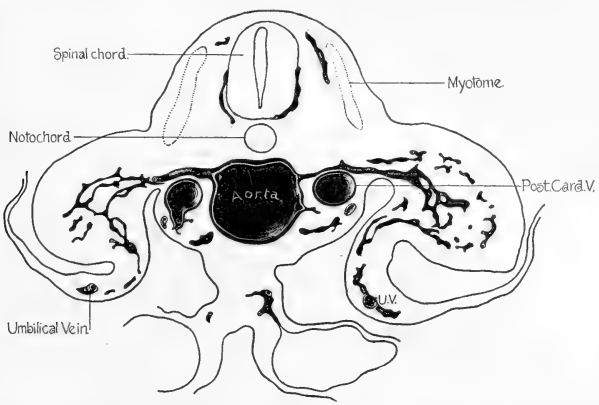

Fig. 8.—Cross section of chick of 33 somites in region of anterior limbs and midway between the sixteenth and seventeenth intersegmental vessels. The section shows the fourth right and the second left subclavian. The section shows clearly the peripheral limit of extension of the limb capillaries and the nonvascular, marginal zone.

U. V., umbilical vein.

Post. Card. V., Posterior cardinal vein.

the third subclavian considerably below the interspace belonging to the eighteenth pair. Here, then, only one of the subclavians is a segmental vessel, and *of the total of seven vessels but three are segmentally placed.* The umbilical vein has been appreciably extended considerably below the region opposite the limb bud by a still further caudal downgrowth of the primary body wall capillaries. Opposite the limb, it receives on each side about ten distinct tributaries, the upper ones, especially, being no longer capillaries in size

but small venules. Thus the earliest drainage channel for the limb does not lose this function during the next succeeding stage, but instead becomes increasingly important as the chief vein of the limb.

I have presented two typical sections through this embryo in the arm region since these will answer well for the relations thus shown in all the embryos belonging to the first subclavian period. Fig. 8 shows a cross-section through the embryo at the region of origin of the fourth right and the second left subclavians. Both vessels are unsegmentally arranged and hence the dorsal segmental vessels do not appear in the section. While on the right side the subclavian arises from the dorso-lateral angle of the aorta, the left vessel emerges from the true lateral side of the aortic wall, and only a short distance above the origin of the nephric capillaries, one of which is shown in the section. Thus in the early stages the place of origin of the subclavians from the aortic circumference varies considerably, and Fig. 4, showing the first left subclavian in this embryo, indicates how far laterally the early vessels of the subclavian series may arise. *In this instance the subclavian is almost a mid lateral derivative of the aorta.* Such vessels must curve dorsally in crossing the posterior-cardinal vein to reach the tissue of the limb, but the early dorso-lateral branches all course in a straight transverse line.[14] The character of the dorsal-segmental vessels and their capillaries has already been mentioned.

A review of the table which has been presented, shows two features of interest in connection with these earliest stages in the vascularization of the limbs. I refer to the high position of the first sub-

[14]Rabl has emphasized this straight course of the early subclavians, pointing out that Sabin missed it, for the stages which the latter studied were all old enough to show the dorsal bend which the subclavians then take in reaching the limbs. This dorsal bending is assuredly a secondary bending of an original straight vessel; but my own specimens have disclosed a number of the very earliest subclavians arising from so low a point on the lateral aortic wall that a primary arching course is necessary to reach the limb tissue. It is not unlikely that these subclavians disclose the more primitive place of origin of the subclavian series, for they do not occur in even slightly older embryos. Good justification thus exists in considering the subclavians as primarily true lateral branches of the aorta.

clavian capillaries—their origin in the neighborhood of the twelfth and thirteenth segments—and to certain differences in the vascularization of the right and left limbs.

Rabl's studies indicated that the subclavians of later stages arose at successively lower levels from the aortic wall. The injections here reported, however, indicate a more cephalic extension of the subclavians than had been previously suspected, and so extend even more the "wandering" of the upper limb and its vessels.

It appears that the right limb bud is the first to receive capillaries, and that this limb in the early stages always possesses a greater number of subclavian capillaries than the left limb. Apparently the two limbs are identical in their relations to the body and in the conditions with which they have to deal in their development save in one respect, namely, that at the time of origin and earliest stages of the limb buds, the embryo is always resting on the left side. This causes a slightly more flexed position of the under or left limb-bud with reference to the body wall and permits a somewhat extended, freer projection of the uppermost right limb. This may be related to the greater speed and profusion with which the first vessels grow into the right limb.

But in summing up the condition found in the five embryos illustrating the first period in the development of the subclavian, nothing is more striking than that we have to deal here merely with an irregular plexus of true capillary vessels which are in no way related to a segmental plan. Thus if the chance arrangement of any irregular capillary plexus obtains here, it should happen that as many of the vessels arise from non-segmental as from segmental points, and this is actually the case.

Embryos of the Second Period.

The first embryo classed in the stage of segmental subclavians (Embryo 8), has almost as high a proportion of non-segmental vessels as has Embryo 7, but two significant changes have occurred. These consist in *the purely rudimentary character of the non-segmental subclavians and the enlargement of that pair of segmental subclavians opposite the eighteenth pair of dorsal segmental ves-*

scls. The figure (Fig. 9) plainly indicates this. On the right side, the segmental subclavian opposite the nineteenth pair of dorsal segmentals is somewhat larger than the uppermost atrophying, non-segmental subclavians of that side. Some influence, then, favors the subclavians at strictly segmental points (*i. e.,* opposite the intersomitic intervals) and is inimical to the growth of those not so situated. Of the segmental subclavians, one, doubtless for purely hydrodynamical reasons, begins to be the chief supply of the limb.

Embryo 9 of the series shows a most interesting condition (Fig. 10). Here all but one of the subclavian series persisting are approximately in harmony with the segmental plan. On the left side, excepting the main vessel, the only subclavians which have survived are those at true segmental points. Thus the dorsal segmental vessels have opposite them at the sixteenth and seventeenth interspaces, two delicate segmental subclavians. *The main subclavian artery on this side does not arise at an exactly segmental point and it is formed by two frequently anastomosing vessels, arising somewhat in front of the eighteenth interspace.* Doubtless this channel is to be shifted by unequal growth and be incorporated with the eighteenth dorsal segmentals in a later stage. The right side has two vestigial vessels, which no longer reach the limb tissue, and a larger channel opposite the eighteenth segment and constructed here also not from one but from several preëxisting capillaries.

Embryo 10 possesses thirty-four somites, and has some six subclavians, four on the right and two on the left side. On the former side, two of the persisting subclavians are segmental vessels, exactly opposite the eighteenth and nineteenth dorsal segmentals, but the remaining two are non-segmental and occur in the interspace between the former two. *These non-segmental subclavians are, strangely enough, large trunks, equally as large as the true segmental subclavians,* plunging into the core of the limb and being important

Fig. 9.—Dorsal view of anterior limb buds and their vessels in a chick of seventy-two hours incubation, × 53½.

15th D. I. V., fifteenth dorsal intersegmental vein, *i. e.,* that of the sixteenth interspace.

Trans. Subcl. Art., transitory subclavian artery.

Chief Prim. Subcl. Art., chief primary subclavian artery.

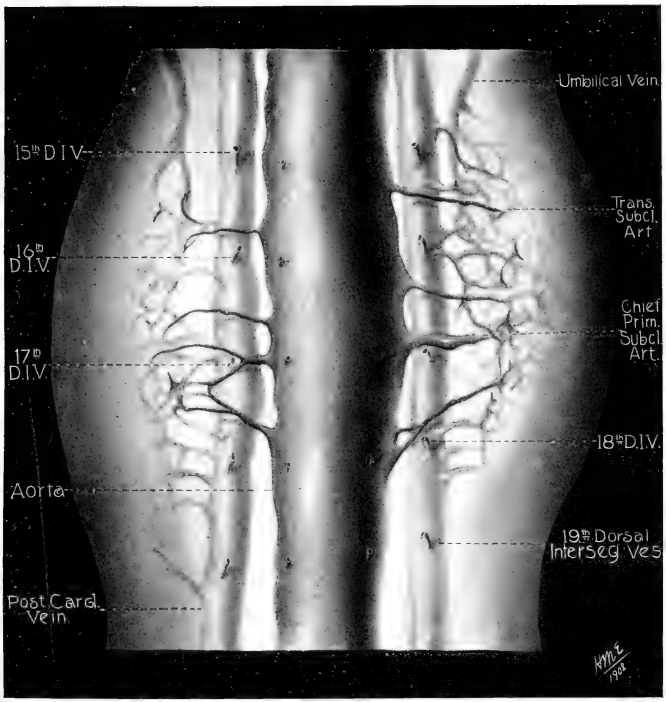
Umbilical Vein
15th D I V
Trans. Subcl. Art
16th D.I.V.
Chief Prim. Subcl. Art.
17th D.I.V.
18th D.I.V.
Aorta
19th Dorsal Interseg. Ves
Post Card. Vein
KYM E
1904

FIG. 9.

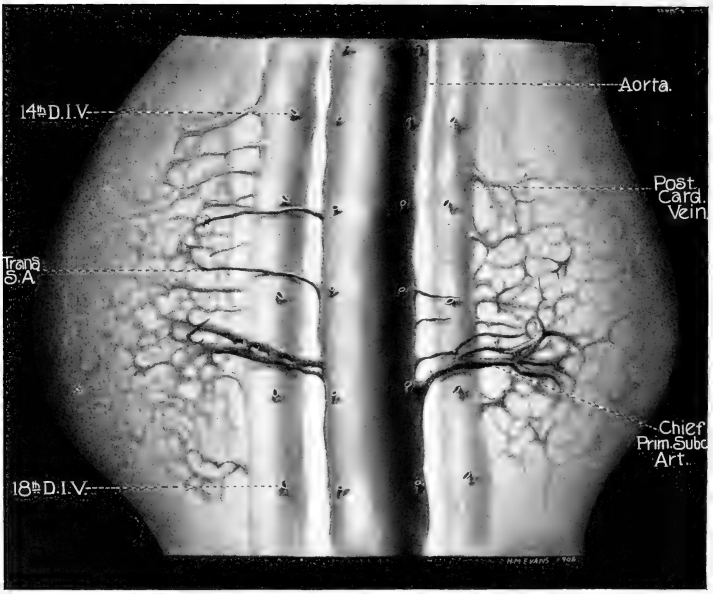
Aorta.
14th D.I.V.
Post. Card. Vein
Trans. S.A.
Chief Prim. Subc. Art.
18th D.I.V.

FIG. 10.

arterial sources in the limb's circulation. On the left side, both of the subclavians existing are true segmental vessels, at the eighteenth and nineteenth segmental points.

The cross section (Fig. 11) shows the pair of segmental subclavians corresponding to the eighteenth segment, and the corresponding dorsal segmentals. The subclavians arise at the dorso-lateral angle of the aortic circumference and, indeed, in a slight local bulging of the aortic wall, from which the dorsal segmental vessels also take origin. The limbs do not project laterally as before but are bent in more, parallel with the main body axis. The aorta is elongated dorso-ventrally with a slight compensatory lateral narrowing more marked ventrally so that in section the whole vessel now appears triangular. The dorsal segmental vessels are still confined in distribution to the spinal cord and chiefly to its lateral aspect. Neither the dorsal nor the ventral surface of the cord are yet supplied with capillaries though these vessels have begun to extend over both of these surfaces. The highest tributaries of the segmental veins are thus now somewhat above the dorso-lateral angle of the cord.

The remaining embryo of the second period—the period of segmental subclavians—is Embryo 11, with thirty-six somites and two segmental subclavians on each side, those of the eighteenth and the nineteenth segments; but the latter vessels are now mere vestigial rudiments. The common origin of the dorsal segmental vessels and the subclavians is somewhat more pronounced.

I need not dwell longer on the four embryos which belong to the period of multiple segmental subclavians. The accounts of

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Fig. 10.—Dorsal view of anterior limb buds and their vessels in a chick of seventy-two hours incubation, × 53½ (embryo 9 of table).

Trans. S. A., transitory subclavian artery, here opposite the sixteenth dorsal intersegmental vessels. The figure shows an interesting stage in the evolution of the limb's vessels. The original subclavian capillaries are now chiefly represented by those at intersegmental points, *i. e.*, the so-called "segmental subclavians." But even here there are atrophying and the chief primary subclavian arteries remain. The latter vessels happen to be constructed from several contiguous subclavian capillaries rather than from a single one as is usually the case.

Rabl and of Müller have already sufficiently emphasized this interesting stage in the limb vessels.

We have seen that most of the subclavian capillaries arising from the aorta at non-segmental points eventually atrophy, and there now remain only the vessels which stand opposite the segmental interspaces. Thus are produced the segmental subclavians, a truly metameric arrangement of the limb vessels.

Two features of some importance in these stages have been previously overlooked. These are:

1. Abundant traces of the earlier capillary plexus stage of subclavians occur in the period of segmental subclavians. These consist in several smaller or atrophying vessels of the subclavian series which do not stand at segmental points. Such vessels are often present to complicate the picture of the segmental subclavians, especially early in this period. When the stage is reached in which the multiple segmental subclavians are carried up as common branches with the dorsal segmental vessels, these non-segmental rudiments rarely persist longer and we have at length a perfect picture of multiple true segmental subclavians.

Most of the non-segmental subclavians of this stage are delicate vessels, but it occasionally happens that some of them are larger sturdy channels of equal value with the segmentals. This was the case, for instance, in Embryo 10 of the series. It was thus surprising to me that Rabl had not found such vessels, but a careful rereading of his descriptions shows that he doubtless saw some instances of them. He attached a peculiar significance to them, however, conceiving that they came about through a splitting of a preexisting single segmental vessel, thus forming a double vessel whose roots wandered apart! It was quite impossible to him that the subclavians should arise at other than segmental points. Even the cases of "insel bildung" he would make come through a similar splitting of single vascular channels.

2. The second point which I wish to make is that it must occasionally happen that even the vessel most favored in the row of subclavians may not be at first at an exactly segmental point as Fig. 10 plainly showed.

The effect of the intrusion of a metameric influence in the plan of the limb's vessels is as plainly marked in the case of its veins as in the arteries, for of the row of venules entering the posterior cardinal vein, those at segmental points are often definitely larger than the remainder. Thus segmental veins as well as arteries exist.

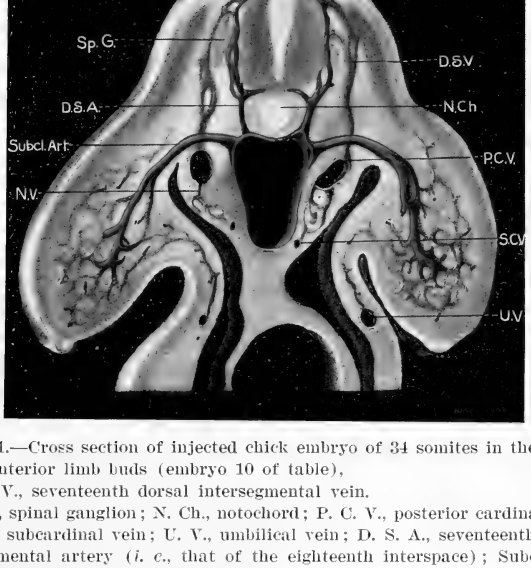

Fig. 11.—Cross section of injected chick embryo of 34 somites in the region of the anterior limb buds (embryo 10 of table),

D. S. V., seventeenth dorsal intersegmental vein.

Sp. G., spinal ganglion; N. Ch., notochord; P. C. V., posterior cardinal vein; S. C. V., subcardinal vein; U. V., umbilical vein; D. S. A., seventeenth dorsal intersegmental artery (*i. e.*, that of the eighteenth interspace); Subcl. Art., subclavian artery; N. V., nephric vein.

The fact that the veins draining more ventrally into the umbilical vein are not effected by the segmental plan would indicate that a metamerism does not pervade the entire limb tissue. One feels that the segmental arrangement of the arteries and more dorsally placed veins is the direct result of the influence of the adjoining myotomes.

I must comment here on the embryo listed as No. 17 in the series.

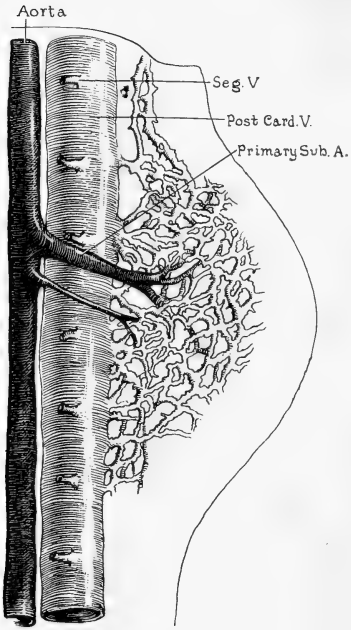

Fig. 12.—Right wing bud of chick of 45 somites, × 53½.

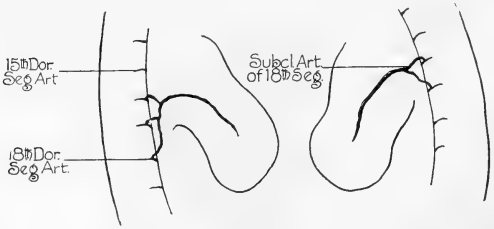

Fig. 12b.—Right and left wing buds of a chick of the fifth day.

I have placed it there since it is the only one which does not fit well into the series, for though its age and number of somites would indicate a more advanced scheme in the vessels of the limb, I found here no less than six vessels of the subclavian series. The embryo may be viewed, as an instance, in which the limb and its vascular system has run slightly behind the normal for this age, or as a case of the persistence of multiple non-segmental subclavians. I have drawn the embryo, as a whole, since it shows splendidly the typical relations of the early limb capillaries and those belonging to the dorsal segmental series (Fig. 3).

Period of Primary Subclavian Artery.

Embryos 12 to 16 all illustrate stages in the growth of the primary subclavian artery. The relation of this trunk to the preëxisting segmental subclavians, has already been clearly indicated in Embryo 8; even there we saw the early exaggeration of one of the members of the subclavian series. The processes of vascular atrophy and death which early eliminate the original non-segmental subclavians, destroy also eventually the segmental subclavians with the single exception of that vessel destined to become the primary subclavian artery.

It is of interest that even in these late stages, there sometimes persists a non-segmental artery. Fig. 12 gives an instance of this. It is the Embryo 12 in the series and possesses some forty-five somites. The large primary subclavian artery has below it and near the middle of the adjoining somite, a narrow rudimentary vessel which has persisted from the primary subclavian series. True segmental vessels, other than the main one, may likewise persist in limbs of this age. This is possible through the early proximal anastomoses between subclavians. The use of several of these paths will give several segmental roots of origin to the primary subclavian trunk. Fig. 12b, is a striking instance of this.

In embryos of eighty-four and ninety-six hours incubation, the primary subclavian artery has attained a large size. The dorsal segmental vessels have also increased in caliber. The capillaries belonging to the latter system have surrounded the spinal cord com-

pletely and grown out as a loose plexus over the outer surface of the myotomes.

Fig. 13, from the embryo of 116 hours (No. 16) show the further growth and elaboration of these changes. The common trunks of the subclavian and dorsal segmental vessels, are themselves being shifted toward the mid-dorsal line, soon to arise from a single common trunk. The segmental arteries and veins have each two main systems of branches which alternately supply and drain the cord at successive points around its circumference. Penetrating arteries extend from the ventral arterial tract into the cord substance at the boundary zone of the neuroblasts and ependyma. They are drained by delicate transverse venules.

The subclavian arteries have two small branches before supplying the limb proper, a dorsal branch which supplies the outer capillary plexus over the myotomes and a ventral twig to the Wolffian duct.

The subclavians are large vessels and control the blood supply to the limb. They must be considered now at the height of functional activity, and with this stage in the history of the primary subclavian the present account closes.

IV. Observations on the Conditions Present at Similar Stages in Embryos of the Duck.

Rabl's research on the development of the subclavian artery was conducted entirely on ducks. In it he failed to find stages earlier than the period of strictly segmental subclavians. It was consequently of some importance that these forms be investigated to see if the early subclavian capillary plexus which was present in chicks was not of fundamental value and hence present here also. Of a series of ten of these duck embryos I shall describe carefully only two typical ones, one, an embryo of thirty-three somites in the stage of an irregular subclavian capillary plexus, and the other,

Fig. 13.—Cross section of chick of one hundred and sixteen hours incubation, in region of fore limbs. My., myotome; Dor. Vein, dorsal branch of the segmental vein; Post. Sp. Art., branches of the segmental artery which contribute to the formation of the posterior spinal artery; Pen. Art., penetrating artery; P. C. V., posterior cardinal vein; Se. V., Seitenrumpfvene, thoracoepigastric veins; Rad. Art., radicular artery.

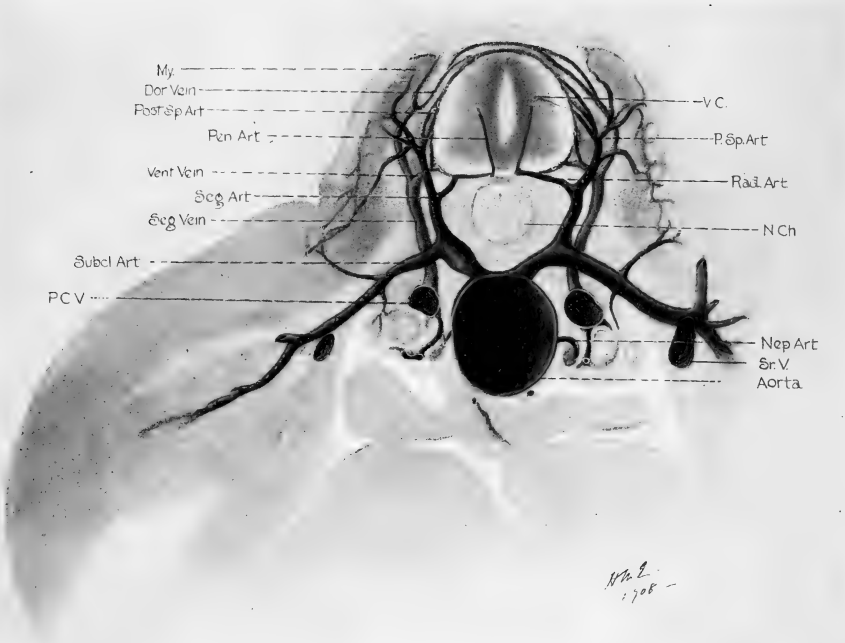
My.
Dor Vein
Post Sp Art
Pen Art
Vent Vein
Seg Art
Seg Vein
Subcl Art
P C V
V C.
P. Sp. Art
Rad. Art
N Ch
Nep Art
Sr. V.
Aorta

FIG. 13.

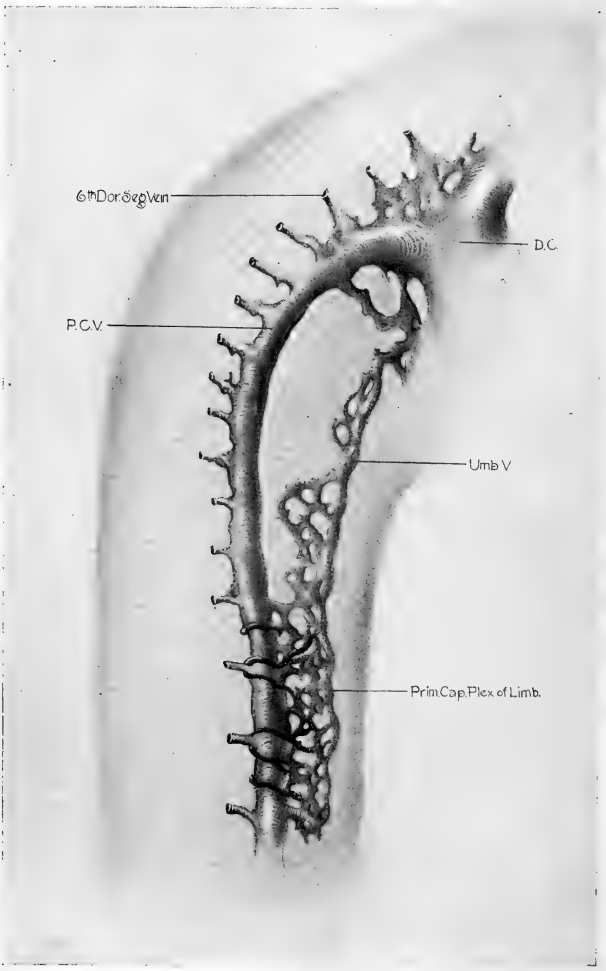

FIG. 14.

an embryo of thirty-eight somites, belonging to the period of segmental subclavians. Both embryos illustrate strikingly the facts observed in these stages for chicks.

Duck Embryos.

No.	Somites present.	Number of Subclavians present.	
		Left.	Right.
1.	33	6	6
2.	38	5	6

The duck embryo of thirty-three somites was drawn carefullly from several aspects before being cut into serial sections. A general view of the region of the anterior limb is shown in Fig. 14. One sees clearly the irregular venous channel through the remains of the primary body-wall plexus, leading now from the capillaries of the limb to the duct of Cuvier. The umbilical vein in the duck has an exactly similar origin as in the chick, and is here also the primary drainage channel for the limb. Between the sixteenth and twentieth dorsal segmental vessels an outgrowth of limb capillaries from the aortic wall occurs. There are six of these vessels. They anastomose promptly and form a continuous irregular capillary plexus extending into the limb, which is as yet a mere swelling of the somatopleure. No one capillary of the subclavian series is larger than its neighbor. The series is in no way arranged in a segmental plan.

Cross sections through the embryo show the topography of the limb region. I have drawn one which shows strikingly the hitherto undescribed origin of these earliest subclavian capillaries from the mid-lateral region of the aortic wall. They are compelled to bend dorsally in growing into the limb bud. The section (Fig. 15) shows the fourth left and the sixth right subclavians. Neither vessel happens to arise at a segmental point.

Fig. 14.—Upper body wall of duck embryo of 32 somites. Lettering as in previous figures. The wall of the aorta is concealed behind the vein.

There are six subclavians on either side in this embryo. The first subclavian on the right side occurs midway between the sixteenth and seventeenth dorsal segmental vessels, the second subclavian opposite the seventeenth segmentals, the third somewhat beyond this point, the fourth just in front of the eighteenth dorsal segmentals, the fifth somewhat beyond this point, and the sixth midway between the eighteenth and nineteenth segmentals.

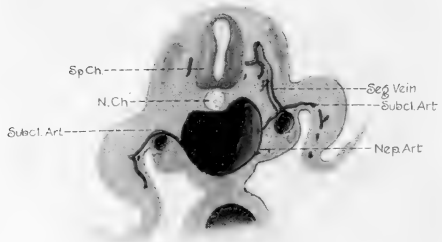

Fig. 15.—Cross section of the duck embryo shown in Fig. 14 in the region of the anterior limb buds. One notes the midlateral origin of the subclavian capillaries from the aortic wall.

On the left side, the first subclavian arises opposite the seventeenth segmental vessels, the second midway between these and the eighteenth vessels, the third just in front of the eighteenth segmentals, the fourth midway between the eighteenth and nineteenth segmentals, the fifth at the level of the nineteenth segmentals, and the sixth midway between the nineteenth and twentieth segmentals.

It is impossible, here also, to see in the arrangement of the subclavians any influence of metamerism. There are as many vessels out of segmental alignment as are in accordance with it and this because, again, in the origin of the typical plexus here as many

capillaries should chance to be opposite the intersomitic spaces as are opposite the somite masses and vice versa.

Duck embryo 2, possessing thirty-eight somites, happens to have almost as many subclavians as occurred in the younger embryo, but in the older stage, besides being larger, these vessels are almost all at segmental points, so that the embryo belongs clearly to the period of multiple segmental subclavians.

On the right side, there are six subclavian vessels arising from the aorta. The first subclavian occurs just in front of the sixteenth segmental vessel, the second and third at the level of the seventeenth segmental vessel, the fourth and fifth at the level of the eighteenth segmentals, and the sixth halfway between the eighteenth and nineteenth segmentals.

On the left side, the first subclavian stands opposite the sixteenth segmentals, the second opposite the seventeenth vessels, the third opposite the eighteenth segmentals, and the fifth opposite the nineteenth segmentals. Thus there are on the left side as many as four segments represented by subclavians.

However the study of even this embryo, with such a complete series of segmental subclavians, shows that here also there persist some vessels not in segmental alignment. The last subclavian on the right side is such a vessel, for it occurs midway between the eighteenth and nineteenth dorsal segmental vessels. The cases of two subclavians existing opposite a segmental point are easily explained by the chance origin of two of the early capillaries opposite one of the inter-somitic clefts. In such cases both vessels are equally favored, and both persist to the stage of segmental subclavians, where they increase the number of vessels to be expected. I have no doubt but that the condition in this Embryo 2, was preceded by a stage of some ten or twelve subclavian capillaries, similar to those seen in chicks 3 and 4, but these interesting stages are so transitory in character that it is only rarely that we have the good fortune to see them. Some capillaries, here as elsewhere in the developing vascular system, push out, function slightly and die in a surprisingly short time.

V. Comparison with the Posterior Limb Bud.

It was of great interest to ascertain whether the leg bud in the embryo had a similar capillary plexus from the aorta in its earliest stages. Such was actually found to be the case. Fig. 16 shows the hind limb buds in a chick embryo of thirty-two somites, No. 3 of the series.

One may see distinctly the dorsal segmental vessels and in addition, independent lateral offshoots from the aorta. At this time in the leg, the posterior-cardinal vein has not yet extended there, and both the dorsal segmental vessels and the lateral capillaries anastomose in the tissue of the limb and furnish its primary plexus. This plexus is dorsal to a more ventral plexus which arises very early; in fact, with the formation of the lower aorta, and is not to be confused with the latter.

The injections demonstrate the later origin and growth of the sciatic artery from this mesh of capillaries, so that we have to do here with an exactly analogous condition as occurs in the upper limb's vessels. In both cases, the chief axial vessel of the limb is preceded by, or may be said to exist in the form of, a simple capillary plexus arising directly from the aortic wall.

VI. Observations on the Early Mammalian Arm Bud.

At the present time we have the most complete history of the earliest limb vessels in the birds, but it is naturally of the greatest interest to compare these findings with the conditions obtaining in mammalian embryos.

Little is known of stages preceding the single axial subclavian in the latter class, save the two human embryos listed by Keibel and Elze, and the instance of early segmental human subclavians described by the writer.

I accordingly undertook a series of injections of young mammalian embryos, choosing on account of the abundance of the material,

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Fig. 16.—The caudal end of a chick embryo of 32 somites (embryo 3 of table), showing the primary capillary plexus in the posterior limb buds.

26th Dor. Seg. Vein, twenty-sixth dorsal segmental vein, *i. e.*, that in the twenty-seventh interspace.

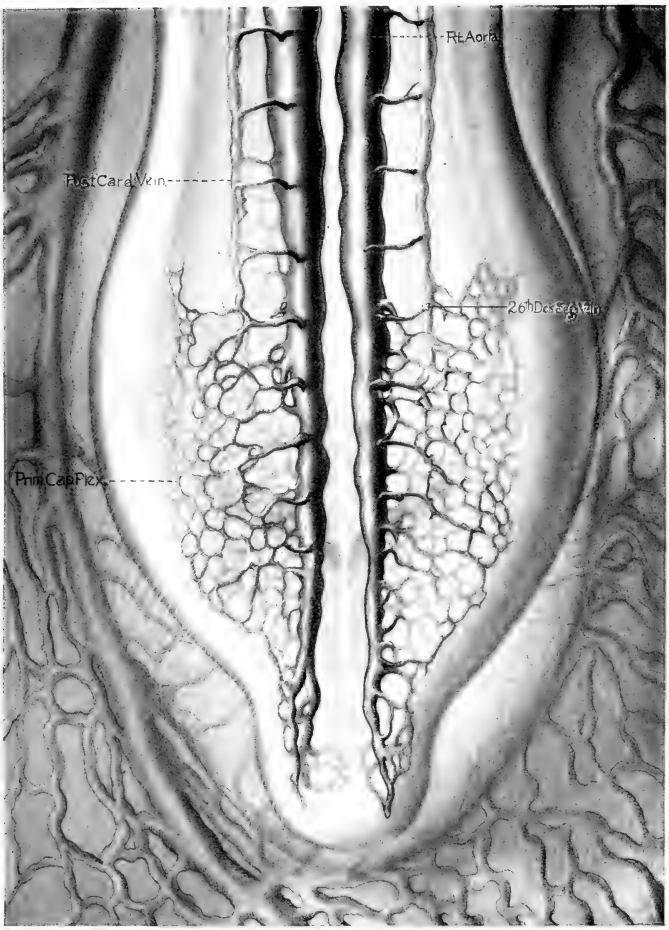

FIG. 16.

embryos of the pig. Embryos young enough to show the earliest conditions in the arm bud are not common, and to supplement this material I have been fortunate enough to examine several perfect series of rabbit embryos from the Harvard Embryological Collection through the kindness of Professor Minot. The latter embryos are all the more interesting since they were the types chosen in the compilation of the "Normal Plates on the Development of the Rabbit" by Minot and Taylor.[15] Thus the stages of development

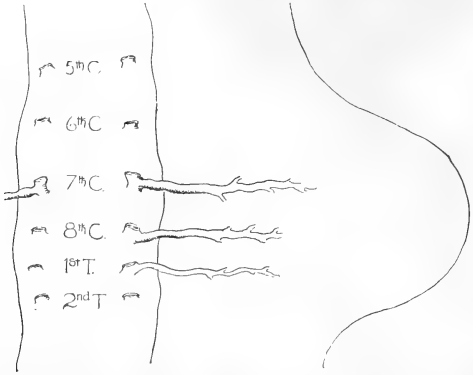

Fig. 17.—Reconstruction of the position and course of the segmental subclavian arteries present in a rabbit embryo of the tenth day, No. 559 Harvard Embryological Collection.

may be accurately known from the various details listed opposite them in the latter work. They comprised embryos designated as Nos. 562, 559 and 556 and in the "Normal Plates" are given the table numbers, 10, 11 and 12. I shall describe these very briefly.

Embryo 562 has the limb buds as mere swellings of the somatopleure. On the left side, one could not be certain of the existence of any subclavian capillaries, but on the right side, a subclavian is present midway between the sixth cervical and the seventh cervical segmental vessels.

[15]Minot and Taylor. "Normal Plates on the Development of the Rabbit." In the series edited by Keibel.

Embryo 559 has three segmental subclavians on the right side and but one on the left. The right subclavians arise from the seventh and eighth cervical and the first thoracic segmental arteries. The left subclavian is a branch of the seventh cervical segmental vessel. Fig. 17 shows a reconstruction of the subclavians in this embryo.

Embryo 556, slightly older, possesses only a single subclavian on the right side, that of the seventh cervical segment, but two segmental subclavians on the left side, those of the seventh and eighth segments.

In both the latter cases (Embryos 559 and 556) the subclavian arteries are already branches of the dorsal segmental vessels, but in the earlier case (No. 562), in which a single non-segmental subclavian existed, this was obviously not the case. There is every reason for believing that this youngest embryo is in the first stage of development of the subclavians, and one feels that the study of more mammalian limb buds at this stage will show more segmental and non-segmental subclavian capillaries.[16]

Not only in its arterial but also in its venous system does the early mammalian arm bud agree strikingly with that of the bird. In mammals, also the first and most important drainage channel for the arm is the umbilical vein. Figs. 18 and 19 show the position and character of the venules which drain the early mammalian arm bud into the umbilical vein. The uppermost or cephalic portion of the mammalian umbilical vein has long been known to persist for a considerable time as a much attenuated channel, still connect-

[16]Since these observations were made, Goppert has published an account of the early blood vessels in the arm buds in white mice and his reconstructions bear this out. He has shown striking instances of a segmental subclavian series, with segmental and non-segmental members, though he does not realize the significance of the latter vessels. Goppert is disposed to view this merely as an evidence of variability in the embryonic arterial system. He has missed the key to the solution, however, for we are dealing here, as my injections show, with the persisting members of an early irregular, capillary plexus. In such fleeting phenomena as the outgrowth and regression of many of these capillaries, we must expect to see embryos from the same uterus in slightly different stages of development. There is as good reason for this interpretation, surely as there is for his of variability.

Goppert, E. "Variabilität im embryonalen Arteriensystem." Verhandlungen der Anatomischen Gesellschaft, Anat. Anz., Bd. XXXII, 1908, pp. 92-103.

ing with the duct of Cuvier after the main vessel has established connections through the liver. *The reason for this persistence of the old upper portion of the umbilical vein is now clear, for it still furnishes an important drainage channel for the arm bud.*

Thus in pig embryos 7½ millimeters long this cephalic part of the umbilical vein still receives some seven or eight tributaries from

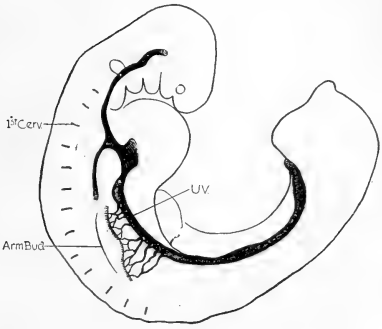

Fig. 18.

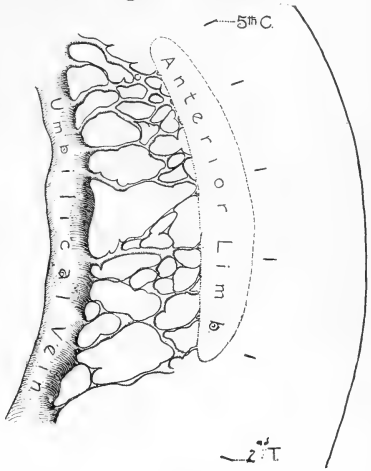

Fig. 19.

Fig. 18.—Lateral view of pig embryo 6 mms. long, showing drainage of arm bud into umbilical vein, × 11.2.

Fig. 19.—Detailed view of venules draining the left anterior limb bud of the pig embryo shown in Fig. 18, × 33⅓.

the arm bud as shown in Fig. 20. The mammalian arm bud is also drained by a series of venules opening into the posterior cardinal vein.

Summary of Results.

The chief facts brought forward in the present investigation may be summarized as followed:

1. *The first blood-vessels supplying the limb buds are capillaries which grow from multiple irregular points of the lateral aortic wall*

and anastomosing, often even before they reach the root of the limb, form a simple and quite typical plexus. In the arm bud, this capillary plexus constitutes the earliest stage of the subclavian artery, in the leg bud, of the femoral artery. The first subclavian capillaries, partaking of the character of any irregular capillary plexus, are thus never arranged in a truly segmental plan.

2. The subclavian capillaries join another plexus of capillaries, which has grown down in the body-wall from Cuvier's duct—the

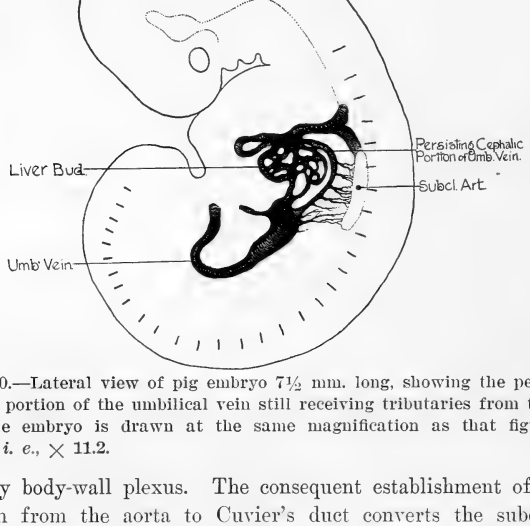

FIG. 20.—Lateral view of pig embryo 7½ mm. long, showing the persisting cephalic portion of the umbilical vein still receiving tributaries from the arm bud. The embryo is drawn at the same magnification as that figured in Fig. 18, *i. e.*, × 11.2.

primary body-wall plexus. The consequent establishment of a circulation from the aorta to Cuvier's duct converts the subclavian capillaries into arterioles and certain of the primary body-wall capillaries into a vein—the umbilical vein. In the birds, the drainage of the early wing bud is thus the sole primary function of the umbilical vein. In the mammals, although the development of the umbilical vein in connection with the chorionic circulation precedes the formation of the limb buds, nevertheless, when the arm buds arise, their capillaries establish, here also, a drainage into the um-

bilical vein. This drainage of the mammalian arm bud into the upper portion of the umbilical vein persists after the latter vessel has established its chief circulation through the liver and is doubtless one of the chief causes delaying the atrophy of the upper or cephalic portion of the umbilical vein.

3. The occurrence of a period of multiple segmental subclavians is brought about by processes of atrophy and doubtless slight shifting. Thus most of the primary subclavian capillaries which are not at segmental points, *i. e.,* opposite the interspaces between the somites, eventually atrophy, leaving as functioning vessels only those members of the early series which are fortunately situated in accordance with this plan.

4. Even during the period of true segmental subclavians, however, there often persist some members of the first subclavian series which are out of segmental alignment. These may indeed get to be vessels of some size. The chief primary subclavian artery itself may not at first happen to lie at exactly a segmental point. The chief determining factors in the persistence of vessels are doubtless hydrodynamical and only secondarily the influence of metamerism.

5. A purely segmental character in the arm vessels is finally secured at the time of inclusion of the subclavian vessels as common trunks with the dorsal segmental vessels. This union is not a process of active fusion of the subclavian and dorsal trunks but is effected by processes of unequal growth which occur in the expansion of the aortic wall. Dorsal and subclavian arteries are carried out together by a local bulging of the aortic wall, which becomes a common trunk.

6. The primary subclavian artery represents the exaggeration of one of the pairs of segmental subclavians, which is most favorably situated as the principal circulatory channel for the limb.

VIII. Application of these Facts to the General Embryology of the Vascular System.

Two conceptions have arisen regarding the method of development of the vascular system. According to the one, arteries and veins grow out to their end beds as development proceeds, but according to the other, vascular activity is always initiated by capillaries

which tend everywhere to form a mesh-work or plexus; arteries and veins are always subsequent formations from such capillary plexuses due to the transforming influence of the circulation.

The former conception would appear to be held by most of the workers in angiogenesis, though most of the descriptions of the development of vessels are so worded as to avoid a lucid statement on this fundamental point. I may refer, for instance, to the many admirable researches of Hochstetter, where, though many important facts concerning the chief embryonic vessels are clearly given, one may look in vain for anything bearing on this point. In the case of the limb vessels, for instance, we must imagine from his description that the single axial vessel grew out into the core of the limb. Very recently Curt Elze[17] has ranged himself with those who would recognize such a process as the means of development of all the body's vessels and as definitely opposed to the idea of a capillary plexus anlage for any of them. On the other hand, Hans Rabl and Erik Müller have supported vigorously the latter idea, the foundation for which had been laid in the great paper of Thoma on the origin of the chick's yolk vessels.

There is plenty of morphological evidence in the adult body for a preëxisting plexus or net-like condition of all the vascular trunks. The remarkable number of variations in the position, system of branching and anastomoses cannot be explained as satisfactorily on any other basis. Thus, without knowledge of conditions in the embryo, Aeby[18] and Baader[19] promulgated such a plexus origin for blood-vessels many years ago.

Professor His did not hesitate to state that the main vessels in the embryo were derived from net-like anlagen, but it remained for Thoma[20] to make the meaning of all this very significant.

Thoma observed that in its early stages the system of the vitelline vessels in the chick formed a strikingly uniform simple plexus of

[17]Elze. "Beschreibung eines menschlichen Embryo von zirka 7 mm. grösster Länge." Anat. Hefte, 1-35, 1907.

[18]Aeby. "Der Bau des menschlichen Körpers." 1871.

[19]Baader. "Ueber die Varietäten der Armarterien des Menschen." Inaug. Diss. Bern, 1866.

[20]Thoma, R. "Untersuchungen über die Histogenese und Histomechanik des Gefässsystems." 1893.

irregular capillaries. He observed that the fortuitous position of some of these capillaries with respect to the aortæ and venous ostia of the heart gave them a more constant and rapid circulation than occurred in other capillaries of the mesh. Later stages showed these capillaries became arteries and veins respectively. As the vitelline vascular system grew, Thoma saw the same laws at work from center to periphery, that the further elaboration of the arterial and venous trees was the result of successive incorporation of adjoining portions of the general capillary plexus. If these processes are at work everywhere in the development of the vascular system, they furnish us with a better understanding of angiogenesis, for the development of a given artery or vein to any portion of the body cannot be due to miraculous predestination but to the definite action of quite definite physical laws. Capillaries first invade a region and the relation of these capillaries to the nearest arterial and venous channels determines always the manner in which the new veins and arteries shall arise. Then elaboration of arteries and veins is always the result of hydrodynamical forces involved in the circulation.

The application of Thoma's work to the development of the blood-vessels in the body of the embryo has never been adequately tested.

The method of injecting completely the embryonic vascular system has furnished much evidence that the capillary plexus anlage can be demonstrated for all the body's vessels. *The preceding account of earliest circulatory conditions in the limb bud gains much significance in this light, for before there can be said to be limb arteries or veins, a primitive plexus of capillaries grows into the limb tissue. From this plexus in later stages, arteries and veins are formed.*

Rabl has shown the origin of several arteries in the fore-limb region from capillary nets, but one must leave this interesting story, the development of the later vessels, to another time. *In the present study, we have been able to see that in the limbs, the main vessels themselves—the femoral and the subclavian arteries—exist originally in the form of a capillary plexus.*

In conclusion I beg to speak with gratitude of the many suggestions and helpful interest in the present investigation which I owe to Professor Mall.

THE CUTANEOUS GLANDS OF THE COMMON TOADS.*

BY

EFFA FUNK MUHSE.

WITH 7 PLATES.

CONTENTS.

*Contribution from the Zoölogical Laboratory of Indiana University, No. 96. being a thesis accepted as in part fulfilling the requirements for the degree of Doctor of Philosophy, June, 1908.

General Introduction.

The purpose of the present paper is to show that there is but one kind of gland in the cutis of the common toads. The writer is convinced that the several kinds of glands described by authors are but different stages in the development of the same gland. Throughout the paper, the reference is to the general integument of the body, exclusive of modification in the region of the head, feet and cloaca. It is principally the conditions in the adult toad that are under consideration, but references are made to conditions in certain stages preceding the adult, when a point may thereby be more clearly discussed. The structure of the epidermis and cutis in Batrachians is more or less a matter of general knowledge, and the writer has considered them very briefly, merely for ready reference while dealing with the glands.

I wish to express my grateful appreciation of the facilities placed at my disposal by the Department of Histology and Embryology of Cornell University, where the first material for this investigation was prepared. The work was finished in the Department of Zoölogy in Indiana University, and I am greatly indebted to Dr. C. H. Eigenmann and Dr. Charles Zeleny for valuable suggestions, and criticism of the final report on the material examined.

Material. Toads have been collected during this investigation from three different localities. The first specimens (Bufo americanus) were taken at Ithaca, N. Y., principally at the breeding

season late in April.[1] Others (Bufo fowleri Putnam) were collected during the summer and autumn in Washington, D. C. Additional toads (Bufo fowleri Putnam) were collected at Bloomington, Ind., at the breeding season in the middle of April, and during May.[2]

The two species differ somewhat in the coloration and in the shape and arrangement of the warts. The brief description of the color pattern given in this paper, and the relation of the wart distribution to the same, applies definitely to fowleri. On the other hand, the detailed description of the skin and its glands has been derived for the most part from the study of sections of americanus, whose skin is thicker and whose warts are more massive. I have noticed no essential differences in the minute structure of the parts and do not deem it necessary to limit my statements to either of the two species. All drawings and photographs of sections from adult toads, except those horizontal to the surface, *i. e.*, transverse of the glands (Figs. 1, 16, 52, 69-76), and except Figs. 55 and 77, are from americanus.

Methods. Pieces of skin from different parts of the body have been killed in the more common fixing agents, Perenyi's, Zenker's, Gilson's, Flemming's, mercuric chloride and 4 per cent. formaldehyde. The last has proved the most satisfactory for the purpose of this investigation.

Many stains have been used. Delafield's hæmatoxylin with Fischer's eosin, and Mallory's stain have been quite sufficient for general detail; Tanzer's orcein has been used to differentiate elastic fibers. The material has not been prepared with the view to studying the distribution of the nerves to the glands, or the nature of the nerve endings.

Photographs give the general relations which are essential to the elucidation of this paper. Drawings have been made when finer detail was desired.

[1]The specimens of americanus found in the National Museum were kindly placed at my disposal by Dr. Stejneger, for observation of external features.

[2]I am indebted to Miss Mary C. Dickeson for the identification of a typical specimen, one from Washington and another from Bloomington, as Bufo fowleri Putnam.

Literature. The skin and the skin glands of Batrachians have been written about very extensively since 1840, when Ascherson dealt with the skin glands of the frogs. The family Ranidae and the families of Urodela have since furnished the favorite species on which work has been done. The skin of the common toad, or the glands contained therein, has in only a few instances been made the subject of a structural study. The toad has been dealt with alone (Eckhard, '49, Rainey, '55, Calmels, '83, Weiss, '99, Bristol and Bartelmez, '08) or incidentally as one of a series of Batrachians (Bolau, '64, Leydig, '67, Schultz, '89, Seeck, '91). With but very few exceptions, the many investigators have been of the opinion that there is present in the cutis of the species of Batrachians dealt with, more than one kind of gland. There is also a diversity of opinion regarding the structure of what is known from the description or classification to be an equivalent type of gland.

General Description of the Skin.

Unevenness. As compared with other Batrachians, the adult toad is very warty over its dorsal surface, and there is a general unevenness over the whole body. Microscopic examination of mounts of the molt, and of prepared sections from various regions of the body, further emphasize the great diversity of elevations in the surface covering of the animal.

Causes. Numerous gland sacs are located in the cutis, each with a duct passing to the surface of the epidermis, where a distinct opening is visible. At many places in the dorsal skin of the toad, the glands are grouped, producing the so-called warts. (Figs. 9, 12-14, 21-24.) The skin lies on a more or less even, unimpressionable surface, the muscles and bone or cartilage, and since the glands can make little or no impression on them, most of the expansion due to these many gland sacs, is outward. The unevenness of the skin is further caused by the presence of elastic fibers in the cutis. The part played by these fibers is evident from two observations. If a piece of skin is cut from any region of the body, the piece becomes smaller and the hole much larger than the original incision. The opposite effect occurs when a toad is frightened. The skin of

the body becomes inflated and is then stretched much beyond its normal size. The grouping of glands on the dorsal surface quite overshadows the wrinkles caused by the elastic fibers; but on the ventral surface the wrinkles are of nearly equal importance with the glands, in producing the unevenness.

To the naked eye, the ventral skin has a rather uniformly flaked or blocked off appearance. In one individual the blocks may be conical in form, in another rounded eminences, while in others they appear as flattened pavement. At the areas of transition into dorsal skin, the flakes increase in size, and the uniformity is gradually broken up by the occurrence of warts. Moreover, the skin at the bend of the limbs does not have the blocked-off appearance. It either lies in creases or is smoothly stretched. In the area of the ventral skin at the union of the thighs, the blocks are larger and quite unequal in size and shape. Sections from the lower side show that the skin between the elevations is relatively very thin, and the great variation is confined largely to the outer cutis layer. The glands are sparsely scattered through the elevations (Fig. 15). They are sometimes grouped but never have the close definite arrangement that is found in the warts of the dorsal skin (Fig. 14), nor do they ever attain the large size of those above.

Classes of warts. The warts of the dorsal surface may be divided into three classes: (1) The most prominent elevations which occur on the dorsal surface are the so-called parotids (Fig. 9). They are a pair of low, elongated, relatively smooth masses. These are located on the back, each in line with and a short distance behind either eye, the cranial crest alone intervening. The outer forward end of each is in close proximity to the tympanum. The two parotids of a given individual are quite alike in shape, color and dimensions. These warts are constant for the species and are, as a rule, proportionate to the size of the animal. The parotids of an average sized adult measure about 6 by 13 mm.

The remaining warts vary and can be described in general terms only.

(2) Next to the parotids the largest warts occur on the back posterior to the parotids, on either side of the median line or groove,

which extends often without interruption from just behind the snout to the tip of the vertebral column. The skin over the dorsal tibular portion of the legs bears warts of a similar nature. This class of elevations measures from 1.5 to 4 mm. in diameter, and there occasionally occur elongated mound-like masses which reach a length of 5 or 6 mm. The warts are often in groups of two, three or more. These warts or groups of warts are not bilaterally symmetrical in their arrangement, nor is there any constancy in the number present.

(3) The remaining warts, those which measure less than 1.5 mm. in diameter, may be put in a third class. The warts on the upper eye-lids, cheeks, flanks, outer surfaces of the thighs, distal edges of the hind feet and in the spaces intervening between the larger elevations previously described, constitute this class. A row of these warts extends along either flank. It begins at the outer posterior edge of each parotid and extends to about the point reached by the knee of the hind leg when folded against the body.

Coloration. Color. The toad follows a general rule of animal coloration. The ventral surface is light and the dorsal some shades darker. The transition region is at the line which separates the exposed from the unexposed part, when the animal lies stretched out on its ventral surface. The color of the lower surface differs with individuals and is either buff, dull white, faint yellowish or pale green. Between the fore limbs a few dashes of pigment occur. Individual toads differ greatly in the appearance of the dorsal surface. All are more or less conspicuously or inconspicuously spotted. In some cases the spots are but slightly darker than the remainder of the surface and may be very few in number. In others there is the greatest contrast possible with the colors present. The ground color of the dorsal surface is, as a rule, in darker shades of the ventral color. The spots appear as if superimposed in still darker shades of the same general color. The spots are, however, often so dark as to appear seal brown or even black. Frequently the spots, many of them at least, are encircled by a very narrow band of jet black, red, yellow or white, which makes the contrast greater.

Arrangement of spots. The more distinct spots occur on either side of the median line of the back in a more or less regular series

of six or eight pairs (Fig. 9). The first occurs over the eye bulges, a second and third close to either end of the parotids. Others are present on back to near the tip of the vertebral column. There exists no uniformity between the shape and size of the spots which occupy a somewhat corresponding position. On either side of the median line of the same individual, an approximately equal area is occupied by spots, even though their number may not be equal. Some animals possess small and numerous spots, others fewer but larger ones. Spots which appear as a continuous band across the legs when they are folded against the body occur with considerable constancy over the thigh and tibular portion of the hind limbs. Darker bands are also present over the cheeks and ulnar portion of the fore limbs. Minor, less distinct spots are found on the remaining dorsal surface of the flanks and limbs.

Relation of warts to spots. The parotids are, as a rule, of the same or of a slightly darker shade than the ground color of the dorsal surface. Only occasionally is one or both of these warts covered by a decided spot. Quite often, however, the darker pigment spreads very faintly over a part of the surface. Far the greater majority of warts forming the second class [(2) p. 325] are grouped in those spot areas of the dorsal surface, which are most decided in color and in the dark band across the tibular portion of the legs. The warts are of a lighter shade than the spots and this fact further increases the complexity of the color pattern.

Except for the one or two days of each year spent in the water, at the breeding season, the toad passes its time on, or burrowing into dry land and to this end its outer covering is adapted. The smooth slimy skin of the tadpole, during metamorphosis and growth into the adult, gives place to a relatively dry and extremely warty skin. This admirably fits the toad, even aside from its characteristic coloration, to harmonize with the loose soil of the garden or road side.

The Epidermis.

Strata. The lower layer of cells, those in contact with the cutis, constitute the germinating stratum. As the cells pass outward from this stratum, they show a gradual change in their shape and character.

After having occupied a place in the two or more cell layers of the transitional stratum, they become lifeless material, the molt, which is periodically cast off. (Figs. 2-4, 19, 25, 33, 48, 49.)

Beaker cells. One celled glands, called beaker cells, aid in this process of molting, as was early stated by Schultze ('67) for Batrachians in general. In the adult toad these beaker cells are the only glands located wholly within the epidermis. They are situated at intervals in the upper part of the transitional stratum and differ from surrounding cells in shape and appearance. (Figs. 1, 2, 3, 48.) The bodies of these cells may lie at any depth among the two or three outer layers of the transitional stratum They connect with the surface of this stratum by means of a more or less slender neck, which has an opening at the upper end. The body of the cell is almost entirely filled by a nucleus whose chromatin is collected in a few masses. Two nuclei in a cell have been observed. The cytoplasm of a beaker cell is much clearer when stained than that of surrounding ones. These cells have been met with in all regions of the epidermis, ventral skin, ordinary skin of the back and the epidermis over the parotids and other warts of the back and legs. They are often so slender that in cross section, the body of such a cell is but little larger than the nucleus of an ordinary cell. They may lie between two cells or at the angle of several. Their distribution varies. A section horizontal to the epidermis, from a parotid, shows them to occur in about the proportion of one beaker cell to three ordinary cells if we consider but one layer of cells. (Fig. 1.) In another individual, a vertical section of ordinary skin showed eighteen such cells within a distance of 5 mm. (Fig. 3.) At the opening of a beaker cell, an accumulation of granules is frequently noted lying between the transitional and molt strata. Occasionally at these points the molt stratum is raised and freed for a considerable distance. Everything points to the conclusion reached by Schultze and later by Schultz ('89) that these cells are one-celled glands, whose secretion loosens the molt from the transitional stratum.

Epidermis relative to the regions of the body. The epidermis reaches its maximum thickness over the parotids. This is due both to the larger size of the cells and to the greater number of cell layers.

The epidermis is here very uniform and even, as compared to other regions. (Figs. 2, 14, 23, 25, 57, 58.) The epidermis is usually thickened and dips down to form a pit about the mouths of the ducts from the large glands. The thickening is due to an increase of cells in the transitional stratum. The ducts of the small glands do not, as a rule, cause a depression.

The epidermis over the larger warts of the back and legs corresponds in the general structure to that over the parotids, except that its thickness varies somewhat with the size of the wart (Fig. 21). The epidermis in the areas between the warts has a thickness equal to only from one third to a half the thickness over the parotids. Each stratum is here proportionately decreased through a change in the size of the cells and in the number of layers. The transitional stratum consists of two or three layers as compared to from four to seven in the same stratum over the warts (Figs. 2, 3, 21). The average thickness of the ventral epidermis is intermediate between that over the warts and that of the areas between them on the dorsal side of the animal (Figs. 4, 15).

The Cutis.

The cutis is far more varied than the epidermis. It is subject to rearrangement occasioned by the increase or decrease of intrusive elements such as the glands, nerves and bloodvessels. Its depth is always greatest where the largest gland sacs are located (Figs. 14, 23, 24). It is least where they are entirely absent (Figs. 17, 18, 21-o.s.). In the latter areas of the back, the cutis is most typical and uniform.

Strata. The primary element of the cutis is the connective tissue fiber. Apparent difference in the strata of the cutis is due to the arrangement of the fibers for a specific purpose. I shall speak of the outer loose, compact and inner loose strata, which are descriptive terms (Figs. 17, 18). The compact stratum consists in large part of bundles of fibers. These are closely associated and extend in different directions. In some areas the bundles occur in successive horizontal sheets, approximately at right angles to each other (Fig. 17). Other regions or individuals show these bundles

somewhat interwoven. The essential feature in the arrangement of the bundles is their compactness. At certain points bundles from the horizontal compact layer turn both inward and outward, to form part of vertical strands which pass through the compact stratum; these bundles subdivide, and together with bundles which branch off from either surface of the compact stratum, form the inner and outer loose strata of the cutis. In either case the fibers are very loosely arranged in a network. Fibers from the outer loose layer terminate on the side toward the epidermis, in very fine numerous branches. These are closely associated with the processes of the cells of the germinating stratum of the epidermis. This gives the appearance of a very thin homogeneous stratum, which for the most part follows intimately the lower border of the epidermis (Fig. 77). The union between the compact layer and the muscles is effected through the inner loose stratum. Here, as in the outer stratum, the fibers are loosely interwoven. Elastic fibers of considerable length and of very uniform diameter are also present. Throughout the three strata they occur widely scattered and without definite arrangement.

The blood-vessels pass from the deeper tissues into the inner loose stratum of the cutis. They form a coarse network parallel to the surface. From this network branches pass directly to the large glands (Fig. 55). Others pass through the vertical strands to the outer loose stratum (Fig. 17). Here, likewise parallel to the surface, is formed a fine network of capillaries, which is just beneath the pigment cells. From this network capillary loops pass outward and lie at the base of endbulbs (Fig. 20). Other loops project for some distance into the epidermis (Fig. 19). The branches previously mentioned as passing from the inner network of blood vessels to a gland break up into a dense capillary mesh. This intimately surrounds the gland acinus, lying at many points in direct contact with the gland wall (Figs. 44, 47, 51-58).

Cutis according to regions. The cutis varies greatly in depth wherever gland sacs occur, according to the number and size of the same. The gland sacs lie, as far as their sizes permit, in the outer loose stratum, but the larger ones lie deeper, for the most part in the compact stratum (Figs. 14, 25, 40-42, 58). Often the latter

sacs are surrounded by a very thin sheath of loose connective tissue which is continuous with the upper loose layer. About the sacs and also the gland outlets, the finely divided fibers may form a homogeneous layer similar or even continuous with that appearing at the inner border of the epidermis. A definite network of elastic fibers surrounds the large gland sacs. That this is not the result of pressure of the gland wall against the cutis is shown by the fact that the fibers about the neck and collar form a stronger, more definite network than about the sacs (Fig. 76). Here pressure could count for very little. In the warts the bundles forming the compact stratum are always more or less interwoven (Figs. 25, 40, 61). Transverse strands are present, but they curve about the glands or are visible as vertical strands through only a part of the depth of the compact stratum. Nothing indicates that they take any special part in the support of the gland sacs.

Wherever warts occur, there is a change in all the strata of the cutis proportionate to the extent and height of the wart. Greater demands are made on the blood vessels, nerves and elastic fibers, and there is an increase in the depth of the inner loose and, to a less extent, in the outer loose strata. A great change likewise takes place in the compact layer, because of the increased need of support. The ventral skin is decidedly, but quite uniformly, uneven. The inner loose and compact layers are relatively uniform in depth, and in great part it is the outer loose layer which varies (Fig. 15).

In the ordinary skin of the dorsal surface, especially at the edge of the warts, papillæ may occur. These are produced by the projection into the epidermis of an end bulb of spirally arranged cells, at the base of which is a blood loop (Fig. 20). In the ventral skin and over the warts are found what I shall call rudimentary bulbs. In this case a small amount of cutis projects into the epidermis for a considerable distance. It bears at its summit a single large cell, the long axis of whose nucleus is perpendicular to the surface.

Cutaneous Glands of the Toad.

Introduction.

The cutaneous glands with which we are dealing consist of three parts: neck, collar and acinus (Fig. 42); each is made up of many

cells. Such a gland is a differentiated part of the epidermis. As the acinus of a gland enlarges, it pushes farther and farther away from the epidermis into the cutis, with the elements of which it has no direct connection. The strata of the cutis increase in depth and are rearranged and adapted to the bodies of the glands. These epidermal glands go by the name of cutaneous glands to distinguish them from the one-celled glands of the epidermis, the beaker cells.

The present investigation has convinced the writer that there exists in the cutis of the toad only one kind of gland. All cutaneous glands, however different they may appear, are developmental stages of the one kind. The climax of the complex, graduated series is reached by the large sacs that hold in readiness a great quantity of granular secretion. Those Batrachians in which the parotids occur present the greatest differences in the size and structure of the glands, and in the location of their sacs in the cutis.

Literature. Many authors, whether considering the glands of Batrachians from a histological, embryological or physiological point of view, have proceeded on the theory that at least two kinds exist. The conclusions of those who have discussed the question of the number of kinds of glands differ. The majority believe that two, even three, or four kinds of glands are present. Many of the earlier writers made their classification largely on the basis of size into large and small glands, or according to the shape of the gland acinus. (Ascherson, '40, Eckhard, '49, Rainey, '55, Hensche, '56, Szczesny, '67, Ciaccio, '67, Leydig, '67.) The more recent classification is into mucus and poison, nuclear, or granular glands, based on a difference in the epithelial structure and in the secretion produced. (Englemann, '70, Schultz, '89, Seeck, '91, Drasch, '92, Weiss, '99, Phisalix, '00, Esterly, '03, Bristol and Bartelmez, '08.) Esterly speaks of the large poison glands and of the mucus variety. He finds that in every large gland there is the fundament of a new gland, which resembles glands of the mucus variety. He, however, shows no relation between the separate mucus glands and the large poison glands. Several investigators (Calmels, '83, Leydig, '92, Vollmer, '93, Nicoglu, '93, Junius, '96, Ancel, '01) have given evidence or expressed their belief in one kind of cutaneous gland.

Calmels is the only one of this group who has dealt with the toad. He has evidently excluded from his discussion the glands of the ventral surface, for he makes the statement that the poison glands which occur only on the dorsal surface differ from those of the ventral side, in that they contain a milky secretion produced by the poison cells. He makes no mention of smooth muscle fibers about the glands. It is evident from his description that he has seen the muscle fibers, but has mistaken them for epithelial cells. Recognizing but one kind of gland, he establishes with the epithelium as a basis four types. According to him, the youngest epithelium is found about the lumen of the largest glands, but he has mistaken the muscle fibers in slightly longitudinal view for young epithelium. What he describes as daughter cells of the epithelium in the other three stages of epithelium found in the small glands are probably the transverse view of muscle fibers. I have not had access to Leydig's article nor to Nicoglu's. Nicoglu and Vollmer are referred to by Esterly as having stated that they saw regenerating forms of glands. Both worked on salamanders. Vollmer figures a small gland arising from the epidermis, but does not give intermediate forms. His study was largely an experimental one. Nicoglu and Heidenhain each seem to have published an article in 1893, as the results of the same investigation. If this is correct, the two reached different conclusions, for Heidenhain divides glands into three kinds—mucus, poison and double. Esterly refers to Nicoglu as an investigator who believed in one kind of gland.

Junius, dealing with the structure of the cutaneous glands of the frog, describes what he believes to be the young and old forms. He, however, merely expresses it as a belief that the forms he describes are developmental stages of the same kind of gland. He did not observe intermediate stages. He further expresses it as his opinion that in the renewal of glands, the process of their embryonic development is probably repeated. He offers no evidence for this opinion.

Ancel deals with the development of cutaneous glands especially in the salamanders. He states that all glands arise in the epidermis, but that certain among them are arrested sooner in their evolution

than others; that the large poison glands are more completely differentiated toward a special function.

Secretion and when expelled. Under proper conditions, the secretion, which is produced by the cutaneous glands of Batrachians, comes to the surface of the animal. The skin of a toad, which has in no way been disturbed, is dry. If it is roughly handled, some of the cutaneous glands expel a colorless fluid and the skin becomes moist. Under a powerful stimulus a milky secretion oozes through the ducts to the surface in the form of small drops. It is probable that in nature, only the most severe shock or torment by a natural enemy causes the expulsion of the milky secretion. The toads, which have been dealt with in this investigation, have in no case expelled it short of chemical, electrical or severe mechanical stimulus, or only after the head has been severed from the body. If the stimulus, *e. g.,* electrical, is localized, only the glands from the stimulated region are discharged. The relation of the transparent and the thick milky secretions to the glands will be discussed later. As far as concerns this investigation we will accept it as an established fact that at least the milky secretion has an irritating effect on mucus membranes. Further, that on certain small animals of other species it is poisonous and even fatal in its results. Investigations of a chemical and experimental nature have shown this to be true of the secretion from a number of Batrachians. (Gratiolet and Cloëg, '52, Calmels, '82, Phisalix, '00.)

Origin of glands in the larva. The development of glands in the larva has been followed in a few Batrachians, notably the salamanders. With one exception (Phisalix, '00) the more recent writers (Maurer, '95, Ancel, '01) agree that they come from the ectoderm. Here and there in the lower cell layer of the primitive epidermis of the tadpole, a single cell becomes differentiated. Rapid cell division follows and a gland bud, *i. e.,* a small solid mass of cells is soon established. This cell collection pushes down into the cutis, retaining a connection with the epidermis. At the point of connection the future duct is developed and a lumen soon arises in the acinus. I have observed gland buds in the toad tadpole, whose body length was 8 mm. (Fig. 6). The hind legs had at this stage reached a

length of 2 to 3 mm. and the fore limbs, while well formed, were not yet visible on the surface. The buds occurred in both the ventral and dorsal epidermis of the body and limbs. A younger series, whose body length was 6.5 mm. and whose hind limbs were merely buds, showed no indication of gland formation (Fig. 5). It is interesting to note that the gland buds arise just after the animal has passed its most typical aquatic form, and appear at the same time with other adult structures.

Gradation of glands in adult. I have found buds of a similar nature in the epidermis of the adult toad (Fig. 26). From this point on a very completely gradated series of glands may be selected (Figs. 27-41). The climax of the series, beyond which point degeneration may set in, we will term the mature gland (Figs. 14, 42, 58-a). The size of the neck, collar and walls of the acinus (excluding for the present the epithelium) reach, in the mature type, their highest development. The elements which constitute the parts are all present in the younger glands. They are developed in proportion to the size and age of the glands. Throughout the series a gradual change in the epithelium and in the secretion can be followed. The production of poison granules in the secretion completes its highest development.

Batteries of glands. The great variation in the size and shape of the glands and differences in location in the strata of the cutis incident to their size, make it difficult to give a general description. I shall, therefore, for convenience designate three strata or batteries of cutaneous glands: (1) inner, (2) outer and (3) transitional (Fig. 25, a, c, b). The glands occur singly in the ordinary skin of the back, and singly or somewhat closely arranged in the ventral skin. In warts, where they reach the largest size, they are closely grouped. The glands in a given battery vary greatly according to the regions of the body.

(1) The glands in any region, whose bodies reach almost to the inner loose stratum of the cutis, constitute the inner battery (Figs. 14, 25-a, 58-a). The lumen of such a gland is completely filled with secretion and is irregularly lined by naked nuclei rather than by a cellular epithelium (Figs. 43-47). The extent of each wart is gov-

erned largely by the size and number of glands of the inner battery. Authors have given to these glands in the toad, and to corresponding glands in other Batrachians, the name of large, contractile, granular or poison glands.

(2) Among or just beneath the pigment cells are found small glands, whose bodies lie wholly in the outer loose cutis, and which make up the outer battery of glands (Figs. 12, 14, 23, 25-c, 58-c). The bodies of these glands never reach a deeper level than the collars of the glands of the inner battery. A lumen is present which is lined by a definite epithelium (Figs. 28-35). These have in general been called small, non-contractile, or mucus glands.

(3) Those glands which show an intermediate condition, both as regards the structure and the location, comprise the transitional battery (Figs. 23, 25-b, 36-41). Such glands are not always present where those of the inner and outer batteries occur. It is probable that when these glands have occurred, most authors have classed them with the mucus, small or non-contractile. In a few instances a separate category has been established to include these glands.

I am convinced that the glands of these three batteries are all different stages in the life history of one kind of cutaneous gland.

The Mature Gland.

Mature glands occur in the skin of the various parts of the body. They reach their highest development and largest size in the central areas of the parotids of the adult toad (Fig. 14). The following description of the mature type applies definitely to those occurring in the above region. A median longitudinal section of such a gland shows that it is relatively an enormous sac-like body with a short, thick outlet which consists of a neck and collar (Figs. 14, 25-a, 42, 58-a). The neck is in connection with the epidermis and the collar is a great accumulation of cells that marks the transition from the neck into the body of the gland (Figs. 78, 79). A duct leads from the cavity of the sac, the lumen, through the neck and collar to the surface. The most conspicuous feature of this type of gland is the great quantity of granular secretion, which completely fills its lumen. In the central areas of the parotids, the vertical diameter of these

gland sacs is always greater than the transverse and often reaches a depth of 1800 microns, and even as great as 1900 microns. If the sacs are not crowded, they are more or less circular in the transverse plane. If they are closely associated and are affected by pressure from neighboring glands, a variety of polygonal shapes arise (Fig. 16). The transverse diameter varies from 500 to 1200 microns.

Wall of acinus. The gland sac consists of a homogeneous substance or matrix. In the periphery are imbedded muscle fibers. To the lumen surface are attached epithelial cells or nuclei. In some cases the wall of the acinus is thin and the muscle fibers form a more or less continuous sheath, and the epithelial cells rest closely against the fibers (Figs. 44, 59, 60). In other instances the wall of the sac is thick, the individual fibers are less closely arranged in it and the fibers and epithelium are separated by a considerable amount of matrix (Figs. 45, 47, 56, 62, 65). The nature of the matrix of the acinus wall has been variously interpreted. Drasch is the only investigator who has made any reference to a substance which encloses the muscle fibers and on which rests the epithelium; he says that the whole is surrounded by a membrana propria. Several others (Schultz, Weiss, Junius) state that the muscles rest upon a membrana propria or basement membrane. Schultz further states that each epidermal cell sends a foot in between two muscle fibers. Mme. Phisalix says that the membrana consists of smooth muscle fibers.

Muscle fibers. The muscle fibers are of the involuntary kind, each elongated and spindle-shaped. An oval or elongated nucleus contains a single nucleolus (Figs. 43-46, 51, 54, 61). In many instances I have followed fibers through their whole extent and found them to be single spindles. Other fibers, with one end split in two, have been observed. Several writers (Drasch, Junius, Ancel) have stated that the muscle fibers are divided into fibrillæ at one or both ends. I have never observed a similar condition in the toad. The fibers are meridionally arranged and adapted to the curvature of the gland body. The outer ends of a limited number of these fibers extend to the outer part of the gland collar and their opposite ends are overlapped by the tapering ends of other fibers. The number of

fibers gradually increases until the greatest circumference of the acinus is reached. From this region they decrease toward the lower pole over which they pass. The average fiber about a large gland has a length of from 130 to 150 microns and in the region of the nucleus a diameter of from 7 to 10 microns. Several fibers are required to complete the circuit of the gland. There is, therefore, no definite arrangement of the nuclei about any given part of the acinus. Seeck describes similar structures, *i. e.*, the spindle cells, and considers them replacement cells for the epithelium. He denies that they are muscle cells. The transverse sections of the fibers appear as a layer of cubical cells (Figs. 47, 51-53, 56, 61, 62, 65, 66, 75). Weiss evidently mistook this view of the fibers for epithelium. A discussion of the action of the muscle-fibers will follow later.

Epithelium. The epithelium of this type of gland consists in large part of naked nuclei. They are attached to the matrix or are partly sunken in the same. They are not uniformly distributed. Over small areas they form a continuous pavement; over similar areas they may be entirely absent (Figs. 43-47, 56, 59-66, 75). The nuclei take in general one of two forms: (1) flattened, circular bodies, which appear quite uniformly dense when stained (Figs. 44, 59), and (2) those which arch out toward the secretion and show a clear area on the attached side (Figs. 45, 47, 60, 65). Mme. Phisalix studied the salamander with special reference to the part played by the nucleus in evolving the secretion. She describes nuclei, which take the form of parachutes and states that they are actively engaged in producing poison grains. The nuclei of the toad are much smaller than those of the salamander. An epithelial cell, that is, a nucleus situated in a small definitely limited cytoplasmic mass, in contact with the matrix, is seldom met with in the mature gland. The body of such a cell may be densely granular (Figs. 51, 52), or it may appear entirely homogeneous, or with but a few granules (Figs. 51, 61). One individual, collected with others at the breeding season, proved an exception in that a large number of cells were present. Even in this instance there was by no means a continuous cellular epithelium. The cells varied greatly in size. They were found in all parts of the acinus, and were here and there greatly

crowded together. The nucleus was situated in that part of the cell which was attached to the matrix. The cytoplasm of the cell was very similar to the secretion in the gland lumen (Figs. 16, 52).

Secretion. The secretion of the mature gland comes to the surface of the skin in drops. It is white or creamy in color, and has a strong disagreeable odor, very similar to that of the so-called Jimson weed (Datura). If the fresh secretion is placed on a slide and examined with the microscope, it is seen to be a liquid densely crowded with small globular bodies. The globules vary in size from 1 to 3 microns, but all are not perfect spheres. When in motion, the globules flow about in the liquid and the mount reminds one very much of a similar preparation of fresh blood, with the corpuscles in motion. The liquid in which the globules float is colorless. In consistency it probably is much like the plasma, for it permits of as free movement as in the case of the blood.

The secretion mass appears differently in different preparations (Figs. 25-a, 42, 59-68). It seems probable, however, that the alveolar appearance in Fig. 64 may be due to the fixing agent. Judging from the fresh secretion, the conditions represented in Figs. 59, 61, 63, 67 seem quite normal, except that it is hard to explain the separation of the more granular from the comparatively homogeneous or finely punctuated liquid. It is possible that in fully mature glands the granules may have drawn closer together in the center of the lumen, leaving near the outlet of the gland and about the periphery a stratum of the liquid secretion. As evidence of this there is often a change in the color and consistency of the secretion during the continuance of the flow, which will be spoken of later.

Both the fresh and the stained globules may show one or more refractive bodies (Fig. 68). Large clusters of globules have been seen in certain preparations (Fig. 61). They are spheres from 15 to 25 microns in diameter, the periphery of which are made up of globules. The whole mass floats in the liquid and is filled with the same. We have no way of knowing if this is a normal condition, since such a cluster, if present in the secretion of the lumen, cannot pass in toto through the mouth of the gland, and appear in the freshly discharged secretion.

I have not aimed in this investigation to make a cytological study of the parts of the skin, and the material has not been prepared with that in view. Consequently the question of the production of the secretion in relation to the parts of the epithelial cell or nucleus will not be treated from that standpoint. Further reference to the mature secretion will be made after we will have discussed the stages of gland development.

Gland outlet. The gland outlet consists of a neck and collar solidly built up from cells, except for a duct which passes through their vertical axis (Figs. 14, 25, 42, 67, 78, 79). In the mature gland there is a gradual transition of the neck and collar into each other. The latter has in general the form of a truncate cone, the base of which rests upon the wall of the acinus, squarely over the lumen of the same. From the top of the cone, the neck continues to the surface at a different angle, assuming more or less the form of a cylinder (Fig. 25-a).

Concentric zones. The cells of the outlet are arranged in two concentric zones (Figs. 78, 79). The inner and outer zones are distally continuous respectively with the molt stratum and with the outer layers of cells of the transitional stratum of the epidermis. Proximally their position corresponds respectively to the epithelium and to the muscular part of the acinus wall. The molt cells continue down the duct to about the level of the lower surface of the epidermis. Their short diameter is in this position perpendicular to the duct. The whole layer is intimately bound together (Figs. 70, 71). At about the place where this lifeless layer ends, definite living cells are found; these continue to the lumen of the acinus, thus completing the inner zone. Their arrangement is radial (Figs. 71-74, 78). Those nearest the molt are more or less polygonal in form, and arranged in a single layer (Figs. 71, 72). As the base of the collar is approached, these cells become more and more elongated and are so shifted in their position as to appear in several layers (Figs. 73, 74, 78). A thick pad is thus formed in the collar to which reference will be made in connection with the emptying of the gland. The cells of the outer zone, beginning at the distal end of the outlet, are but little modified from surrounding cells of the epidermis, except

that they are somewhat flattened (Fig. 69). In the proximal part of the neck, the cells are polygonal in form. They are radially placed in at least two irregular layers (Figs. 70, 71, 78). In the collar the cells elongate, shift their position so as to appear as several layers, but maintain their radial arrangement (Figs. 73, 74, 78).

Cog-wheel-like structure. In the outlet just below where it passes through the pigmented layer of the cutis, the cells form a cog-wheel-like structure (Fig. 72.) The cells of the inner zone, thirty or more in number, are radially arranged about the duct which at this point is more or less circular. The cells of the outer zone are in groups of ten or more, and the sixteen to twenty groups in turn radiate from the inner zone. This structure is a constant feature of the outlets of the mature glands, and I have selected it to arbitrarily mark the outer limit of the gland collar. The distal ends of the first sixteen to twenty muscle fibers extend to this point. The spaces alternating with the groups of cells appear relatively clear when stained, for at this level only the cytoplasm of the muscle fibers is present; the nuclei are at a lower level. Toward and passing into the acinus wall, the fibers constantly increase in number, overlapping in part those that have preceded them. At any given level in the outer zone between the cog-wheel and the acinus, the muscle fibers and the cells of the collar proper are intermingled with one another (Figs. 73, 74). This arrangement provides a firm attachment for the muscle fibers. Several authors (Schultz, Seeck, Phisalix) describe the collar of the species dealt with as consisting of elongated cells, circularly arranged. Schultz even states that it approaches the nature of a sphincter muscle. As we have seen above, all the cells of the collar have in the toad a radial arrangement. Esterly describes dilator and constrictor muscles in cells of the neck, which lie against the duct. I have not been able to verify this in the toad.

Duct. The ducts of the mature glands vary both in their shape and caliber. In the region of the epidermis, a duct has a slit-like form, simple or branched (Figs. 69, 70). Often the epidermis is depressed and appears in surface view as a pit or furrow at the opening of the duct (Figs. 25, 67). But occasionally there is no

such modification in its surface (Fig. 42). A very large number of cells, forty or more in the layer surrounding the molt at the opening of the duct, may be counted in one section (Fig. 69). The slit in the region of the neck becomes more compressed as the base of the neck is approached (Fig. 70). For some distance its walls may even be pressed together and the duct may thus be closed (Fig. 71). At the beginning of the collar it becomes more or less circular (Figs. 72, 73, 76). Just before the gland lumen is reached, the inner series of cells frequently entirely obliterate the duct (Figs. 74, 78). This is due in part to the action of the network of elastic fibers which surrounds the collar (Fig. 76).

Relation of outlet to epidermis and cutis. Seeck, referring to the toad, states that the Malpighian layer of the epidermis passes directly into the neck of the gland. I find that the neck, except where it is continuous with the outer transitional layers of cells and with the molt, is in most cases easily distinguishable from surrounding cells. In the first place, the epidermis is usually somewhat increased in depth about the gland outlet. A limited amount of cutis often penetrates between the neck and the inner third or more of the epidermis (Fig. 78). We have above noted the radial arrangement of the cells in both zones, especially about the lower part of the neck. This likewise differentiates and separates the neck from the lower stratum of the epidermis. I do not believe that the germinating stratum of the epidermis, after it has given rise to the cell that becomes differentiated into a gland bud, has any further connection with that developing gland. A definite network of elastic fibers surrounds the collar and the lower part of the neck, as we have earlier seen, but there is no direct connection between the cutis and the neck and collar of the glands.

The mature glands of different regions. The glands, which I have described above and which I have called the mature type, comprise, except for degenerate sacs, the inner battery. They occur over the whole body ventral as well as dorsal (Fig. 15-m). Mme. Phisalix states that these glands, poison as she calls them, are found only occasionally on the ventral surface of the salamander. All other investigators, who believe in more than one kind of gland and

who say anything about the distribution of the so-called poison gland, say that they are found only in the dorsal skin. The mature glands wherever occurring in the toad presents the same epithelium and muscle structure, but they vary greatly in size according to their location. With the variation in size, the neck, collar and acinus change proportionately. The largest glands are found in the central areas of the parotids. As many as 130 large sacs have been counted in one parotid. About the edges of these warts the glands are smaller and may be very irregular in shape (Fig. 14). In all other warts the glands are smaller and fewer in number, but they bear to each other a similar relation as in the parotids (Figs. 21). The smallest mature glands occur singly and only occasionally in the ordinary skin of the back, and in the ventral skin. The effect of pressure from adjacent glands is here eliminated and the acinus tends to assume a shape more nearly spherical, seldom larger than 175 microns in diameter. Those glands that are somewhat compressed at the poles range in depth from 175 to 290 microns and in transverse diameter from 280 to 360 microns.

Expulsion of the secretion. I have for several reasons spoken of the above as the mature glands. They contain the secretion in its final form, *i. e.,* a finely punctuated liquid in which float countless poison grains. The nuclei may continue to produce secretion and the lumen of such a gland acts as a reservoir for the secretion until needed by the animal for its protection. Bristol and Bartelmez, who have recently stated that there are both mucus and poison glands in the toad, say that "when a poison gland has reached its full development, it is simply a reservoir of poison." I have not been able to learn if all or only a part of the mature glands are discharged as the result of a natural stimulus. The results of artificial stimuli differ. Electricity applied to a limited area of the skin causes a flow of secretion from that part alone. The same is true of mechanical stimulus. Pithing the animal in no case caused a discharge, but decapitation or killing with chloroform, in many instances, produced a general expulsion of secretion.

Authors have quite generally agreed that the expulsion of the secretion is due to the contraction of the smooth muscles about the gland

sacs. It is merely the nature of the cells that has, as a rule, given rise to this conclusion. Calmels and Seeck do not attribute the expulsion to smooth muscle fibers about the individual glands. Calmels does not describe or figure, as such, a layer of smooth muscle fibers in the acinus wall. His statements regarding muscles are not clear, and I shall not attempt to state his position.

Seeck, on the other hand, describes the layer of spindle-shaped cells in the gland wall, but definitely states that they are not muscular in nature. According to him, the expulsion of the secretion is caused by the subcutaneous muscles. Also that the attachment of the skin to the underlying tissues differs according to the species, and thus the secretion flows differently from the toad than from the salamander. Further than this statement of Seeck's, those who have held that the expulsion is due to the action of subcutaneous muscles have not stated in what way they act.

The skin in the toad is very loosely attached over much of the body. While the glands are discharging, I have not been able to detect any change in the tension of the skin. I have experimented with several toads, the results of which prove beyond a doubt that the expulsion of secretion from the glands is not due to the action of subcutaneous muscles. Toads were killed by pithing, and no secretion was expelled. At once a long piece of skin, containing a parotid, was cut on three sides, lifted and entirely freed, except at one end. Electrical stimulus was then applied to either the upper or under side of the parotid, and the secretion poured out in just the same way, and just as freely, as in those animals where the stimulus was applied to the normal skin.

Furthermore, there are present in the cutis of the skin of the toad no smooth muscle fibers, other than those definitely arranged in the acinus wall of the gland.

It will be recalled that the collar of the outlet consists of two zones of cells radially arranged, and that the cells of the inner zone form a pad which rests upon the lumen, thus giving considerable diameter to the acinus in this region. Also that the more or less continuous sheath of muscle fibers in the matrix of the acinus is firmly attached to that part of the collar which rests against and

is in connection with the pad of cells. Furthermore, that the duct is usually closed at several points, especially in the base of the collar, against which the secretion presses. As the smooth muscle fibers contract, the cells of both zones of the outlet are drawn apart, and the duct is thus opened. Pressure is exerted on the periphery of the lumen, causing the secretion nearest the duct to escape first. As the effect of continued contraction, a large gland may be entirely emptied.

Stages of the Gland other than the Mature.

Developing stages. Glands younger than the mature cannot be classified into distinct types. It is safe to say that no two glands are alike in every way. The degree of development is the principal reason for the great variation. But among other things that may modify the shape, and relative size and development of the glands, is the region in which a gland may occur, whether in the ventral skin, in the ordinary skin of the back, or in the parotids or other warts.

I shall accompany the general description of the glands of the outer and transitional batteries with descriptions of specific glands, which represent certain stages in the development toward the mature glands. These have been chosen from the parotids, with the exception of the first stage (Fig. 26), which was in the ordinary skin near the parotid. The stages of the outer battery (I) may be divided into those that are not differentiated into neck, collar and acinus (a and b), and those that are (c, d, e). The latter condition is also true of the glands constituting the transitional battery (II), of which I shall describe but three stages (f, g, h).

I. (a) The youngest stage which I found in the adult toad is a bud, consisting of a group of cells contained in the epidermis, and producing a slight bulging toward the cutis (Fig. 26).

(b) Slightly more advanced is a mass of cells which has partly pushed into the cutis. The cells forming the connection between the mass and the epidermis are slightly modified (Fig. 27).

Later developmental stages show all the essential parts of the mature type. The acinus of the glands of the outer battery is spherical or somewhat compressed at the poles. The walls of the

acinus consist of a matrix in which are imbedded muscle fibers and on which rests the epithelium, whatever form it may assume. The number of muscle fibers increases with the size of the acinus. In the smaller glands, the fibers may be few and scattered. Compared with the depth of the acinus, the fibers are relatively very long, sometimes reaching even through half its circumference (Fig. 34). The most apparent variation in the glands of the outer series is in the epithelium.

Glands with a definitely branched acinus and a common collar and neck, others with both a branched acinus and collar, and the neck divided for part of the way, are found occasionally. Sacs more or less oval, or spherical, with a definite outpocketing or bud at one side, are common. This last has also been observed in the mature type.

Two stages, a and b, have been described.

(c) In the third stage a lumen is forming and is lined with a cubical epithelium (Fig. 28).

(d) Fig. 29 shows that in this section of a given gland, the cells of the epithelium are uniformly cubical, each with a large centrally placed nucleus and homogeneous cytoplasm. In an adjoining section toward the center of the gland, four homogeneous cells have changed into reticular cells and have projected somewhat into the lumen. Part of the contents of these cells has streamed as an indefinite threadlike mass into the otherwise empty lumen. Fig. 30 shows a somewhat similar condition, except that relative to the other cells the reticular ones have enlarged more and projected further into the lumen. Fig. 31 shows a gland similar to the preceding ones, except that the cytoplasm of the enlarged cells has a granular appearance. The nucleus of the enlarged cells of the above glands has remained in contact with the base of the cell which rests upon the matrix.

(e) In a more advanced stage, the outer lower hemisphere of the gland is lined with enlarged cells. These are crowded together and all assume slightly polygonal forms (Figs. 32-35, 50). The cytoplasm of the enlarged cells may be homogeneous, reticular, or granular. All of these cells may be of one sort, or one cell may be

homogeneous, another reticular and another granular. Even a cell may exhibit one kind of secretion in its upper region and another in its lower (Fig. 50).

I have found many stages between what I have described under d and e. The glands referred to as d and e, and which make up the larger glands of the outer battery, measure on the average 96 microns in depth and 160 microns in transverse diameter.

II. The glands of the transitional battery are often not present in an area at a given time. But when these intermediate forms are present, there is no sharp dividing line between them and the outer and inner batteries. As said before, I have divided glands into these three batteries merely for convenience. In shape, the acinus of transitional glands tend to elongate in depth. The collar becomes somewhat conical in shape. The neck becomes relatively shorter. But it is more particularly in the epithelium, secretion and lumen, that the transition from young to mature glands is apparent.

(f) A stage in advance of the last spoken of (e), is shown by a gland in which the acinus is somewhat elongated, and the epithelial cells in its lower half are greatly increased in height. The nucleus of each cell is crowded toward the edge and the cytoplasm of the cell is granular, presenting the same granular appearance as the secretion of adjacent mature glands. The cells about the upper part of the acinus remain cubical in form with homogeneous cytoplasm. The lumen is still free from secretion (Figs. 36, 37). What appears as drops of secretion in the lumen, and probably what has been so described, are but the cut ends of cells, which have extended further into the lumen and because of the weight of the secretion are bent over at an angle.

(g) Further advance is shown by a gland in which even the cells of the upper part of the acinus have elongated. The cytoplasm of the epithelial cells about the neck is reticular, and stains exactly as do part of the cells in neighboring glands of the outer battery; that of the remaining cells is faintly granular. The lumen is almost filled with a secretion similar to the cytoplasm in the last-mentioned cells (Figs. 38, 39).

There is considerable difference in different individuals. A

transitional gland (Fig. 40) from one animal presented conditions similar to those found in the periphery of adjacent mature glands. The cells in the lower two thirds of this gland were greatly enlarged and very irregular, those of the upper part remained cubical. Many of the large cells appear vacuolated, as is true of the periphery of the mature glands of this individual. The secretion in the lumen and the cyptoplasm of part of the cells is identical with that found at the edge of the lumen of the mature glands. Further, certain parts of this gland showed granules, similar to those of the mature secretion.

(h) The last stage preceding the mature resembles the preceding in size and shape. There are no epithelial cells, but the lumen which is almost filled with secretion is lined by naked nuclei (Fig. 41). Such glands are really but small mature glands. A mature gland, occurring elsewhere than in a wart, is very similar to this stage in the parotids.

A network of blood vessels has not been observed about the acinus of a small gland, but the glands are in close association with the lymph spaces of the outer loose cutis stratum.

Degenerate forms. The developmental stages above described precede the mature type. It, in turn, is followed by different degrees of degeneration. Certain sacs, situated among the inner battery of glands, are in a completely collapsed condition. All of the secretion or the greater part is missing. The gland wall is greatly thickened, but, except for contraction, the elements appear normal when compared with those of the filled glands of the same region (Figs. 24, 53). The gland proper has drawn away from the network of capillaries, which have somewhat crowded together. A further degree of degeneration is shown by collapsed glands, in which the normal structure of the parts is no longer preserved. The walls are not continuous, blood corpuscles are scattered about in the gland area, and a general breakdown is apparent (Figs. 23, 57, 58). The neck and collar are also in a very degenerate condition.

One Kind of Cutaneous Gland—Its Purpose and History.

Purpose. Wright ('84) figures and describes in the epidermis

of Amiurus, mucus cells which produce the slime that covers the surface of the skin. These cells, he says, are common to all Pisces. Each is a one-celled gland, which, when fully grown, opens to the surface and may measure from 20 to 25 microns in length.

Ayres ('93) has described for Bdellostoma dombeyi, a row of thread glands whose pores open on either side of the body. These glands produce grains which, when poured into the water, unroll and an enormous transparent gelatinous mass is formed about the animal for its protection. This acts merely in a mechanical way. These glands are sunken in the muscles of the body. He does not say if one-celled glands are present generally in the epidermis.

Maurer ('95) figures single mucus cells in the epidermis of Bdellostoma. He also figures such glands for many higher fishes, including the eel, and also for the larvæ of Batrachians.

We have earlier seen that the only one-celled glands in the epidermis of the adult toad are the beaker cells, which open below the molt and not to the surface. All the cutaneous glands are, however, in connection with the epidermis from which they arose. The smaller of these have frequently been called mucus glands in contrast to the larger ones which have been called the poison glands. But we know that the adult toad differs from fishes and certain Batrachians in that it is not adapted to permanent water conditions. Each year, during the breeding season, the adult toad spends but a day or two in the water. For this, the toad requires no more of an adaptation to the water than does the mammal, for example, which occasionally swims in the water. The adult toad is adapted to a drier environment than any other Batrachians of a similar distribution. It is highly improbable, then, that in the adult toad, whose skin is normally dry, the large number of small glands, with numerous secreting cells, should function solely in the production of mucus.

We have seen that a reticular mass is sometimes present in the lumen of the smaller glands (Figs. 29, 33). Others, especially the transitional glands, may contain in the lumen a homogeneous or slightly granular secretion (Fig. 40). In the outer part and about the periphery of the acinus of the mature glands, either a homogeneous or finely punctuated secretion is occasionally present (Figs.

44, 59, 61, 63, 67). The bulk of the secretion in the lumen of the mature glands is, however, densely granular (Fig. 61). Artificial stimulation causes the secretion to flow from the lumen of the small glands, and in small amounts from the outer parts of the mature glands. It appears as a transparent liquid on the surface. If the stimulus is continued, the liquid on the surface becomes milky, due to the expulsion of the densely granular secretion from the mature glands. This mixes with the secretion already on the surface. Further stimulation causes a continuation of the flow and the discharged liquid becomes thick and creamy, even yellowish. If this is wiped away, the last liquid to appear from the gland just before exhaustion is again milky.

The concentrated liquid, bearing the poison granules, is poured out and spreads into the more transparent liquid that has already come to the surface. The granules of the mature secretion are known to have an irritating or poisonous effect on the mucous membranes of other animals. If, therefore, the secretion is poured out when the animal is stimulated or irritated by an enemy, the advantages that must accrue to the toad are apparent.

Production of secretion in relation to age. The mature glands of the adult toad may differ greatly in the relative time of their existence. The first gland buds that arise in the tadpole develop quickly into glands with mature secretion (Fig. 10). As compared to the large glands of the parotids of the adult, these first glands are very incompletely developed. There is, however, a gradual growth and it is possible that in many instances the mature glands of the adult are the end results of the first buds to arise in the tadpole. Such glands may, however, have been called upon to fully discharge at some time, and in that case will have degenerated. Since buds may arise at any time, after their first appearance, during the life of the animal and develop into mature glands, the places of discharged glands are filled in this way. Consequently we see that there may exist in the same region of two individuals of the same age, or even side by side in one individual, mature glands which differ greatly in their relative age.

I have previously traced the development of a bud that has arisen

in the epidermis of the adult (Figs. 26-42). I will speak briefly of the stages through which a mature gland of the adult has gone, that has arisen as one of the first buds of the tadpole. The bud arises in the epidermis and pushes into the cutis as an undifferentiated gland (Fig. 10-a). A later stage is the formation of a definite acinus with a lumen which is lined by uniform cubical epithelium (Fig. 10-b). Soon certain epithelial cells enlarge (Fig. 10c) and finally, small glands with the lumen completely filled with granular secretion and lined by epithelial nuclei, result (Fig. 10-d). In the small toad which has just completed metamorphosis (Fig. 7), such a gland is no larger than certain epithelial cells that may occur in the mature gland of the adult (Fig. 52). If the reader will take into consideration the difference in magnification, reference to a few photographs will illustrate the gradual change in the mature gland with the growth of the animal. Fig. 10 represents the condition in the earliest transformed toad (Fig. 7). Fig. 11 represents the parotid region of a small toad of the first summer (Fig. 8); Fig. 12, the parotid of a toad of the late fall or after the first hibernation (Fig. 9); Figs. 13, 22, 43 and 46, the parotid of a toad of the second year, and Figs. 14, 23, 24, 44, 45 and 47, the parotid of an adult toad. A comparison of Figs. 43 and 46 with Figs. 44, 45 and 47 shows that the naked epithelial nuclei increase in size. Schultz states that he observed many mitotic figures among the epithelial nuclei of the poison glands, as he called them. I have never observed epithelial nuclei in a state of division, but it is evident from the constant growth of a gland that division, either direct or indirect, must take place. Moreover, it is probable that the naked nuclei, which were left attached to the matrix, when the body of the cells became secretion, continue to produce secretion during the life of the gland. It would be difficult otherwise to account for the large amount of secretion in the lumen of the large glands as compared to the secreting surface. Thus we see that in all probability three conditions—nuclear division, increase in the size of nuclei, and continued activity of the same—combine in the production and increase of the secretion.

I have said that epithelial cells occur only occasionally in the

wall of the mature glands (Figs. 51, 52). The appearance of the cytoplasm in the cells is exactly similar to the secretion in the lumen. This is evidence that the granules are produced by the same nuclei that furnish the liquid secretion. If these are poison cells they must necessarily have remained latent for a long period. On the other hand, it is possible that under certain conditions, while the nuclei are secreting, a difference between the density of the old and new secretion may cause the new secretion to be limited to a small area for a time. If this is so, these are sacs of poison rather than true cells.

Life history of the one kind of gland. Since the mature glands, occurring in large numbers in the warts, pour out their secretion at times, the questions arise: how is the secretion perpetuated and how are the glands replaced when worn out or destroyed?

Literature. Heidenhain and Esterly have each stated concerning certain salamanders, that a gland bud is present in the neck or collar of every poison gland. When the latter is emptied of its secretion, the bud begins to grow. Heidenhain states that he observed intermediate stages and that some cells of the ingrowing gland were granular in nature. Esterly says that the bud is always mucus, but he has never observed transitional stages between the germ and the poison glands. I have observed an ingrowing gland in different stages of cutaneous glands in Amblystoma jeffersonianum, but not in the case of the toads.

Schultz says that epithelial cells of the poison glands increase by mitotic division, and that only a few cells reach their development at the same time. Drasch states that completely emptied poison glands are replaced by small poison glands which grow rapidly. Junius suggests that the old glands are probably replaced by entirely new glands.

Mme. Phisalix states that emptied glands refill. Weiss says that the protoplasm of the inner part of the epithelial cells furnish the secretion of the poison glands. He further says that the poison glands of the toad, which have been artificially stimulated to exhaustion, refill completely in from 24 to 36 hours. He considers that this opposes the idea that the secretion arises through cell growth and succeeding dissolution.

To test the effects following gland discharge, I selected six toads for an experiment. The left parotid gland of each was stimulated with electricity, till apparently exhausted of its milky secretion. Viewed externally, the elevation of the stimulated wart was greatly reduced in every case. The right parotid was in no case stimulated, and remained unaffected. The toads were killed at different intervals and both the right and left glands were sectioned. No. 1 was killed immediately, No. 2 in 6 hours, No. 3 in 12 hours, No. 4 in 30 hours, No. 5 in 52 hours, and No. 6 in one week from the time of stimulation. The right glands in every case appeared normal. With the exception of a few sacs in the left glands of Nos. 1 and 6, the large sacs of all the individuals were completely empty. The few filled sacs do not seem to have discharged at all. In every case, many of the emptied glands had completely collapsed, and, in most instances, all of the sacs had collapsed. It is evident from sections of a given pair of parotids that they were before the emptying of the left very much alike in the shape, arrangement and development of the different batteries of glands. After the stimulation the smaller glands, *i. e.,* those of the outer battery, had remained intact as far as their shape and epithelium were concerned. The small mature glands had emptied.

It is certain that emptied gland sacs have not refilled with secretion seven days after emptying. Further than this I do not wish to draw definite conclusions from this experiment, since only one individual was killed at each interval. It is, however, my belief that a collapsed gland sac, one that had lost its shape, never refills. Further, that a completely emptied gland that has not collapsed at first will ultimately do so, from the force of surrounding growth and pressure.

According to Bristol and Bartelmez, poison glands are found only on the upper surface of the toad, and mucus glands over the whole surface. "When the poison is discharged, the remains of the gland are resorbed, and at the same time one of the five or six undeveloped glands, grouped about the mouth of the functioning gland, grows down alongside the remains of the discharged gland pushing it aside to occupy its former place." The authors make no statement as to the nature of these replacing glands other than the above.

In the species dealt with in this investigation, I have never seen small glands that were closely and definitely associated with the necks of large glands. The small glands occur without any relation of position to the necks of the mature glands.

How glands are replaced in the toad. I have shown that the mature glands may break down and degenerate. New gland buds may arise in the epidermis at any time during the life of the individual, in just the same way as the first glands arose in the tadpole. This is the *only* mode of gland renewal in the toad. Buds do not arise in the neck or collar of the mature type. An occasional nucleus and poison sac is seen in the collar (Fig. 77). This condition might without careful examination lead to the conclusion that a small gland is forming, but it is simply a secreting cell out of place.

Gradation a proof of one kind. The series of stages in the adult toad, beginning with the gland bud in the epidermis to the mature gland, has been described (pp. 345-348). There is a complete gradation from the gland buds in the epidermis through stages called mucus by authors(Figs. 26-35), located immediately below the epidermis, and through the transitional glands (Figs. 36-41), to the large typical, mature poison glands (Figs. 14, 42, 58-a), extending deeper and deeper into the cutis as they develop. There is also a series from the mature gland to the shrivelled, empty sacs (Figs. 24, 53, 58), and finally to a vanishing fragment (Figs. 23, 57), lying below the largest mature glands. There is no evidence that the poison glands are developed in any way except through the so-called mucus glands, which are nothing more nor less than young immature poison glands. There are never any degenerate glands, except through injury, seen in the outer battery to which the so-called mucus glands are confined. They do not degenerate except accidentally, but are stages in the development of the large mature glands.

Distribution of glands, a proof. Facts regarding the distribution of warts, and of different stages of glands over the whole body, strengthen the evidence that but one kind of gland exists in the cutis of the toad. The glands develop into the mature stage in all regions of the body. They reach that stage in greater numbers on the dorsal surface where they crowd together and form the parotids and other

warts. On the ventral surface, and between the warts of the dorsal surface, they only occur occasionally (Fig. 15). Excepting the parotids, the warts and hence the mature glands, vary in number and their exact distribution is not constant for the species. The same is true of mature glands which occur singly. This is conclusive proof that the so-called poison glands, *i. e.*, the mature glands, are not destined to arise in identical regions of the skin of all individuals of the species.

The small glands are more numerous than the mature glands. In either the warts or in the ordinary skin of the back, they are about proportionate to the number of mature glands occurring in the region. In the ventral skin, the ratio between the younger stages and the mature glands is considerably greater. The younger stages are larger than a similar stage in the dorsal surface. The epithelial cells of many of these glands seem to have been arrested in their development, while the acinus continues to enlarge (Fig. 15).

Proofs from warts with degenerate glands and from growing warts. Certain warts consist of a quite uniform inner battery of glands, which show no degenerate glands. The outer battery is then quite uniform in size, structure and apparent age, and there exist no transitional forms (Figs. 13, 14). On the other hand, there are warts which show degenerate glands in certain areas, or quite generally in the lower part of the wart. The glands of the outer battery are in these areas varied in their appearance, transitional forms exist, and even the mature glands may be very different in size and shape (Figs. 23, 24, 57, 58).

Certain warts seem to be enlarging by the addition of glands, and show some of the previous conditions, especially as to the irregularity of the inner series (Figs. 21, 22).

Conclusions.

1. There are present in the epidermis many beaker cells, each a one-celled gland, the mouth of whose slender neck opens between the transitional and the molt strata. They are found in the epidermis of all regions of the body, and may occur in large numbers. They

produce a granular secretion which helps to loosen the molt from the underlying stratum.

2. The so-called cutaneous glands arise as buds in the germinating stratum of the epidermis. The first gland buds arise just as the tadpole has passed its most typical aquatic form. From this time on during the life of the toad, new ones arise in a similar manner.

3. Very early the bud becomes differentiated into neck, collar and acinus. As a gland enlarges, it pushes deeper and deeper into the cutis, but a direct connection is not established between the cutis and the glands. Before the first glands reach maturity, new glands arise and develop, and even a third series may be developed. A very complete series of stages arranged in two or three strata may be found between the earliest stage and the mature glands. The climax is reached by the large mature glands, whose granular secretion is irritating or poisonous in its effects on other animals.

4. The elements of all the parts of a mature gland appear in the very early stages, but they increase in size and number as development proceeds. The shape of the acinus and collar gradually changes as the lumen fills with secretion.

5. The wall of the acinus of a mature gland consists of a matrix *in* the periphery of which are *imbedded* smooth muscle fibers meridionally arranged. Attached to the lumen surface of the matrix is the epithelium, which for the most part consists of naked nuclei, irregularly arranged.

6. The collar and neck of the mature gland consists of two concentric zones of cells *radially* arranged. At the outer limit of the collar the cells of both zones are arranged in a *peculiar cog-wheel-like structure*. The outer ends of the more distal muscle fibers, which have extended from the wall of the acinus in among the cells of the outer zone of the collar, reach to the cog-wheel. An effective attachment for the action of the muscle fibers is thus established.

7. The duct of the mature gland is slit-like and irregular in shape in the region of the epidermis. Through the remainder of the outlet it is more or less circular in form, and is often closed for part of the way.

8. The first gland buds that arise in the epidermis of the tadpole

develop quickly into small mature glands, *i. e.,* glands filled with granular secretion and lined with naked, epithelial nuclei. Likewise the gland buds that arise in the epidermis of the adult develop when needed into small mature glands. With the growth of the glands, the future production and increase of the secretion is due to the division of the naked, epithelical nuclei, increase in their size and continued activity of the same. The globules and the liquid part of the secretion are both produced by the same nuclei.

9. The expulsion of the secretion is accomplished by the contraction of the smooth muscle fibers in the walls of the individual gland sacs. The contraction serves the double purpose of opening the duct by pulling on the radially arranged cells of the outlet, and of concerted pressure on the secretion of the lumen. The expulsion is not due to the action of the subcutaneous muscles.

10. When a wart has been stimulated to exhaustion, with few exceptions all the gland sacs are completely emptied and most of them have collapsed. Contrary to the statement of one writer that the glands of the toad refill in 24 to 36 hours, I have found that at the end of seven days there is no evidence of refilling. Such gland sacs degenerate and are replaced by younger ones. With the degeneration of a few or most of the mature glands of a parotid, for example, and their replacement, the type of the wart changes, *i. e.,* younger glands become transitional in form, new ones arise, and there is considerable irregularity in the size and shape of the new mature sacs.

11. The mature glands of the toad are not replaced by the development and ingrowth of a bud situated in the neck of the old glands, and which has been said by some writers on other Batrachians to have remained latent, ready for development as soon as the pressure of the old gland is removed. Neither are there small glands *definitely* arranged *about* the neck of the gland for its ultimate replacement.

12. Glands reach maturity *both* in the *ventral* and dorsal skin. They reach the mature stage in much greater numbers, on the dorsal surface, where they develop to enormous sizes, and where they are often closely grouped, producing warts.

13. The pair of parotids is the greatest accumulation of glands. Their position and relative size are constant for the species. All the

other warts are variable in size and location. The largest usually occur in groups of two, three or more and are located in the darker pigmented spots of the back and legs.

14. The glands vary greatly in size and appearance, *but there is only one kind of cutaneous gland in the common toads. All the glands are but different developmental stages of the one kind.*

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PLATE I.*

FIG. 1. Outline drawing of epidermis of parotid. Section near surface and parallel to it. Circular bodies = beaker cells. × 280.

FIGS. 2-4. Outline drawing of epidermis to show relative thickness, shape of cells and number of cell layers in different regions of the body of the adult toad. × 310.

FIG. 2. Parotid. Two beaker cells.

FIG. 3. Ordinary skin of the back. Four beaker cells.

FIG. 4. Ventral skin.

*The illustrations of all the plates refer to the adult toad unless otherwise stated.

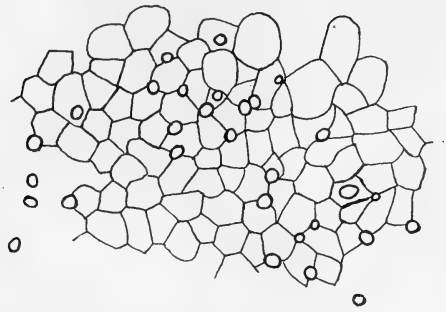


Fig. 1

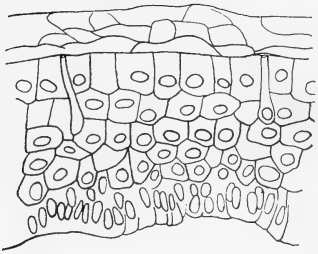


Fig. 2

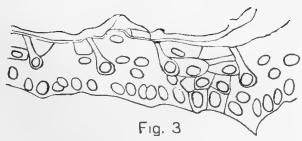


Fig. 3

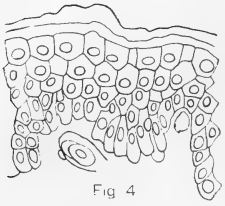


Fig. 4

PLATE II.

The cutaneous glands and the warts, both with regard to the regions of the body of the adult, and with regard to age. General structure of the skin.

Fig. 5-9, developmental stages of the toad. × ¾.*

Fig. 5. The tadpole just previous to the appearance of gland buds.

Fig. 6. The tadpole with the first gland buds.

Fig. 7. The metamorphosed toad just after leaving the water.

Fig. 8. Young toad of the first summer.

Fig. 9. Toad of the late fall, or just after the first hibernation.

Fig. 10. Glands from the dorsal surface of the toad just metamorphosed. (Fig. 7) a-d, gradation. × 210.

Fig. 11. Parotid of first summer toad (Fig. 8). Gradation. × 45.

Fig. 12. Parotid of late fall toad (Fig. 9). Gradation. × 18.

Fig. 13. Parotid of second year toad. Outer and inner batteries. × 10.

Fig. 14. Parotid. Outer and inner batteries. × 10.

Fig. 15. Ventral skin. m-mature gland. × 10.

Fig. 16. Parotid. Transverse of glands. × 10.

Fig. 17. Ordinary skin of the back; epidermis, cutis: a-outer loose, b-compact, c-inner loose strata, pigment, vertical strand; nerves and blood vessel. × 45.

Fig. 18. Ordinary skin of the dorsal side of hind legs. Epidermis and cutis. × 45.

Fig. 19. Ordinary skin of back. Three strata of epidermis. Processes of germinating stratum. Blood loops. × 210.

Fig. 20. Papilla, ordinary skin of the back. Capillary at base of end bulb. × 210.

Fig. 21. Wart, and ordinary skin—o. s.—of the back. × 10.

Fig. 22. Parotid of second year toad, showing growth and transitional glands. × 10.

Fig. 23. Parotid showing replacement of degenerate glands. Transitional glands are present. × 10.

Fig. 24. Parotid. Degeneration and replacement. × 10.

*The magnification of all figures takes into account the plate reduction. Plates II, III, V, and VII are the result of a one-fourth reduction, Plate IV of a one-third reduction, while for Plate VI there was no reduction.

EFFA FUNK MUHSE.

Fig. 5 Fig. 6 Fig. 7 Fig. 8 Fig. 9

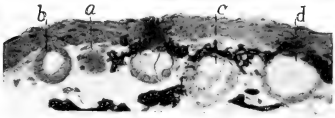


Fig. 10

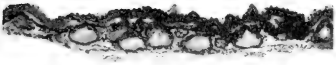


Fig. 11

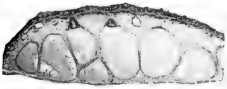


Fig. 12

Fig. 13

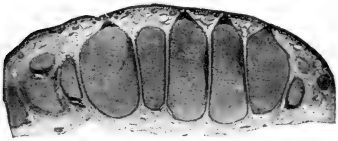


Fig. 14

Fig. 15

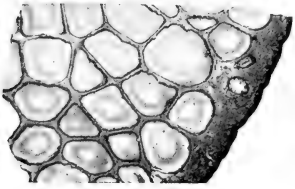


Fig. 16

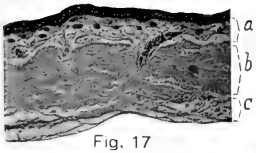


Fig. 17

Fig. 18

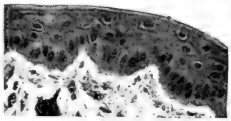
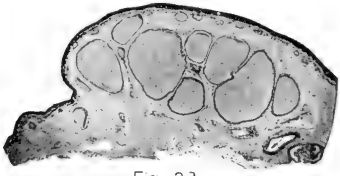


Fig. 19

Fig. 20

Fig. 21

Fig. 22



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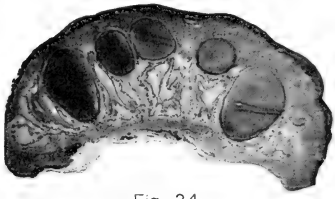

Fig. 24

PLATE III.

Developmental stages of the cutaneous glands, found in the adult; batteries and gradation.

FIG. 25. Parotid. Batteries: a-inner, b-transitional, c-outer. Epidermis; cutis; outlet of glands. × 34.

FIG. 26. Gland bud, ordinary skin of the back near parotid. × 210.

FIG. 27. Undifferentiated gland, parotid. × 210.

FIGS. 28-32. Glands of outer battery, parotid. Gradation, × 210.

FIGS. 33-35. Gland of outer battery, parotid. First (34), third (35), fifth (33), sections of same gland. Fig. 34 shows the longitudinal view of smooth muscle fibers, and in the other two the muscle fibers are cut transversely, and appear in some cases as triangular cells at the base. × 210.

FIGS. 36 and 37. Glands of transitional battery, parotid. × 210.

FIGS. 38-41. Glands of transitional battery, with sections of large mature glands of inner battery, parotid. Figs. 38 and 39 are different sections of the same gland. × 45.

FIG. 42. Mature gland of medium size, inner battery, parotid. × 45.

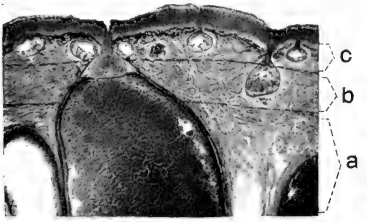

Fig. 25

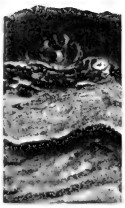
Fig. 26


Fig. 27


Fig. 28

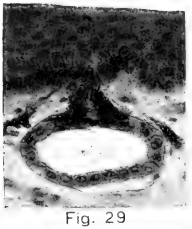
Fig. 29

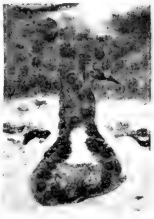
Fig. 30


Fig. 31


Fig. 32

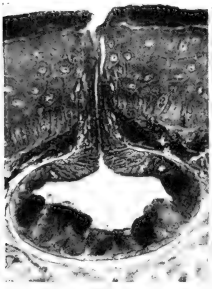
Fig. 33

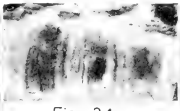
Fig. 34

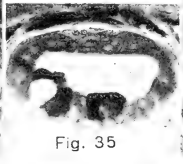
Fig. 35

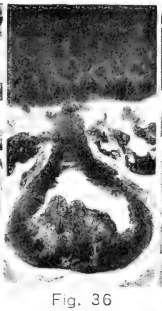
Fig. 36


Fig. 37

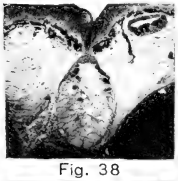
Fig. 38

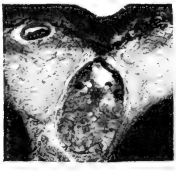
Fig. 39

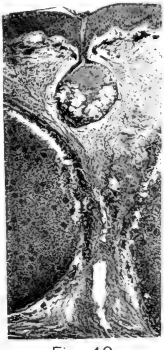
Fig. 40


Fig. 41


Fig. 42

PLATE IV.

Detail of wall of acinus, mature glands of parotid. Detail of epidermis. Detail of gland of outer battery (Camera lucida).

a. e. c.—epithelial cell with cytoplasm alveolar in appearance.
b. c.—beaker cell, superimposed against an ordinary epithelial cell.
c.—capillary and corpuscles.
c. t.—connective tissue fibers.
e. n.—epithelial nuclei, naked.
g. e. c.—epithelial cell with granular cytoplasm.
g. s.—germinating stratum.
i. b.—intercellular bridges.
m.—matrix.
m. f.—smooth muscle fiber, and nucleus.
m. s.—molt stratum.
p.—processes.
s.—secretion.
t. s.—transitional stratum.

FIG. 43. Second year toad. Vertical section of gland. × 413 1/3.

FIG. 44. Vertical section of adjacent glands, with intervening connective tissue. × 413 1/3.

FIG. 45. Vertical section of gland. × 413 1/3.

FIG. 46. Second year toad. Vertical section of adjacent glands, with intervening connective tissue. × 413 1/3.

FIG. 47. Vertical section of gland, near lower pole. × 413 1/3.

FIG. 48. Vertical section of epidermis. × 553 1/3.

FIG. 49. Second year toad. Vertical section of epidermis. Parotid. × 553 1/3.

FIG. 50. Vertical section near centre of gland of outer battery, parotid. × 413 1/3.

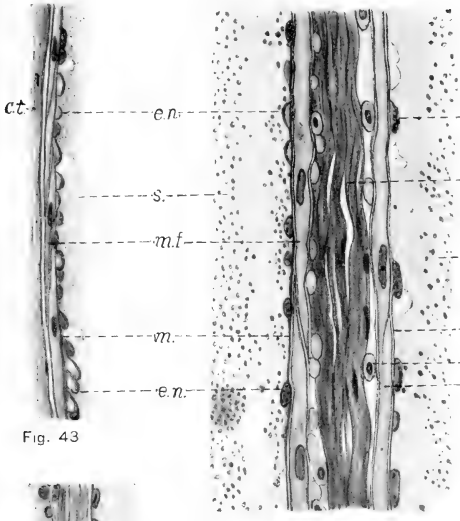

Fig. 43

Fig. 44

Fig. 45

Fig. 46

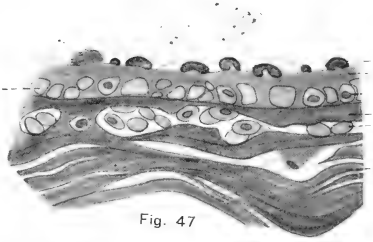

Fig. 47

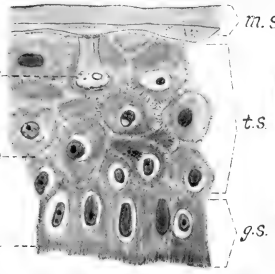

Fig. 48

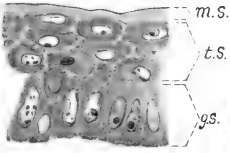

Fig. 49

Fig. 50

PLATE V.

Structure of wall of acinus, mature glands of parotids: capillaries, muscle fibers, epithelial nuclei, and epithelial cells or poison sacs. Degenerating forms of glands.

Figs. 51 and 54. Vertical, tangential sections of glands. Beginning at periphery of photograph,—connective tissue, network of capillaries, muscle fibers, naked epithelial nuclei. Fig. 51, in addition, shows secretion and three epithelial cells or poison sacs. The muscle fibers of the two glands appear in different states of contraction. × 210.

Fig. 52. Section horizontal to glands. Walls of two adjacent glands and intervening connective tissue. Upper gland shows a cluster of epithelial cells or poison sacs. Lower gland shows well the transverse view of muscle fibers. × 210.

Fig. 53. Vertical section of collapsed gland between two filled glands. Note the capillary network, and the similarity of the transverse view of the muscle fibers at the base, to a cubical epithelium, for which several writers have mistaken it. × 210.

Fig. 55. Vertical section of glands and cutis, showing blood-vessels in lower cutis layer, with branches leading to base of glands, and capillary network about same. × 45.

Fig. 56. Vertical section of gland at lower pole. From below upward, connective tissue, capillary and corpuscles, matrix with imbedded muscle fibers and attached epithelial nuclei, and secretion and detached nuclei. × 210.

Figs. 57 and 58. Vertical sections. In the inner battery, appears part of a mature, completely filled gland, and degenerating forms, the lower one of which in 57 opens to the inner side of the cutis and is filled with corpuscles. In the region of the degenerating forms, occur a relatively greater number of small glands, which show considerable gradation. × 45.

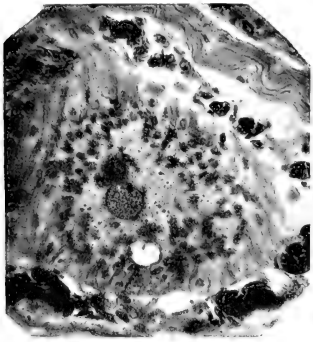

Fig. 51

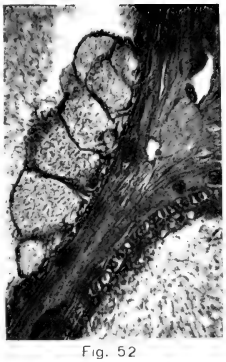

Fig. 52

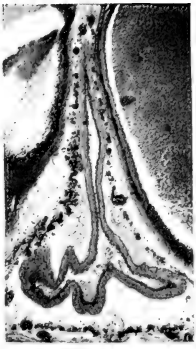

Fig. 53

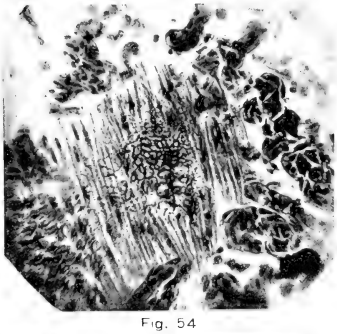

Fig. 54

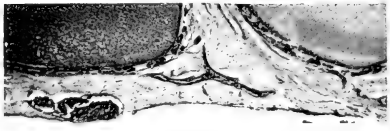

Fig. 55

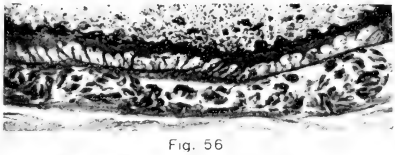

Fig. 56

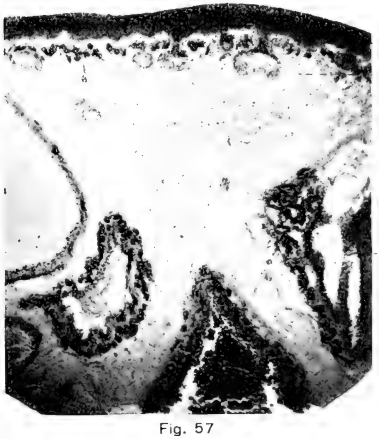

Fig. 57

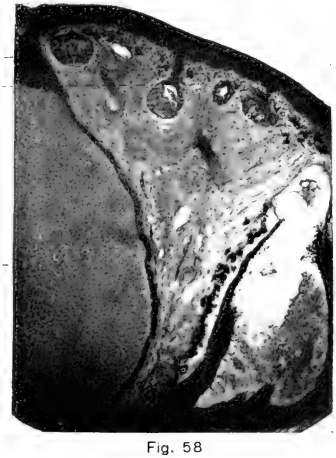

Fig. 58

PLATE VI.

Structure of wall of acinus and of secretion, mature glands of parotid. Vertical section of gland.

FIG. 59. Walls of adjacent glands and intervening connective tissue. Longitudinal view of muscle fibers. Note stratum of finely granular secretion in right gland. × 280.

FIG. 60. Walls of adjacent glands and intervening connective tissue. Note the arched appearance of the epithelial nuclei with enclosed clear area. × 280.

FIG. 61. Parts of three mature glands: granular secretion with globular bodies, peripheral stratum of finely granular secretion, and muscle fibers about central gland. Cutis. × 60.

FIG. 62. Walls of adjacent glands and intervening connective tissue. Gland to left shows an oblique view of muscle fibers imbedded in matrix, that to right a longitudinal view. × 280.

FIG. 63. Secretion—finely granular peripheral layer and the coarsely granular, together with a few detached nuclei. (Same as Fig. 67.) × 280.

FIG. 64. Secretion. × 280.

FIG. 65. Wall of acinus and secretion. Imbedded in the matrix are several muscle fibers seen in transverse view, two of which show a nucleus. Attached to the inner side of the matrix are three arched, naked, epithelial nuclei. × 670.

FIG. 66. Wall of acinus near lower pole of mature gland—muscle fibers, matrix, epithelial nuclei,—and secretion. × 280.

FIG. 67. Shows difference in appearance of secretion in a given gland. × 60.

FIG. 68. Secretion. Black and white dots in the grains represent the refractive bodies. × 670.

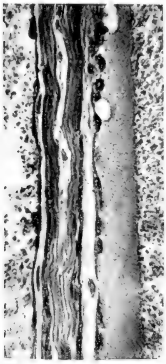

Fig. 59

Fig. 60

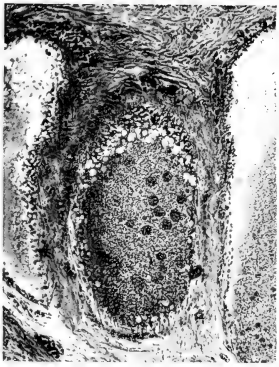

Fig. 61

Fig 62

Fig. 66

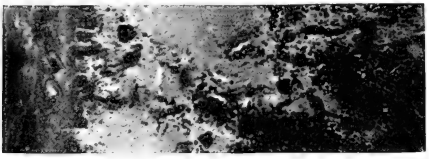

Fig. 63

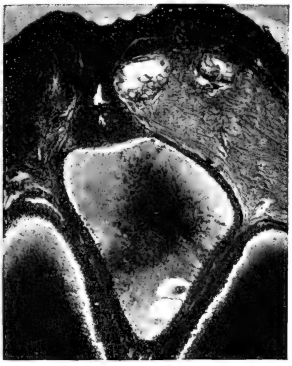

Fig. 67

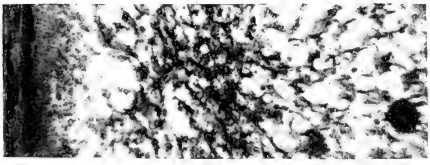

Fig. 64

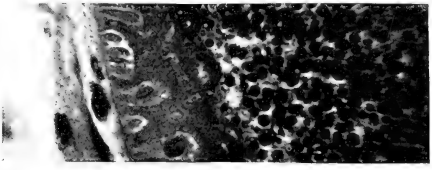

Fig. 65

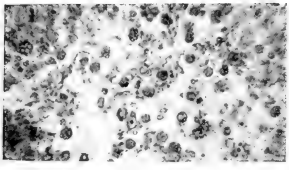

Fig. 68

PLATE VII.

Structure of the neck and collar of the mature gland of the parotid.

FIGS. 69-75. Transverse section of the outlet and acinus of the same gland at different levels, the outlet similar in shape to that of the mature gland in Fig. 25, Pl. III. × 210.

FIG. 69. View of neck, third section from surface (sections 10 microns). a.—neck of small gland.

FIG. 70. View of neck, seventh section from surface.

FIG. 71. View of neck, ninth section from surface.

FIG. 72. View of cog-wheel-like structure, twelfth section from surface.

FIG. 73. View of collar, seventeenth section from surface.

FIG. 74. View of collar, twenty-second section from surface.

FIG. 75. View of acinus (upper gland).

FIG. 76. Oblique section of collar, showing surrounding network of elastic fibers. × 210.

FIG. 77. Oblique section of collar of mature gland, showing two secreting cells. Note thin homogeneous stratum at lower border of epidermis. × 45.

FIG. 78. Outlet of mature gland (same as in Fig. 42). Note the separation of the neck from the lower half of the epidermis. × 210.

FIG. 79. Outlet of mature gland of late fall toad (Fig. 9), showing the two concentric zones of cells. × 210.

Fig. 69

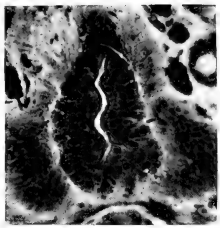
Fig. 70

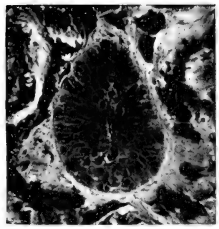
Fig. 71

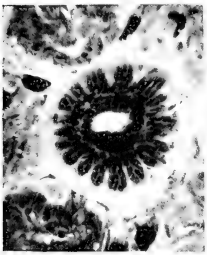
Fig. 72

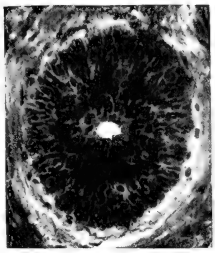
Fig. 73

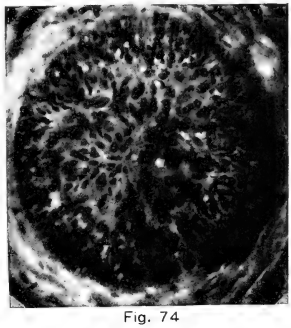
Fig. 74


Fig. 75

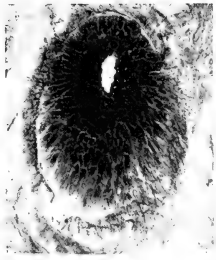
Fig. 76


Fig. 77

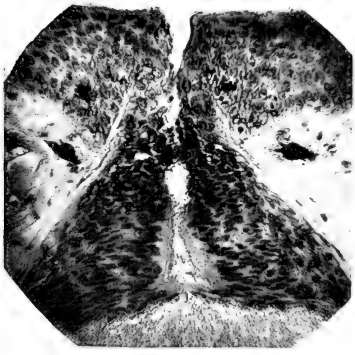
Fig. 78

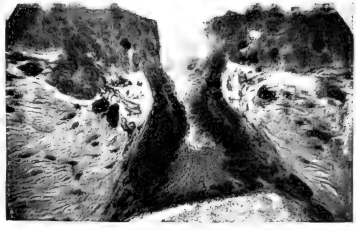
Fig. 79

A CONTRIBUTION TO OUR KNOWLEDGE OF THE EARLIEST KNOWN STAGES OF PLACENTATION AND EMBRYONIC DEVELOPMENT IN MAN.

BY

MAXIMILIAN HERZOG.

From the Laboratory of Pathology of the Michael Reese Hospital, Chicago, Illinois.

With 30 Illustrations.

CONTENTS.

Introductory Remarks.

The most important original contribution to our knowledge of the earliest stages of human placentation was made less than ten years ago by Peters, whose monograph, "Ueber die Einbettung des menschlichen Eies," Wien, 1899, is well known to every student of the subject.

The present author, a number of years ago, became interested in human placentation, primarily in connection with the morbid anatomy and histopathology of ectopic gestation, and he has for years, as a matter of routine, searched for very young human ova in every uterus and Fallopian tube which fell into his hands, either

as an operative or as a post-mortem specimen.[1] He has finally been fortunate enough to obtain a uterus from an absolutely unobjectionable case, containing an ovum, approximately like the Peters' ovum, but including a well-preserved embryonic shield, younger, as it appears, than any human embryo heretofore satisfactorily described. The specimen was obtained in July, 1904, during his term of service as pathologist in the Government Bureau of Science, Manila, P. I.

The number of very young human ova unobjectionably adapted for a reliable study of the earliest stages of placentation is still exceedingly limited, and it may be said that we have not up to date a single specimen which is ideal and which will compare favorably with what can be obtained from the lower animals by timing conception accurately, removing its product from the living, and fixing it at once by the best methods at our disposal. Jung,[2] in a recent monograph, to be mentioned more fully below, says in this connection: "The ideal specimen would be a uterus extirpated from a live woman, containing *in situ* an ovum of the first week after conception. This ovum *in situ,* after removal from the uterus, should then be treated by aproved technical methods. The uterus itself should be free from those morbid changes which, according to common experience, pathologically alter the implantation of the ovum (myomata, chronic metritis, etc.). We are yet far removed from this ideal. Not a single one of the specimens heretofore described satisfies even approximately these requirements, and it is at

[1]Herzog: Description of an Early Placenta *in situ*, obtained from the living. The Am. Gyn. and Obst. Journal, April, 1898, and Transactions Chicago Pathol. Soc., Vol. III, p. 317.

Herzog: Superfetation in the Human Race. Chicago Medical Recorder, 1898, Vol. 15, p. 1.

Herzog: The Pathology of Tubal Pregnancy. Am. Jour. of Obstet., 1900, Vol. 42, No. 2.

Herzog: Placentation in a Uterus Duplex Bicornis Gravis—Menses 1-2. Trans. Chicago Pathol. Soc., 1904, Vol. 6, p. 51.

[2]Jung: Beiträge zur frühesten Ei-Einbettung beim menschlichen Weibe: Berlin, 1908.

This author has very fully gone into the recent literature of the subject, and I have repeatedly quoted from his monograph.

least doubtful whether we will ever have such a specimen at our disposal."

Jung then points out the great rarity of young human ova obtained *in situ*. He mentions those of Peters, von Spee, Leopold, van Heukelom and Frassi-Keibel, and he finds objectionable features in all of them.

The youngest and undoubtedly most valuable of these specimens is that of Peters. Without trying to detract from the great value and fundamental importance of the Peters' specimen, it is proper to point out that it came from a case which strongly suggests the probability of pathologic changes.[3] Peter's ovum was obtained from a woman who committed suicide by swallowing a large quantity of caustic potash solution, and who died three hours later. Such a rapid death after the ingestion of the fixed mineral alkalies is extremely rare, and must be connected with marked changes of the blood itself and of its circulation. I can find only the very incomplete record of a single similar case, that of a boy, said to have died three hours after swallowing lye.

The embryo in Peters' specimen is said to be less well preserved than the chorion. It was described by Count von Spee as showing two very small epithelial cavities (the amnion and yolk sac), embedded in the chorionic mesoderm. The connection between the embryo and the chorion was thus so extensive that it was impossible to speak of an isolated body-stalk. Count von Spee was uncertain whether "the first little *anlage* of an entodermal diverticulum (allantoic duct)" was present or not. The amniotic cavity was completely closed. It was lined in part by the very thin amniotic epithelium and in part by the tall columnar cells of the embryonic plate. A thin layer of mesoderm extended between the plate and the yolk sac, crossing the median line except toward one end of the embryo (thought to be the cranial end). Nothing is said of a neurenteric canal, or of blood vessels. Peters estimated the length of the embryo to be 190 microns.

A reconstruction of Peters' ovum was made by Keibel, from nine-

[3]The conditions found, which are probably pathologic, will be pointed out below.

teen outline drawings on 2 mm. wax plates prepared by Selenka, and found after his death. Nothing is reported concerning the model except the following.[4]

"Neither an allantoic duct nor an amniotic duct was found. The external surface of the yolk sac appeared uneven (höckerig), as if the 'anlagen' of vessels and blood had already been formed, but naturally this point could not be definitely decided from the wax plates and the model."

After Peters' ovum, the next youngest embryo enumerated by Keibel and Elze in the "Normentafeln" is the one described by von Spee in 1896. It had a longest diameter of 1.84 mm., and a longest yolk sac diameter of 1.054 mm. Length of amnion and allantoic stalk, 0.76 mm.; greatest width of the two latter, 0.76 mm.; greatest width of yolk sac, 1.083 mm.; length of embryonic shield, 0.37 mm.; width of ectoblast plate, 0.23 mm.; length of allantoic duct, 0.35 mm. No amniotic duct was present. Between mesoblast and entoblast there were blood islands which caused the mesoblast to project strongly.

The authors of the "Normentafeln" believe that the embryo 1.74 mm. long described by Beneke, is older than von Spee's embryo. They consider that the description of Beneke's specimen is unsatisfactory.

The Frassi embryo, the youngest examined in compiling the tables which accompany the "Normentafeln," is described as follows: Length of embryonic shield, 1.17 mm.; width, 0.6 mm.; diameters of yolk sac, largest, 1.9 mm.; smallest, 0.9 mm. The embryonic shield is flat, with a long primitive streak (0.5 mm.) and a shallow medullary groove. The medullary folds are not yet sharply defined At the anterior end of the primitive streak the canalis neurentericus is indicated (whether it is pervious is questionable); at the posterior end is the "anlage" of the cloacal membrane. There are no myotomes. "Anlagen" of vessels and blood are found on the yolk sac, and there are vessels in the allantoic stalk and adjacent chorion. There are no vessels in the embryonic shield. The amnion is

[4]Keibel and Elze, Vol. 8 of Keibel's Normentafeln zur Entwicklungsgesch. d. Wirbelth., 1908, p. 9.

closed, and there is no amniotic duct. An allantoic duct is present. The embryonic cœlom is not yet indicated.

Three embryos not considered in the "Normentafeln" are worthy of note; these have been described by Leopold, Teacher and Bryce, and Jung, respectively.

Leopold's small ovum, which forms the basis of a monograph published in 1906, was obtained from a young woman who committed suicide by taking phosphorus. After a prolonged search of the interior of the uterus, Leopold found at the posterior wall of the corpus a small point, somewhat lighter than the surrounding tissue. A cubical piece of tissue containing this lighter point was properly fixed and cut into serial sections, each five microns thick. One hundred and sixty sections showed a very small cavity, which presented the following measurements: Length, 1.4 mm.; height, 0.9 mm.; thickness, 0.8 mm. In no section could Leopold find anything like an embryonic shield or an amnion, and hence he himself raises the question whether this ovum may not be a pathologic specimen.

We most decidedly believe that Leopold's small ovum must be looked upon as pathologic. The absence of the embryonic shield must favor such a suspicion. We are either, it appears, dealing with some profound changes in an ovum, due to phosphorus poisoning, or possibly with the first stages of what would have been a hydatid mole.

Since Leopold published this monograph, it has generally been the tendency of those writing on the first stages of human placentation to discard his specimen from the list of young human ova. This is done by Jung, and we have one year previous to this author placed ourselves on record in the same sense.

Bryce and Teacher[5] have recently published an account of an ovum which they believe to be between thirteen and fourteen days old, and one day younger than Peters' specimen. They received their specimen in "a mixture of urine and blood clot," in which

[5]Bryce, Thomas H., and Teacher, John H.: Contributions to the Study of the Early Development and Embedding of the Human Ovum. Glasgow, 1908.

it had been immersed for twenty hours. It had been discharged by a healthy woman on November 5th, presumably as a result of the disturbance effected by coitus November 3d to 4th. The specimen was hardened in absolute alcohol and sectioned in paraffin.

Attached to the inner surface of the chorionic vesicle, two smaller vesicles were found. The larger, "torn and collapsed," was attached to the chorion "definitely only at one point," but the smaller was bound to the chorion by mesoblast strands, with which it was closely surrounded. The two vesicles were separate from one another. Bryce and Teacher consider that "after careful consideration of the sections and model the conclusion is inevitable that the larger vesicle represents the amnio-embryonic cavity and the smaller the entodermic vesicle or future yolk sac." (Page 26.) However, the embryo is so badly preserved that even this conclusion may be questioned, and on page 34 Bryce and Teacher discuss a different interpretation. In the very young embryo to be described in the present communication the yolk sac vesicle is larger than that of the amnion, and is attached to the chorion only at one point.

Jung's ovum, fully and lucidly described in his recent monograph already quoted, is one of the very youngest and best preserved now on record. It was, however, not obtained *in situ*, but as the result of a curettage of the uterus, and was preserved in 80 per cent. alcohol, which, according to Jung's own comparative studies, is not very favorable for the fixation of young human ova. In spite of this, its fixation has been very satisfactory, and karyokinetic figures have been preserved in the trophoblast, the decidua, and the embryo itself. According to the description given of the latter (p. 102, Jung, l.c.), the "Embryonalgebilde" was found in nineteen sections each on an average of 13 microns thick, making a total length of 247 microns. However, as Jung states, some of the sections containing the embryo had been lost and an attempt at reconstruction had to be given up as futile. The whole "Embryonalgebilde" is said to be enclosed within a thickening of the mesoblast in the interior of the ovum, attached to its basal surface. "Within the mass of mesoblast we see the ectoderm shield ('Keimscheibe'). In transverse section it is a crescentic formation, with its concavity towards the basal side

of the ovum. It is composed of high cylindrical cells, some of which show mitoses. The extremities of the crescent are continuous with the closed amnion, which is composed of one layer of flat cells. The cavity formed by the embryonic shield and the amnion in transverse section has a lenticular shape, and it is filled with a finely granular coagulated mass. Externally the amnion and embryonic shield are surrounded by a thin layer of mesoblast, . . . which separates them from the yolk sac. The latter can only be seen distinctly in twelve sections. The yolk sac shows a simple layer of flat entoderm cells. Toward the embryonic shield this entoblast rests directly upon the mesoblast which separates the ectodermal shield from the yolk sac; toward the other side the entoblast is strongly curved, and quite a distance removed from the mesoblast. The yolk sac, as a whole, has a somewhat hemispherical shape, with its base directed towards the embryonic shield.

"The mesoblast, as already mentioned, extends from a broad base attached to the chorion towards the 'Embryonalanlage,' and forms at its basal side the allantoic stalk. The latter is composed of mesoblast cells only, and shows no trace of an epithelial-lined duct. . . . There are no distinct vessels. However, one sees here and there at the periphery of the mesoblast accumulations of cells, occasionally arranged in a circular manner, with a lumen in the middle. Similar formations are seen on the lateral portions of the allantoic stalk. I am unable to decide whether we are dealing with the first 'Gefässanlagen.' The circular rings do not show in their lumen anything like blood corpuscles. Numerous mitoses are seen in the embryonic shield and in the allantoic stalk."

History of the Case From Which the Ovum Came.[6]

Our own ovum, while not coming up fully to the most ideal requirements, approaches them so closely that it must indeed be a

[6]The first more extensive communication concerning our specimen was made before the Section of Embryology of the Seventh International Congress of Zoology, held in Boston, in August, 1907. It was then the privilege of the author to demonstrate the sections to such well-known European and American embryologists as Professors A. A. W. Hubrecht, F. W. van Wijhe,

very uncommon combination of circumstances which will furnish an investigator with a still better, equally young, human specimen. The ovum was obtained *in situ* from a healthy woman, with absolutely normal and healthy genitalia, who was almost instantly killed by a most peculiar accident.

The body of the woman was brought at once to the public morgue, and there placed in a large refrigerator. The post-mortem examination was made by the author, and the findings, as usual were recorded in the shape of a post-mortem protocol, which here follows, verbatim, as written down at the time.

Mrs. M. R., from Intramuros, Manilla; Filipina, aged 25, died July 17, 1904. The post-mortem examination was made July 18, twelve hours after death. Immediate cause of death not known. It was stated that she had been struck by a small carriage (called carromata in Manila) shortly before she died.

Body of a well-developed young native woman, twenty-five to thirty years of age. Post-mortem rigidity strongly marked. Post-mortem lividity quite noticeable. Abdomen somewhat distended. A repeated careful inspection fails to show any signs of external violence. No wounds, contusions or abrasions of any kind to be seen. On opening the thoracic cavity, the pericardium is found to be much distended, and shining through it there appears to be a firm, dark, blood coagulum. On opening the pericardium, it is found that it contains a large amount of dark, coagulated, gelatinous blood, and blood-tinged serum, distending the pericardium *ad maximum* and compressing the heart. A careful examination fails to show any perforation in the pericardium. The heart, which weighs 226 grams, presents a perforation, which begins two centimeters to the left of the anterior border of the interventricular septum. The perforation extends almost horizontally toward the left, being a little downwardly inclined. It forms a slit 2.2 centi-

Charles Sedgwick Minot, Franklin P. Mall, A. Playfair McMurrich, T. G. Lee, and F. T. Lewis.

Previous to this communication, the specimens had been demonstrated in June, 1907, before the German Medical and the Gynecologic Societies of Chicago.

meters below the sulcus of the heart. The edges of the perforation are almost clean-cut where they enter the myocardium, as if they had been produced by a dull, somewhat serrated knife. The cut takes a somewhat downward and inward course, traveling through the whole thickness of the myocardium. Where the cut enters the cavity of the heart, the margins are not very smooth, but rather irregular and ragged. The consistency of the myocardium is good. Its color is pinkish-brown, and all the serous surfaces are smooth. There are no atheromatous changes. The heart is covered with a very moderate amount of epicardial fat. In short, the whole organ, except for the wound, is absolutely normal. After the removal of the lungs (the apex of the right one showing a very few tubercles, and a little caseous nodule not larger than a lentil), it is seen that the second, fourth, and fifth ribs of the left side are fractured. The fracture of the second rib is found to be 7.5 centimeters posterior to the sternal articulation, that of the fourth one 9.0 cm., and that of the fifth one 9.5 cm. The anterior fragments are directed inwards. The fragments of the fourth and fifth ribs are very sharp and are surrounded by an area of subpleural blood extravasation. However, these fragments have not perforated the pleura costalis. The extravasated blood is strictly subpleural and no free blood is found on the surface of the pleura. *The uterus appears somewhat enlarged, and the left ovary shows a fresh but already closed corpus luteum. On opening the uterine cavity a little hemorrhagic mass, about one-half centimeter or less in diameter, is found embedded in the mucosa of the posterior wall, near the entrance of the left tube. This mass is carefully cut out and placed in Zenker's solution, as it may contain a very young ovum.* All the organs of the body, with the exception of the apex of the right lung, and the perforated heart, are found to be absolutely normal. They are all more or less congested with dark fluid blood. It appears clear that the woman must have been struck at the side of her body, or in the back, by a swiftly moving force. This force, however, did not produce any signs of external violence, particularly no contusions, abrasions, or wounds. The force traveled through the soft parts, and, meeting the resistance of the ribs, fractured them. The

anterior sharp fragment of the fourth or of the fifth rib was evidently driven into the wall of the left ventricle, producing a complete perforation. A highly interesting point is that the sharp fragments neither perforated the pleura costalis nor the parietal layer of the pericardium. Only when the resistance of the firm wall of the ventricle was encountered did a rupture or perforation occur. A hemorrhage took place, and when the pericardium was completely filled, and the myocardium much compressed, the heart's action came to a sudden standstill. Death occurred from syncope at once.

Anatomical Diagnosis.

Fracture of the second, fourth, and fifth ribs of the left side. Complete perforation of the wall of the left ventricle. Hemorrhage into the pericardium. Compression of the myocardium. Beginning tuberculosis of the apex of the right lung.

Microscopic examination of the myocardium showed it to be perfectly normal.

To this history may be added the statement that it was later learned through the police reports that the woman had indeed been struck by the swiftly-moving shaft of a small carriage; that she fell forward, got up, staggered, fell again, and was dead within a very short time.[7]

The small piece of tissue removed from the uterus was placed in Zenker's solution, later washed in running water, and was then embedded in paraffin. Since our facilities for cutting serial sections were not the very best at this time (July, 1904) in Manila, and since the writer was then engaged in the study of Bubonic plague, a few sections only were prepared, and the bulk of the block was preserved for future work. From the few sections examined, the firm impression was gained that the uterine mucosa presented the picture of a very early decidua, with cystic hemorrhagic gland spaces

[7]The history of this case has previously been published by the author in a paper: "Peculiar Cases of Traumatism of Internal Organs, Some Due to Tropical Conditions and Practices." Surgery, Gynecology and Obstetrics, Vol. IV, No. 6, p. 741, Chicago, 1907; and Philippine Journal of Science, Vol. 2, 1907.

of the type of those of the Peters' ovum. However, the first sections did not show either the cavity of the ovum or a trace of the trophoblast.

General Description of the Ovum.

On June 15, 1907, the block of tissue was finally divided, and I am obliged to Dr. Day, Pathologist in the Chicago Laboratory of the Bureau of Animal Industry, for assistance in preparing a complete series of sections. The individual sections were all 7 microns thick; in all they numbered 303. About three sections were lost on the microtome; several subsequently floated off partly or entirely from the slides during the process of staining in hematoxylin and eosin, but fortunately none of the important sections containing the embryonic shield were lost.[8]

The general outlines of the ovum are those of a bi-convex lens, a somewhat flattened elliptical body. The ovum was found in sections Nos. 63 to 235; the embryonic shield in sections 142 to 164. The measurements obtained by micrometer are the following:

Ovum (chorionic cavity-exocœlom): Greatest length (section 153), 2.326 mm. Greatest width (section 153), 0.804 mm. Greatest thickness (172 sections at 7 microns each), 1.204 mm.

The trophoblast begins to show in section No. 47, and ends in section No. 264. Since the chorion mesoderm begins to show in section No. 63 and ends in section No. 236, the trophoblast on one pole is 16 sections or 112 microns thick, and on the other 29 sections, or 203 microns. In section 150 the trophoblast, towards the muscularis, is a little over one millimeter thick; towards the upper surface, 0.9 mm.

A list of the smallest human ova described, compiled from Peters' and Jung's collection, follows:

[8]The author has in the case under discussion and in a few cases of early tubal pregnancies attempted to obtain complete and perfect series of ova *in situ* by the paraffin embedding method. However, experience has taught him that young human ova *in situ* presenting very heterogeneous tissue elements, including masses of maternal blood, are not best adapted for the paraffin method, but should preferably be embedded in celloidin.

Bryce and Teacher: 0.77, 0.63, 0.52 mm.
Peters: 1.6, 0.9, 0.8 mm.
Herzog: 2.326, 0.804, 1.204 mm.
Graf von Spee: 2.5, 1.5 mm.
Jung: 2.5, 2.2 mm.
Mertens: 3.0, 2.0 mm.
Beneke: 4.2, 2.2., 1.2 mm.
Leopold: 4.0, 3.7 mm.
Graf v. Spee: 4.0 mm.
von Heukelom: 4.5, 5.5.
Beigel-Loewe: 4.0, 5.0, 2.5-3.0 mm.

It appears from the above table that the author's ovum is somewhat larger than Peters' ovum. However, the real difference in the cubical contents of the two ova cannot be much, since the Manila ovum, while markedly larger in its longest diameter, is indeed not strictly elliptical, like the Peters' ovum, but has the shape of a biconvex lens. It is useless to speculate whether or not the Manila and the Peters' ovum are of exactly the same age, or whether the former is a little older than the latter. We have in our case not made any efforts, at the time when the ovum was obtained, to get data as to menstruation and probable time of conception. Such an attempt would have been useless in the case of a full-blooded native from the lower walks of life. Even in those reported cases of very young ova, where these data were obtainable, the estimates as to the age of the embryo are not very correct. Peters estimated the age of his ovum as of three to four days; Leopold, an older ovum (4.0; 3.7 mm), as seven to eight days old. Jung thinks that the Peters' ovum is considerably older than three to four days; Bryce and Teacher estimate its age at thirteen to fourteen days. Von Spee and Minot believe that seven to eight days intervene from the time of fertilization in the tube till implantation occurs in the uterus. The age of a human ovum, like that of the lower mammalian animals, whose early embryology has been sufficiently studied, can probably be best estimated from the stage of development of the embryo proper, but the data in this respect as to very young human embryos are insufficient.

Description of the Embryo.

The sections of the embryo have been accurately drawn under my direction by Miss Grace Amadon, and are shown in Plates I to IV, Figs. 1 to 22. The embryo is first seen in section 164 (Fig. 1 of the embryo), and the ectoderm cells of the embryonic shield were last encountered in section 149. Hence the whole length of the embryonic shield proper is 16 by 7 microns equals 112 microns. However, the mesoderm extends as a thickened mass beyond the ectodermal limit of the embryonic shield from sections 150 to 143, or over a distance of 8 sections, equal in all to 56 microns. We have here what appears to be an extension of the shield-mesoderm beyond the shield ectoderm and entoderm—a mesoderm "Vorhof." Including the mesoderm "Vorhof," the embryonic shield extends through twenty-two sections; its whole length therefore is 22 by 7 microns equals 154 microns.

The first section (164) has hit the embryo in a tangential manner, and shows only a single layer of cells. The three following sections (Figs. 2 to 4) consist of an inner layer of ectoderm and an outer layer of mesoderm. In the next section (Fig. 5) a delicate strand of cells is clearly seen between the ectoderm and mesoderm. This must be interpreted as a subdivision of the mesoderm. It continues into the eighth section, in which the entoderm first appears. The entoderm is represented by a small group of cells between the two layers of mesoderm, seen on the upper side of Fig. 8. In Fig. 9 the entoderm has expanded so that the cavity of the yolk sac now appears. It lies in the upper half of the figure.

The embryo is anchored to the chorion by an allantoic stalk, composed externally of mesoderm and traversed by a rather slender, somewhat curved canal of entoderm cells (Figs. 9 to 14). The connection between the entoderm of the allantois and of the yolk sac presumably occurred in sections 156 and 155, but the continuity of the entoderm has been destroyed.

Around the allantoic stalk (Figs. 10 to 13) where its mesoderm is continuous with the yolk sac mesoderm, there are found some solid and some open circular masses of mesoderm cells. The open

rings are composed of three to four to five mesoderm cells; the solid round or oval cords contain a larger number of cells. These formations undoubtedly represent the earliest "anlage" of the yolk sac blood vessels. The chorion mesoderm and the mesoderm where it extends somewhat into the trophoblast do not yet show any traces of blood vessels.

The cavity of the yolk sac, which first appeared in section 156 (Fig. 9) remains small and slit-like through sections 157 and 158. It then gradually increases and attains a transverse lateral diameter of about 176 microns in sections 152 and 150. In section 143, where the last of the "Vorhof" mesoderm is seen, the transverse diameter of the yolk sac is 192 microns. From here on it hangs down free for a considerable distance into the exocœlom. It can last be seen in section 122. Its entoderm and mesoderm layers are there very distinct. The yolk sac must have extended somewhat beyond section 122, but from 121 on it has been lost in the sections. It extended through at least thirty-four, and probably through forty sections, hence its greatest sagittal diameter was between 250 and 300 microns.

The cavity of the amnion, which has a diameter of from 100 to 160 microns in the first sections, becomes reduced to a canal in section 153, having a lateral diameter of 45 microns and a dorso-ventral diameter of 30 microns. This canal terminates in section 149 (Fig. 16). In the first sections of the embryo (Figs. 2 to 8) the amniotic cavity is almost circular. Internally, it is bounded below by the thick embryonic shield, which is curved so as to form a deep crescent. Its concavity is toward the chorion. The ends of the crescent are continued as the very thin amniotic epithelium. However, the amnion is not complete in this region because its two lateral wings stop short and do not meet in the median sagittal plane. This seems clearly due to the artificial rupture of the very thin membrane. With equal certainty it may be said that the great concavity of the ectoderm shield is not artificial, but was present ante-mortem. So thick a layer is not liable to distortion, and its cells show no evidence of disturbance.

Some embryologists have expected to find an inversion of the germ

layers in early human embryos. The "Blattumkehr" in monkeys has been defined by Selenka as follows (Biol. Centralbl., Vol. 2, p. 552):—

"Der Embryonalbezirk . . . ist gezwungen sich . . . im Innern der Keimblasse einzustülpen, wobei das Entoderm zur kappenartigen Hülle ausgeweitet wird, die Keimblätter sind daher an dieser Stelle umgelagert, umgekehrt, invertirt."

It is now known that no true inversion of the layers occurs in man, but, as stated by Bryce and Teacher, "there is no doubt that the plate of embryonic ectoderm is inturned, and there is strong probability that the condition is a primary one and not due to a precocious formation of amnion folds." (Page 34.) The primary infolding of the embryonic shield is strikingly shown in Figs. 2 to 8.

Where the embryonic shield forms the floor of the amniotic cavity in sections 156 to 153 (Figs. 9 to 12), there is an opening through the ectoderm in the median sagittal plane of the shield. No other indication of a neurotic canal was found. The opening, however, is presumably an artefact. Similar ruptures through ectoderm are seen in sections 13 and 15. Around the median aperture the ectoderm is not continuous with the entoderm, as would occur if the structure were a true neurenteric canal.

The cells of the three germ layers as seen in this embryo may be described as follows: The ectoderm of the shield is composed of more or less cuboidal or cylindrical cells, which are quite epithelial in character. The nuclei are round or oval, with a finely granular chromatin network, and with generally one, occasionally two, distinct, deeply-stained nucleoli. The cell protoplasm is generally very finely granular and stains moderately deeply with eosin. The chromatin network can be particularly well seen in the first sections of the ectoderm shield where the cells have been cut very favorably for this observation and where they are not so densely crowded as elsewhere. The karyokinetic figures are generally in the monaster stage. No distinct diasters were found. One cell was seen with two small vesicular nuclei containing densely but finely granular chromatin, and one pair of cells with the same kind of nuclei and an incomplete division of the protoplasm.

The ectoderm cells of the amnion are in general of the same description as the shield ectoderm cells; however, they have smaller protoplasmic bodies, which are not cuboidal, but rather elongated.

The mesoderm cells of the embryonic shield have generally oval nuclei and exceedingly little protoplasm; they are connected with each other by very thin, filamentous, bipolar processes. The yolk sac and amnion mesoderm cells are elongated and connected with each other by bipolar processes. But where the mesoderm cells are most numerous, namely, at the allantoic stalk, their nuclei are larger and their protoplasm, which is fairly abundant, is generally irregularly polygonal.

The entoderm cells of the shield have nuclei very much like the ectoderm cells, but the protoplasmic bodies are smaller and often somewhat elongated. These cells are likewise more or less connected with each other by bipolar though shorter and coarser processes. The entoderm cells of the yolk sac have rather small nuclei, rich in chromatin, and, where they can be seen at their best, are almost geometrically cuboidal. They are rather small in size in comparison with the shield ectoderm cells. The entoderm cells of the allantois are like those of the yolk sac.

Description of Chorion and Decidua.

Nomenclature.

In order to facilitate the description of the chorion and of the decidua in which it was found embedded, as well as to avoid any misunderstanding on the part of the reader, it is well to outline shortly the nomenclature used.

That part of the uterine decidua on which the inner pole of the ovum rests and which is characterized in our case by the presence of large cystic gland spaces and lacunæ, densely crowded with blood, we will call the *decidua basalis.* That thin strip of decidual tissue which separates the ovum from the uterine cavity will be designated as *decidua capsularis,* and that part of the decidua surrounding on all sides the equator of the ovum we will call the *decidua vera.*

In the latter at some distance from the ovum an inner *spongiosa* and an outer *compacta* can generally be easily distinguished. The mesoderm lining the interior of the chorionic cavity or exocœlom will be designated as the *chorion mesoderm,* while the chorion ectoderm will be called *trophoblast.* This term has been proposed by Hubrecht, and it is understood and used so generally that it is perhaps well to retain and not to replace it by other terms.[9] The trophoblast is composed of two kinds of tissue, the inner cell masses and an outer covering of syncytium. We want to state here that the examination of our young ovum has confirmed our opinion, expressed a number of times previously, that both the cells proper of the trophoblast (the future Langhans cell layer) and the syncytium are derived from the fetal ectoderm. We have never previously in hundreds of placentæ in normal and in ectopic gestation, nor in our present case, found anything which would seriously suggest a derivation of the trophoblast syncytium from maternal cells. The ovum under discussion nowhere shows a possibility that the syncytium is derived either from surface or glandular uterine epithelium, from vascular endothelium, or from decidual cells. Hence the term *ectoblast shell* for the combined trophoblast cells and the syncytium is correct. We retain the well-known term trophoblast in spite of the fact that we consider it as to its etymology a misnomer. We have previously expressed ourselves on this subject, as follows:

"The term trophoblast has been given by Hubrecht to the extra-embryonic ectoblast shell under the impression that it had to do a good deal with the nutrition of the early embryo. We doubt, however, that

[9]Minot, in an address on "The Implantation of the Human Ovum in the Uterus," delivered in 1904 before the Gynecological Society and printed in its transactions, has proposed the term trophoderm, but he states himself: "In the address the term trophoblast was used in accordance with my understanding of Professor Hubrecht's views and consent; but, as Professor Hubrecht has objected to this application of his term, it has been necessary to propose a new one. I regret that so good a name as trophoblast has to be dropped." With this address the author only became familiar after the completion of the manuscript of this contribution. He now finds that his views of the physiology and the mechanism of the implantation of the human ovum given are in many respects identical with the previously published explanations of Minot.

this is the case. The mass of the trophoblast in our case is certainly many thousand times that of the embryo. It does not stand to reason to assume that nature in the phylogenetic development would provide, so to speak, at an enormous expense, a very large apparatus for the nutrition of a very small embryo. It appears more reasonable to assume that the trophoblast with its great proliferative energy, which we have likened to the growth of a malignant tumor, has more exclusively the function to provide the means for the embryo to safely implant itself at the very earliest date into the maternal tissues. The reaction of the maternal tissues in contact with the proliferating trophoblast must not be looked upon as due to mechanical causes only, but to fermentative action of enzymes secreted by the trophoblast cells and diffused into the neighboring maternal tissues."

The above statement we still hold to be correct on the whole. However, we agree with Bonnet[10] that the syncytium presents features, namely its property to stain very deeply with eosin, which suggest the possibility that it takes up hæmoglobin from the maternal blood for the benefit of the nutrition of the embryo.

The term syncytium in connection with placentation has been used very promiscuously and has been inaccurately applied to degenerating confluent cell masses of maternal origin. According to Bonnet, the term syncytium has been introduced into histology by Haeckel, who designated by it a nuclei-containing plasma, formed by the confluence of previously separate and distinct cells. Taken in this sense, the term syncytium as appied to the human trophoblast is probably a misnomer. It is very likely—though nothing about this is known from actual observation—that the syncytium of the human placenta is formed "ab origine," as an outer strip or capsule of protoplasm which is provided with expelled nuclei from the cells of the inner cellular trophoblast. In a publication "On the Pathology of Tubal Pregnancy," quoted in a footnote above, I have considered the syncytium of the human placenta as the homo-

[10]Bonnet: Ueber Syncytium, Plasmodien und Symplasma in der Placenta der Säugethiere und des Menschen. Monatschrift für Geb. u. Gyn., 1903, vol. 18, p. 1.

logon of the periblast of transparent pelagic fish eggs, such as those of *Fundulus,* which I had a chance to study in the Summer of 1899, in the Woods Hole Marine Biological Laboratory, under the direction of Professor C. O. Whitman. In these fish eggs the formation of the periblast—an outer capsule of plasma without cell boundaries, but with numerous nuclei—can, of course, be studied from stage to stage under the microscope, and the expulsion of nuclei into the plasma can be seen.

In spite of the fact that the syncytium of the human placenta does not deserve this name in the sense as originally applied, it is well to preserve its use, since it has been universally applied to the outer covering of the trophoblast, and of the later chorionic villi. For degenerative confluent cell masses in the placenta, whether they be of maternal or fetal origin, Bonnet has proposed the term *symplasma,* and he distinguishes between symplasma maternum and symplasma fetale. These terms have been accepted by Jung in the description of his ovum, and we will likewise introduce them into our considerations. Some of the German writers on placentation, following Bonnet, have come to use the terms "Grundschicht," for the cellular part of the "Trophoblast" (the later Langhans layer), and "Deckschicht," for the syncytium.

The General Position of the Ovum and its Mode of Entrance into the Decidua.

At the time of the autopsy, as stated above, the ovum, or rather the small hemorrhagic spot, was found at the posterior wall of the corpus uteri, comparatively high up in the fundus, and near the entrance of the left Fallopian tube; that is, on the same side where the corpus luteum ovarii was noticed. The dark, hemorrhagic spot which contained, as was later on found, the ovum was only very slightly prominent over the remainder of the thick, velvety mucosa. The uterus, after the careful removal of the dark spot, which was excised as a cubical mass, was preserved in the pathologic collection of the Government Laboratory, but I have not had a chance to re-examine it during my stay in Manila, and I do not know whether it has been preserved permanently or not.

The mass removed was sectioned from above downward, and the ovum, which can best be seen as a whole in photomicrograph (Fig. 24), was found situated with it long axis parallel to a line drawn from one ostium internum tubae to the other.

A glance at the photomicrograph (Fig. 24) shows the following points as to the position and surroundings of the ovum in general: The ovum, including its trophoblast and the thin strip of decidua capsularis, protrudes very slightly above the surrounding surface. From the cavity of the uterus it is separated by a very thin decidua capsularis. This in the very center of the upper line of photomicrograph (Fig. 24, section 125) is slightly deficient, and here we see a teat-like process of the chorion mesoderm extending almost to the surface. The inner pole of the ovum rests on a wedge-shaped mass of very large cystic gland spaces densely filled with blood. The decidua vera near the ovum shows densely crowded hypertrophied gland spaces. They are somewhat cystic towards the muscularis, and towards the free surface present enlarged tortuous tubes, separated by intervening septa. The differentiation into a decidua compacta and spongiosa, which is not well shown in Fig. 24, appears more clearly in the photomicrograph Fig. 26.

Large cystic glandular blood-filled spaces are found not only at the base of the ovum, but also towards one side. Such spaces were found also in the first set of sections examined, *i. e.*, in sections which showed neither the trophoblast nor the chorionic cavity. If one studies sections 11 to 39 of the complete series, which likewise do not yet show any trophoblast, the following can be seen. Near the surface, under a thin strip of decidual tissue composed of large, partly-degenerated, decidual cells, there is an opening or hole surrounded on all sides by profoundly degenerated decidua. The decidua here is least degenerated at the upper stratum (capsularis), and most markedly degenerated at the inner (basal) aspect. The decidual cell masses are infiltrated with maternal blood, and are surrounded by large cystic gland spaces (Fig. 27) filled either with blood, with hyaline, eosin-staining balls or masses, with degenerated, dropped-off glandular epithelium, or with networks of fibrin. The hole described is not empty, but more or less filled with blood

and dropped-off degenerated decidual cells. In section 11 the canal or hole has no covering towards the uterine cavity, but the mass of blood and degenerated, chaotically distributed, decidual cells reaches to the very surface.

It is clear that this canal, which is more or less circular in outline, with diameters varying from 1.5 to 1.0 mm., indicates the route over which the ovum traveled to the place where it was found in the decidua. It is probable that the ovum after having been fertilized in the tube was brought to a spot indicated by the surface opening in section 11, or thereabouts. The ovum when less than one millimeter in diameter (this measurement, including, of course, the chorionic cavity and the whole then existing trophoblast) began to make its way into the decidua. It did, however, not eat its way deeply in at all, but traveled under the surface almost parallel to it, being separated from the uterine cavity only by a very thin strip of decidual tissue, in some places so thin that it became slightly defective, or at least apparently so. After the ovum had traveled through the canal described, of which there are about 200 to 250 micra in length left, it must have become stationary and must have begun to expand inwardly, towards the muscularis, and also laterally. It is clear that the ovum as found *in situ* in the decidua is much too large to have traveled through the canal, and it must have been much smaller, probably considerably smaller than one millimeter, at the time when this migration took place.

The canal, as stated, was found moderately well filled with blood corpuscles and degenerated decidual cells. We have, therefore, in our case, unlike Peters, who had in his specimen a mushroom coagulum, a still patent, though partly filled, small canal, running almost parallel to the uterine surface, through which the ovum by its own inherent destructive tendencies made its way to the place of final implantation. While the outer end of the canal described communicates with the surface and is practically open towards it, except as to the presence of maternal blood and degenerated decidual cells, the inner end is closed by the trophoblast of the ovum. On the opposite point of the ovum, which is found in section 264, where

the trophoblast is last seen, we likewise have evidences of its great destructive tendencies. The next sections show numerous degenerating, dropped-off decidual cells, mixed with blood. Thus, there is formed here also a kind of cavity filled with blood and detritus. However, it does not reach the surface, but remains separated from it by decidual tissue. We see the ovum then everywhere more or less surrounded by cystic blood spaces or free blood. In this respect the early human ovum is very much like that of the hedgehog and mouse, as described by Hubrecht and Bonnet, surrounded on all sides by maternal blood, floating as it were in a lake of blood. The conditions in our ovum are more or less identical with what had been found by Peters in his case.

It can be seen in Fig. 25 that the embryonic shield is anchored by its allantois to the chorion mesoderm near the inner pole of the ovum and farthest away from the outer pole and the thin capsularis. The position of the embryo with reference to its outer and inner poles is thus identical with that of Peters' ovum. In the Jung ovum likewise the embryonic shield appears to hold the same relations to the maternal organism, through orientation in this last-mentioned specimen, which was not obtained *in situ,* but by curettement, is not absolutely certain. The apparently identical position of the embryonic shield in the three young human ova is very probably not at all a matter of chance or accident, but is due to a very early automatic orientation of the ovum for the benefit of the developing embryonic shield—from a biogenetic standpoint the most important part of the ovum.

Examining the sections which show the beginning and the course of the canal, the following conditions can be ascertained: The entrance of the canal is evidently not in a gland duct, but in an interglandular septum, through a decidua which here, in spite of profound degenerative processes, shows well the character of a compacta. On both sides of the entering canal we see the enlarged tortuous necks of uterine glands. Enormously enlarged capillaries reaching from the sides of the entering canal to the very surface can also be seen. Both at its entrance and along its course the canal is surrounded by enlarged gland spaces. The glandular epithelium every-

where shows profound degenerative changes, and the large cystic glands are partly or completely filled with blood and detritus, as already mentioned. There is no trace of glandular or surface epithelium left at the site where the canal takes its origin from the surface. Nor can one find any gland ducts opening into the canal, or into the cavity in which the ovum now is situated. All of the conditions point unmistakably to the fact that our own ovum, like that of Peters, penetrated into the decidua not through a gland space, but by eating its way through interglandular tissue of the compacta. It does not appear necessary to us to assume that the ovum can make its way into the decidua only through a spot denuded of the surface epithelium. Since the enlarged gland spaces, even where not in direct contact with the trophoblast, exhibit most marked degeneration of the lining epithelium, we may well assume that the trophoblast, in contact with the surface epithelium, can destroy it easily and make its way into the connective tissue of the decidua.

In our preliminary communication, read in August, 1907, before the Zoological Congress of Boston, we said: "We cannot conclude this preliminary report without pointing out what we might call the pathologic aspect of the early stages of placentation in man. The proliferation of the trophoblast, the manner in which it invades the maternal organism, pushing aside, destroying and changing maternal tissue elements, vascular and other structures, is the exact picture of malignant tumor proliferation, while the reaction of the maternal tissue, taken for itself alone, reminds one forcibly of a profound destructive hemorrhagic inflammation. It is very striking to the pathologist to behold in early placentation, in the apparatus and the phenomena which enable the young ovum to anchor and implant itself firmly into the maternal organism, the very paradigma of two such important pathologic processes as malignant tumor growth and hemorrhagic inflamation."

A further study of the sections has only strengthened the impression gained previously. The destructive tendencies of the early trophoblast of the ovum are certainly very marked. If some hypothetical speculations may be here permitted, we would like to express our opinion that the trophoblast at a certain stage of its

development secretes an enzyme which diffuses into the surrounding maternal tissues and here causes coagulation necrosis and complete degeneration of cells. The trophoblast cells, as represented by our specimen, are certainly not phagocytic in the ordinary sense of the word. We have in vain examined and re-examined our sections to find included in the trophoblast cells or the syncytium maternal blood corpuscles, fixed tissue cells or fragments or remnants of the same. We must, therefore, conclude that the effect of the trophoblast upon the maternal tissue is brought about not by true phagocytosis, but through the action of an enzyme. If the latter destroys maternal cells to a large extent, and this destruction, of course, takes place, as can be seen, we have those conditions which under any circumstances would lead to violent inflammatory reaction, including enormous dilatation of veins and capillaries; free hemorrhages; and if the process take place in a glandular mucosa, with hypertrophy of the glandular apparatus. And, indeed, if we look upon the decidua in our specimen particularly, as seen in Fig. 24, to the left of the ovum, and in Fig. 26, in the whole section, the resemblance between them and a typical, well-marked case of endometritis glandularis hypertrophica is very striking. In fact, when the set of sections represented by Fig. 26 was shown to a very competent pathologist with the statement that it was very probably a very early decidua and that an ovum would be found in the block of tissue, he ridiculed the idea and firmly held that the section simply showed a typical strongly-marked case of endometritis glandularis hypertrophica.

If we behold the great destructive tendencies of the early trophoblast, the question presents itself, Why does this process evidently come almost to a standstill somewhat later in the course of gestation? Two possibilities present themselves. Either the secretion of the supposed destructive trophoblast-enzyme is limited as to time, or there is established a temporary immunity of the maternal tissues, These speculations, while at present entirely hypothetical, might perhaps be supported by experiments in which the effect of filtered extracts of animal placentæ of various stages of development, and in repeated applications, upon the uterine mucosa, would have to be studied.

Peters, in his case, has shown how the ovum did not make its way into a gland duct, but had eaten its path into the decidua and he opposed the old theory as to the formation of the decidua reflexa. He, however, conceded the desirability of demonstrating the mode of entrance of the ovum in more than one case examined *in situ*.

Our own case, offering in some regards even better conditions, namely, almost absolute certainty of the absence of all pathologic deviations from the normal type, fully confirms the view of Peters of the mode of implantation of the ovum and of the erroneousness of the older theories. Both the Peters and our ovum show the correctness of Count von Spee's[11] hypothesis that the human ovum would be found to behave in its method of implantation into the uterine mucosa like the ovum of the guinea-pig. It was shown by von Spee that the dividing blastoderm of the guinea-pig eats its way through the uterine epithelium, into the connective tissue, causing here edema and hyperemia.

Exocœlom and Chorion Mesoderm.

The chorionic cavity or the exocœlom, as to its size and shape, has been sufficiently described. The position of the embryonic shield, yolk sac and allantois have also been indicated. Aside from the "Keimanlage," the exocœlom shows in its interior a finely granular, irregularly lumped material, which has stained intensely with eosin. This material can be well seen in photomicrographs, Figs. 24 and 25. It is responsible for the fact that the sections of the embryo could not be photographed so that they appear on a clear homogeneous background. What is seen in the sections with reference to the eosin-staining granular material proves that the exocœlom "inter vitam" was filled with a watery fluid rich in coagulable proteids. The strongly eosin-staining properties of the granular material may perhaps be due to an absorption of hemoglobin indirectly derived from maternal sources. Towards the periphery, that is, towards the lining chorion mesoderm, and running parallel

[11]von Spee: Die Implantation des Meerschweincheneies. Zeitschr. f. Morph. u. Anthrop., 1901, Vol. 111, p. 130; and Ueber die menschl. Eikammer, etc., Verh. der Anat. Ges. zu Kiel, 1898, p. 196.

with it, are seen long slender fibers, more or less mixed with granular detritus. These fibers are evidently the remnants of degenerated, dropped-off lining mesoderm cells. The latter themselves are comparatively long and fusiform, with very gradually tapering long bipolar processes. Their protoplasm is finely and distinctly granular, well eosin-staining. The nuclei are oval, sometimes almost rod-like, with rounded ends like the nuclei of involuntary muscle cells. They have a fine but darkly-staining chromatin reticulum; often one or two nucleoli can be seen. The chorion mesoderm in most sections has slightly retracted from the trophoblast ectoderm and we here can see distinctly along the outer margin of the mesodermal lining a fine, sharply-cut membrana limitans, as described by Bonnet, in a more advanced older ovum as separating the mesoderm of the villi from their ectoderm. The chorion mesoderm forms teat-like or finger-like processes arising from the periphery and extending outwards into the ectodermal trophoblast. These processes likewise show the fine limiting membrane. Sometimes these processes arise near each other, but they do not yet show any dichotomous division. Here and there are seen floating in the exocœlom bands or filaments of mesoderm cells. They are interesting from the standpoint of the pathologist, because their occasional growth and persistence may lead to the formation of those so-called amniotic bands responsible for disturbance in the normal development of the embryo. These mesodermal bands and strands, crossing the chorionic cavity, have also been described for their respective specimens by Bryce and Teacher, Peters and Jung. They are important because they have been interpreted as the remnants of a once solid mass of mesoderm, existing before the formation of the cœlom and exocœlom.

Keibel (Normentafeln, Vol. 8, p. 12) in discussing the early mesoderm and the formation of the exocœlom says: "In man at a stage when a primitive streak cannot yet be demonstrated with certainty or even does not exist, we find the whole embryonic shield, yolk sac and amnion richly surrounded by mesoblast, as is also the internal surface of the chorion. Spee, in describing his embyro H, says: 'It appears almost inconceivable that the region of the still

smaller primitive streak of an earlier period has furnished these masses (of mesoblast). Probably a small mass of mesoderm is produced from a primitive streak at a very early stage, and later this mass proliferates independently.'" "Although this view of Spee," Keibel continues, "appears to be the most probable, it is well to point out that it is only an hypothesis, which encounters many difficulties. We cannot at present state anything more definite as to the origin of the mesoblast in man. Something more certain may be said of the origin of the cœlom, although its earliest stages have also never been observed in man. It is certain, that, as in mammals generally, the extraembryonic cœlom (the cavity of the ovum) is formed earlier than the embryonic cœlom, and we are probably correct if we assume that the extraembryonic cœlom is formed by cleavage (Spaltbildung) in a mesoblast which has already developed. This would be in accordance with what is known of the other mammals.

The Trophoblast and Its Syncytium.

The early characteristic trophoblast of the human ovum, though first correctly described by Peters from his specimen, had previously been hypothetically constructed from the observation of an older ovum *in situ,* in which the peculiar ectoblast shell had been differentiated by the formation of the villi. We owe this description to van Heukelom[12], who gives it in the following words: "One can best get a conception of these conditions if one imagines all villi connected at their periphery by beams of ectoblast, so that they form an ectoblast shell full of small and large holes. This shell is unevenly thick and rests directly on the maternal compacta. . . ."

The trophoblast as found in our specimen surrounds the chorionic cavity or exocœlom of the ovum like a thick shell. It is, however, not equally powerful on all sides, as has been already indicated by the measurements of the trophoblast. Nor is it a solid mass of cells. It is, on the contrary, honeycombed by irregular communi-

[12]von Heukelom: Ueber die menschliche Placentation: Archiv f. Anat. u. Physiologie (Abth. Anatomie), 1898.

cating spaces contained in a network of irregular bands, strands and masses of trophoblast material. The cavities in the trophoblast are not empty, but well filled with maternal blood. There is a certain regularity of those trophoblast cavities which are situated next to the chorionic cavity. Here they are somewhat regularly cuboidal and they are placed around the chorion mesoderm like blocks of stone in a pavement. It is evident that this arrangement is brought about by two factors working in a certain sense against each other, namely, the pressure of the maternal blood and the growth energy of the chorion mesoderm. Towards the chorion mesoderm, these cavities are lined by a thin layer of trophoblast, composed of one cell layer of the "Grundschicht" and one layer of "Deckschicht" or syncytium. Towards the periphery these cavities are lined by more powerful masses of trophoblast. In the middle stratum of the trophoblast there are irregular cavities of moderate size, and in the outer stratum the open, blood-containing spaces become very large and very irregular. In this outer zone we find the trophoblast material much diminished, and where it is in contact with the thin capsularis, it dwindles down to isolated thin threads or pillars of cells.

The cells of the "Grundschicht," or that part of the trophoblast which later becomes the Langhans layer of the villi, are most characteristic in the middle zone where they are present in the shape of bands and beams and irregular masses, and where they have not been exposed to considerable pressure, as in the zone next to the chorionic cavity. In the middle as well as in the outer zone the cells have the following characters: The protoplasm is generally almost spherical, or in consequence of mutual compression of the cells irregularly polygonal. The cell boundaries are so very distinct that it appears as if the cells had membranes. The protoplasm has stained very lightly with eosin and gives the impression that the cells "inter vitam" must have been very "saftreich." The nuclei are large, round and vesicular, with a finely granular chromatin network. Frequently one or occasionally two nucleoli can be seen; these likewise are more or less distinctly vesicular in appearance. The nuclei are about twice the diameter of the maternal red blood

corpuscles, and the whole cells are three to four times as large in diameter as an erythrocyte. Karyokinetic figures are occasionally, though not very frequently, seen. (Fig. 29.)

The trophoblast cells next to the chorionic cavity are cuboidal and compact. Their protoplasm is rather scanty, and it stains much deeper with eosin than that of the cells of the middle trophoblast stratum; the nuclei are somewhat smaller, more oval and slightly richer in chromatin. Towards the uterine cavity the trophoblast cells form thin bands which in sections present themselves as slender bridges connecting the trophoblast with the structure mentioned before as "the thin strip of the decidua capsularis." Where they lead up to the surface the trophoblast cells have often broken through the syncytium. Here the cells become fusiform, bipolar and while not showing any marked features of degeneration, it becomes difficult to distinguish them from what appear to be decidual cells. It has previously been stated that the decidua capsularis separating the outer pole of the ovum from the uterine cavity is deficient in some portions, so that the ovum, in fact, is not yet entirely separated from the uterine cavity. This impression is very strongly conveyed by one of the sections which for some reason was cut much thinner than the others (it is certainly less than 5 micra). Here one can see that the trophoblast cells have proliferated outwardly, have broken through the syncytium and have become fusiform. They reach to and form the very surface. In this section decidual cells appear to be absent from the strip which borders upon the cavity of the uterus. However, even in this strip some undoubtedly maternal cell elements can be recognized, namely, mononuclear lymphocytes and polynuclear leucocytes, and also, of course, infiltrating erythrocytes.

The trophoblast cells, whether they be present in a single layer, as towards the chorionic cavity, or whether they form larger or smaller irregular masses honeycombed by blood spaces, are covered by the syncytium. This consists of a layer of protoplasm in which cell boundaries are not demonstrable. In sections the protoplasmic layer is generally rather narrow, but there and there it is thickened, forming projections. These are seen particularly in the most peripheral

parts. However, the numerous and large syncytial buds present in somewhat older placentæ are not seen. The protoplasmic strip is deeply eosin-stained, but it shows a tinge as if it had also taken up some of the nuclear stain (hematoxylin). The very margin, however, is purely eosin-stained. The protoplasm is finely vacuolated. The distinctly eosin-stained outer strip consists of a cuticle and cilia. The cuticle can only be distinguished here and there in favorable places, but the cilia are almost everywhere easily seen. (Fig. 30.) The syncytium in our specimen fully conforms to the description given by Bonnet as found in an early human ovum, fixed like ours in Zenker's solution. This author says: "Towards the periphery the plasma of the syncytium is condensed into a stratum frequently staining very intensely with eosin, rubin or Heidenhain's iron hematoxylin. This outer strip, variable in thickness and distinctness, in fact appears like a cuticle. Its free surface in all sufficiently thin sections (3 to 5 micra) shows a very distinct and beautiful lining with cilia ("Bürstenbesatz"). This lining has also been described by Marchand[13] and Lenhossek. According to the latter, these cilia or rods are not motile (they were studied by Lenhossek in a fresh preparation), and in specimens stained with iron hematoxylin they exhibit basal granules in the cuticle."

Bonnet has not been able to see such basal granules, nor are they shown in our specimen, which, however, has not been stained with iron hematoxylin. The cilla in our sections present themselves as stiff, fairly slender, moderately high rods, which form very regularly parallel rows. They are deeply eosin-stained and of the same color as the cuticle. As stated, they can be seen without the least difficulty almost everywhere in the sections where syncytium is found. These rods are unlike the cilia of the glandular epithelium, which can still be well seen in the innermost portion of the decidua spongiosa towards the muscularis.

Since cells provided with a cuticle and cilia or rods generally have

[13]Marchand: Beobachtungen an jungen menschlichen Eiern. Anat. Hefte 1903, Vol. 21, p. 217.

a secretory function, it is possible that this apparatus of the syncytium has something to do with the secretion of the hypothetical enzyme of the trophoblast mentioned above. The nuclei of the syncytium are generally oval, elongated and rather densely provided with chromatin. I have, like Bonnet, not been able to find any cuticle or basement membrane between the syncytium and the cells of the trophoblast. In speaking about the outermost processes of the trophoblast, we described above how the trophoblast cells have invaded the narrow strip of outer polar tissue designated as decidua capsularis. Such processes of proliferating trophoblast elements, including both cells and syncytial masses, are found extending into the maternal tissues around the whole circumference of the ovum. This zone directly surrounding the ovum, forming the soil into which such invading processes extend, has been called the "Umlagerungszone," by Peters, a term which perhaps may be best translated by the "Border Zone."

The Border Zone.

The tissue which forms the bed of the ovum (Eilager) surrounding it more or less from all sides may be divided into three parts, the decidua basalis, the decidua capsularis, and the equatorial zone or decidua vera. The border zone at the base and around the equatorial planes of the ovum is characterized by the presence of large blood sinuses, originally formed from the capillaries and small veins of the uterine mucosa. Some of these blood lacunæ have retained the outlines of vessels; others have become irregular spaces which have no resemblance to ordinary vessels. The largest of these blood sinuses in our specimen are found in the decidua basalis, near the inner pole of the ovum. But very large thin-walled blood spaces surround the ovum on all sides. They proceed from the basal decidua into the equatorial border zone, bend around the upper or outer hemisphere of the ovum, and very nearly reach the thin polar cap of tissue, the decidua capsularis. No real blood spaces are, however, found in this thin cap of tissue, but only free blood corpuscles mixed with cells either of maternal origin (decidual cells) or derived from the ectoblast shell. Very much enlarged capil-

laries and veins can also be seen at a distance from the border zone in the spongiosa and compacta of the decidua extending nearly to the surface. In the border zone of our specimen near the inner pole of the ovum there is a large irregular blood sinus about 1 to 1.5 mm. in diameter, which has been completely opened by the trophoblast and is in free communication with the blood-filled cavities of the trophoblast. On its inner side (towards the muscularis) this sinus is still lined by vascular endothelium. The wall towards the trophoblast has been extensively destroyed, so that the large blood space is lined on one side by much stretched but still fairly well preserved endothelium, and on the other by the irregular, ragged trophoblast. The border zone at the base of the ovum also shows many large cystic gland spaces filled with blood. Most of them can be recognized as derived from glands by remnants of dropped-off, degenerating epithelium, while the densely filled blood spaces, on the other hand, can be identified by their endothelial lining and the remnants or dropped-off floating portions of the same. But there are some cystic blood-filled spaces which cannot be readily identified as being originally glands or blood vessels. Nowhere in the border zone does the glandular epithelium or vascular endothelium show any proliferative processes; degenerative processes only are seen.

At the base of the ovum in the layer of the border zone nearest to the trophoblast a small, rather delicate strip of canalized fibrin is found. This strip consists of a network of fibrin in which are embedded decidual cells, red blood corpuscles and polynuclear leucocytes. There is a more powerful mass of fibrin found in the equatorial tract of the border zone. This mass (Fig. 28) contains rather coarse threads of fibrin and great numbers of red blood corpuscles. It appears that this mass has formed in and is filling out the lumen of an enlarged blood space.

We have described how the trophoblast in approaching the capsularis sends out bands and filaments of cells which become fused with and are lost in the thin capsular strip. The same process can be seen around the whole periphery of the trophoblast. Particularly around the equatorial plane the syncytium can be seen to

take part extensively in this process of fusion. It appears that the syncytial masses after penetrating into the border zone have a tendency to break up into cells. Individual detached pieces of syncytium can often be recognized as such by the deep eosin stain of the protoplasm and by the rods lining the external surface. However, other portions of what appears to be detached syncytium in the border zone have lost their characteristics. It is in the border zone and only in it, in our sections, that marked degenerative processes are seen, and these appear to be mostly confined to cells and tissues of maternal origin. In the border zone are also seen larger protoplasmic masses containing several generally pyknotic nuclei. We think that these are detached degenerating portions of the trophoblast; whether they are portions of the "Grundschicht" or the "Deckschicht," we are not able to decide. We believe that the large, irregularly round cells with vesicular nuclei are derived from the syncytium, since their protoplasm stains very deeply with eosin.

Peters describes numerous and profound changes in the trophoblast. These changes Marchand has already considered to be pathologic and probably due to the fact that the woman from whom this ovum came died from the effects of a rapidly fatal dose of caustic potash. However, Marchand also believes that the extensive presence of blood in the trophoblast of Peters' ovum is abnormal. In this respect he is mistaken, since our own ovum shows the identical condition.

It appears from Peters' monograph (p. 50 and p. 51) that he found in plasmodial masses and in the syncytium more or less normal and also much changed fragmentary red and white blood corpuscles. He describes this quite fully, and he draws from this observation the remarkable conclusion that the maternal blood with its own corpuscular elements contributes to the formation of the syncytium. Neither Bonnet nor Jung have found anything like this.

Not a trace of any such process or condition can be found in our own ovum. Nowhere were red blood corpuscles *in toto* or in fragments seen included in the trophoblast elements. It is quite probable that in Peters' case the profound intoxication with fixed alkali had so changed the red blood corpuscles, had, as we would express it to-day,

so "opsonized" them, that they became liable to be taken up by the phagocytic action of other cells. We know that red blood corpuscles, in consequence of the action of certain bacterial toxins, are so changed that this phagocytosis occurs. We want to mention in this respect what occurs in typhoid fever when numerous red blood corpuscles are taken up by the pulp endothelial cells of the spleen. That the elements of the trophoblast of the human ovum under absolutely normal conditions do exhibit towards the maternal blood corpuscles truly phagocytic properties is certainly not proven. Our own specimen, which we consider perfectly normal, shows absolutely nothing which would justify such a conclusion.

We see in the border zone, particularly around the equatorial planes, cells which show already almost all of the characteristics of the later decidual cells. These cells exhibit a large vesicular nucleus, with rather scanty, finely granular chromatin and obtusely fusiform or irregularly polygonal protoplasm. Between them are found small mononuclear cells and polynuclear leucocytes. This is the picture seen in places a little distant from the ovum. Towards the very interior of the "Umlagerungszone," the outlines of almost completely destroyed gland spaces with dropped-off degenerating epithelia, red blood corpuscles and fibrin are seen abundantly. Here also are seen these cells or cell remnants with pyknotic, irregular, shrunken nuclei, and a very dense, deeply eosin-staining protoplasm. The latter we consider as detached portions of the ectodermal trophoblast. Protoplasmic masses containing several nuclei, which we also take to come from the trophoblast, have already been mentioned. We have, however, not found larger masses of fused degenerating cells, either of maternal or fetal origin, hence we have no occasion in our case to make use of either one of the terms, symplasma maternum or fœtale.

The Decidua.

The character of the decidua as it exists in our ovum is well illustrated in Figs. 24 and 26. In the "Umlagerungszone" and right next to its periphery the degenerative processes and the hemorrhages predominate. At some distance, however, we find a decidua well

differentiated into a compacta and a spongiosa. How far distant from the ovum these characters have already been established we cannot say, since only the ovum and its next neighborhood have been sectioned and examined.

Jung, who, in his ovum, likwise found a distinct differentiation into a compacta and spongiosa quoted Hitchman and Adler's observation of the uterine mucosa before and during menstruation. These authors, even in the absence of gestation, found in the premenstrual period a temporary formation of a compacta and spongiosa. We have ourselves studied the menstrual changes on several specimens obtained per operationem and at once properly fixed, and we have previously (The Pathology of Tubal Pregnancy), summarily described them as follows: "The capillaries of the inter-tubular connective tissue are enormously dilated and densely filled with red blood corpuscles. Many of the latter are also found free, outside of the capillaries, between the connective tissue cells of the interglandular spaces. The whole mucosa is edematous and the connective tissue cells are pushed apart by the edematous and hemorrhagic infiltration. Some of the connective tissue cells, which in the intermenstrual periods are normally all of the type of small lymphoid cells, are enlarged, oval or fusiform. They assume a type found in certain forms of endometritis interstitialis and approach the type of decidual cells. It may really be said that the uterine mucosa in menstruation shows to a very slight extent the beginning stage of a decidua. Most of the surface epithelia of the mucosa are preserved; only a few are missing here and there. Changes similar to those described as characteristic for the menstruating uterine mucosa I have twice observed in the tubal mucosa during menstruation."

The decidua spongiosa is considerably thicker than the compacta (Fig. 26). In the former we find irregular gland spaces, much crowded, and separated from each other by small bridges of tissue. The proliferative energy of the glandular epithelium is shown by the fact that it is found inside of the gland space proper, in the shape of projecting papillary ridges, septa and digit-like processes. All of these masses of epithelia are lined up on a slender basis of con-

nective tissue. The interglandular connective is composed of distinctly fusiform cells with elongated deeply-staining nuclei. In some places the gland spaces project considerably into the muscularis. Towards the latter there are occasionally found between the glands groups of small round lymphoid cells of the type of the cells seen in the interstitial tissue of the non-pregnant uterine mucosa. In the spongiosa at some distance from the ovum there are no very markedly enlarged veins or capillaries seen. But they appear towards the zone where the spongiosa goes over into the compacta. In the latter we see the more or less straight or decidedly tortuous ducts of the glands leading to the surface. Here also the epithelial lining projects in ridges and bands. Between the gland ducts solid septa are present. In the direct neighborhood of the ovum these septa contain enormously enlarged blood spaces (capillaries or veins); at some distance they show the tortuous cork-screw arteries, characteristic for the decidua compacta. The edema existing in the decidua is demonstrated in the spongiosa even at a distance from the ovum by a coagulated granular material found in the gland spaces. In the compacta the edema can be recognized in the solid septa. The cells here are distinctly pushed apart, they are embedded in a granular coagulated material. In the compacta are found cells which exhibit already quite well the characters of decidual cells. They possess a large oval nucleus, with distinct nuclear membrane, scanty chromatin and one or two nucleoli. They have a large protoplasmic body, oval, fusiform or irregularly polygonal in outlines, finely granular and well eosin-staining. Among these larger cells, small mononuclears of the type of lymphocytes or young connective tissue cells are quite numerous.

The epithelium lining the glands is best preserved in the deeper layers of the more distant compacta. It is high columnar with nuclei near the basement membrane. The cilia in favorable locations are still preserved. The profound degenerative processes seen in the glandular and surface epithelium towards the ovum have several times been referred to. No karyokinetic figures were seen anywhere in the glandular epithelium. However, a few cells with two small densely stained round nuclei were found. I am, of course, well

aware that the specimen described is one obtained post-mortem. However, the rapid cooling on ice and the subsequent proper fixation had well preserved it. This, among other things, is attested to by the fact that mitotic figures were found in the embryonic shield and in the trophoblast.

None of the decidual cells, either large or small, show any karyokinetic figures.

Jung, who describes mitoses in the decidua of his specimen on page 29 of his monograph, says: "Marchand and Bonnet are the only authors who have heretofore described mitoses in the decidua. The present author in a paper published in July, 1898 (Superfetation in the Human Race), described among others an aborted specimen of superfetation. Both embryos were contained in the intact fetal membranes. One embryo was 8.6 cm. long; the other 16.5 mm. The superfetation was proven by a microscopic examination of the embryos and of their placentæ, showing in both the different stages of development. In the description of the placentation of the small ovum the following passage occurs: "Large apparent islands of decidual cells. In some places the decidual cells present a very beautiful feature which I had not observed before in the deciduæ of many other placentæ examined. As is well-known, the large vesicular, round or oval nuclei of decidual cells are, as a rule, quite poor in chromatin, which is distributed in the form of small granules in a peripheral manner near the nuclear membrane. In some places of the young placenta of this case the nuclei are rich in chromatin, consisting of coarse granules and masses, arranged in an aster-shaped manner, occasionally a disaster is fairly well recognizable. We have to deal with karyokinetic figures. That this is really the case, and that we are not dealing with a degenerative process of the nuclear chromatin is proven by the fact that the aster stage can be well recognized, and, secondly, by the observation that leucocytes are absent at the place where the karyokinetic figures in decidual cells are found. I have previously pointed out that where a degenerative process—coagulation necrosis—is going on in the decidua we find great numbers of polynuclear leucocytes, many of which show nuclear fragmentation."

The paper in which this statement was made shows a photomicrograph of these cells with karyokinetic figures. In re-examining the photograph now, I find that these cells are comparatively small cells. This would agree with the description of dividing decidual cell as given by Marchand.

The muscularis uteri, as far as it is included in the sections, appears to consist of muscle fibers already somewhat hypertrophied. Measurements by micrometer were not made.

Summary.

From the object described in the preceding pages, an ovum almost identical in size and type with the Peters' ovum, one may draw the conclusion that these two ova represent the *normal type* of the earliest known stage of human placentation. The mode of placentation and implantation in both are practically alike. Certain retrograde changes described in the cells and in the syncytium of the Peters ovum must be looked upon as histopathologic changes, probably due to the poisoning of the mother. This conclusion is justified not only from our own case, but alike from the studies of Marchand, Bonnet, Jung and others.

If we now attempt a summary description of the ovum and its manner of implantation and placentation, we have to make a few hypothetical statements, but, on the whole, we can give a resumé based upon actual facts, as they are clearly represented by the specimen studied.

A human ovum at the earliest stage of normal development hitherto known, a stage which perhaps represents one to two weeks after fertilization, is found interstitially embedded in the decidua. It is incompletely separated from the cavity of the uterus, because it is very superficially embedded and its outer pole is protected by a thin, incomplete decidua capsularis or a coagulum only. The ovum, after having been fertilized, has been transported to or near to the place where it is found embedded. By the aid of an ectodermal trophoblast shell, which probably secretes an enzyme destructive to the epithelial cells and the connective tissue of the uterine mucosa, which is then in a premenstrual or menstrual

condition of congestion and glandular hypertrophy, the ovum produces necrobiosis or coagulation necrosis in the structures of the mucosa. At the same time the trophoblast exhibiting great proliferative energy now penetrates through the necrotic tissue, into the connective tissue of the mucosa. Here the phenomena of edema and of a violent hemorrhagic inflammation are now established. Veins and capillaries become enormously dilated, the blood current becomes sluggish, edematous infiltration becomes pronounced. The ovum automatically orients itself, so that the embryo comes to be situated towards the muscularis. The proliferating trophoblast with its syncytium, provided with cilia or rods, at this time begins to break into and to open up dilated maternal capillaries. Maternal blood now makes its way into the trophoblast, whether it here finds preformed cavities or whether it forms these cavities in a loose protoplasm in consequence of hydrostatic pressure, we do not know. While the trophoblast opens up the enlarged maternal blood lacunæ, the hypertrophy of the mucosa, as a whole, goes on. The gland spaces become large and cystic, their ducts lead to the surface in a tortuous manner. A separation into a spongiosa and compacta becomes early established, and in the latter some cells early assume a marked decidual character.

The ovum is now interstitially embedded in the mucosa and surrounded by a border zone which is composed of an admixture of still attached or detached trophoblast elements, degenerating fixed maternal cells, both connective tissue decidual cells and glandular epithelia. In this border zone ("Umlagerungszone") are also contained enormously enlarged maternal blood vessels, cystic blood-filled gland spaces and free blood. The ovum almost floats as it were in a lake of blood partly contained in the trophoblast cavities, partly in the cystic maternal gland spaces, partly freely infiltrating more or less all of the tissue in the direct neighborhood of the growing germ.

When the preliminary paper was read in Boston, Mass., August 20, 1907, before the Section on Embryology of the Seventh International Zoological Congress, Professor A. A. W. Hubrecht, the Chairman of the Section, in the discussion of the paper, called

attention to a misconception of the writer as to certain relations of the amnion and yolk sac. It is needless to say that the conception of Professor Hubrecht proved to be the correct one. This necessitated on the part of the author a careful re-examination of the sections and some changes in the manuscript for the Transactions of the Congress.

Professor C. S. Minot then kindly placed at my disposal his laboratory and his library to enable me to make the necessary re-examinations and changes. To him, as also to Professor F. T. Lewis, I am under obligations for the great kindness shown on this occasion, and in the final revision of the manuscript.

The literature on human placentation is extensively given in Keibel's Normentafeln; in Peters' monograph; in Webster, "Human Placentation"; in Pfannenstiel's article in Winkel's "Handbuch der Geburtshülfe." The most recent contributions are quoted in Jung's monograph and Bryce and Teacher (l. c.). Stahl, in his article on "Die Embryonalhüllen der Säuger und die Placenta," in Vol. I, Part 2, Hertwig's "Handbuch der Entwickelungslehre der Wirbelthiere," gives the literature on placentation among mammals in general.

Fig. 23.—Colored plate drawn by Miss Katharina Hill, artist of the Department of Anatomy of the University of Chicago, from an enlargement of Fig. 25, photomicrograph from section 153. The embryo shown in this plate is drawn after section No. 155, because in it the yolk sac, embryonic shield proper, allantois and allantoic duct are best seen.

All. S.—Allantoic stalk.
C. A.—Cavity of amnion.
Can. F.—Fine fibrin threads and leucocytes (early canalized fibrin).
Cho. M.—Chorion mesoderm.
D. C.—Decidua capsularis.
Exoc.—Exocœlom.
Gl.—Gland space (epithelium partly preserved).
Mat. Bl. S.—Large cystic gland spaces filled with blood.
S. V.—Yolk sac.
Syn.—Syncytium.
Tr.—Trophoblast.

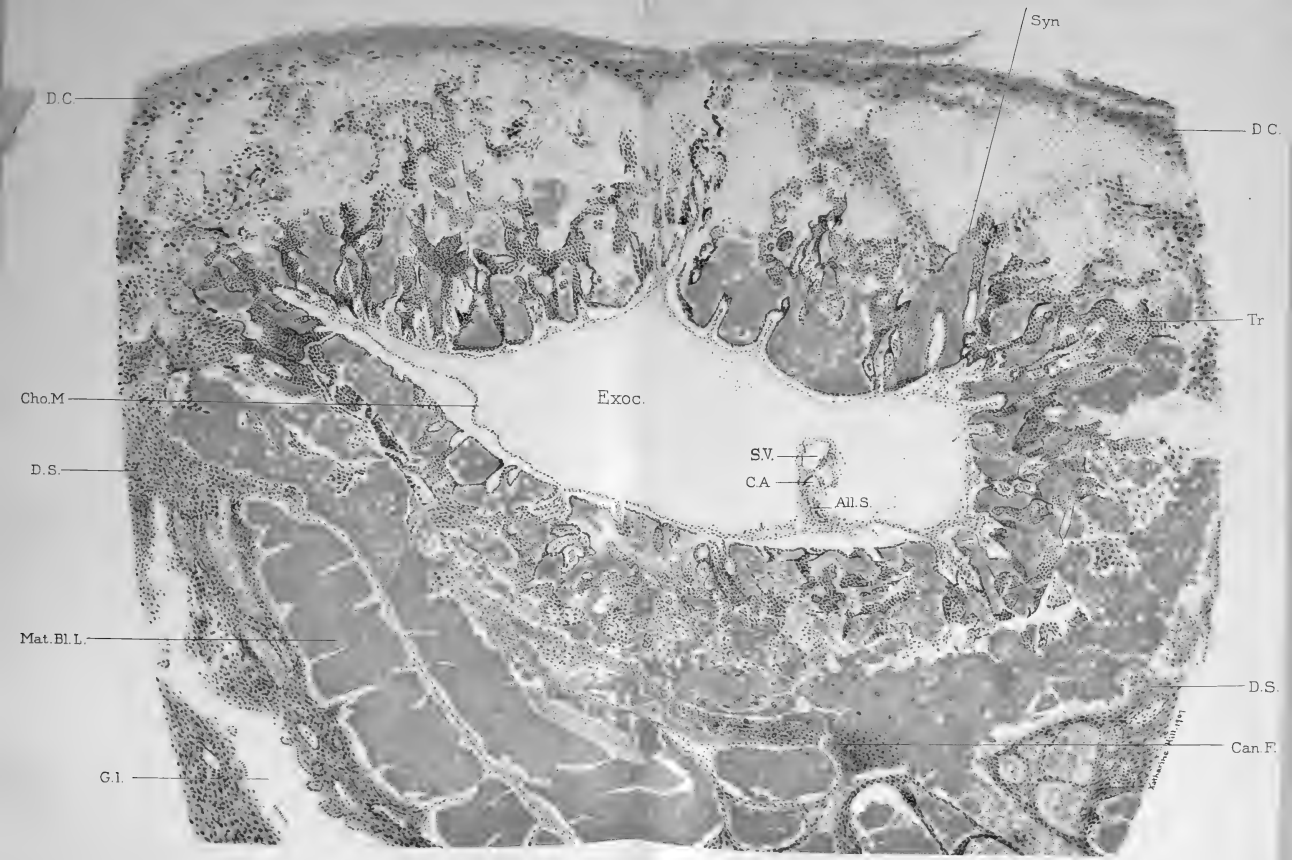

Fig. 23.

FIGS. 1 to 22.—Drawings from twenty-two serial sections of the embryo (Sections Nos. 164-143 of the entire ovum).

C. A.—Cavity of amnion.
C. M.—Chorion mesoderm.
D. A.—Duct of allantois.
Em. S.—Embryonic shield.
Exo.—Exocœlom.
Mes.—Mesoderm.
P. A.—Allantoic stalk.
S. V.—Yolk sac.
V.—Anlage of blood vessels.

FIG. 1.

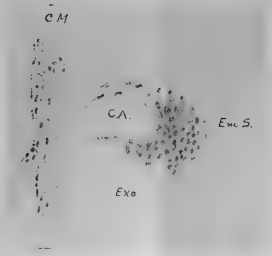

FIG. 2.

FIG. 3.

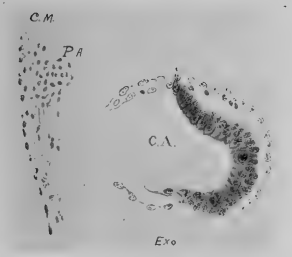

FIG. 4.

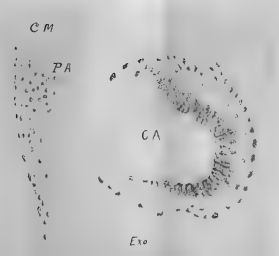

FIG. 5

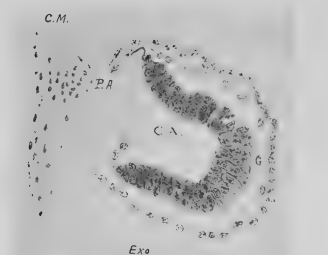

FIG. 6.

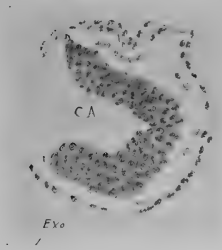

FIG. 7.

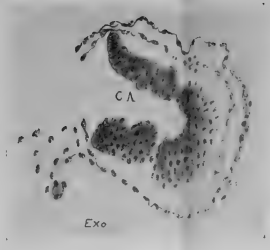

FIG. 8

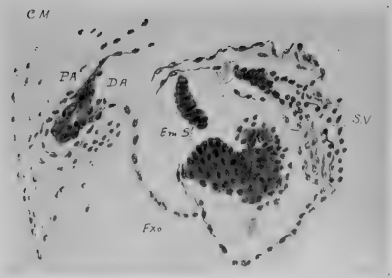

FIG 9.

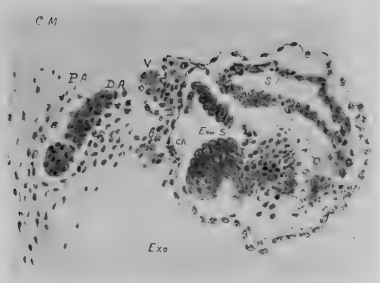

FIG. 10

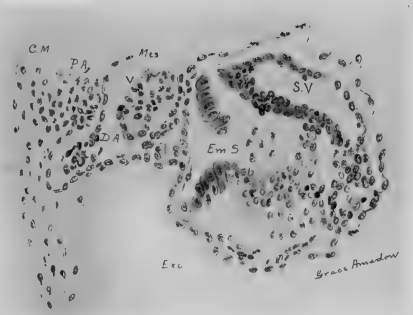

FIG. 11

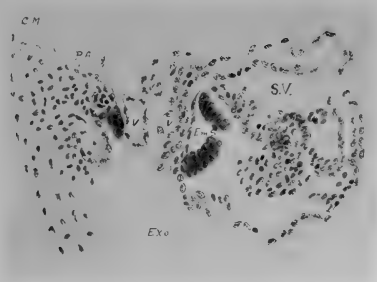

FIG. 12.

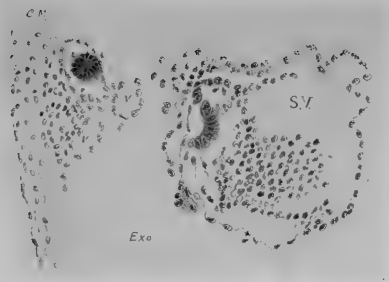

FIG. 13.

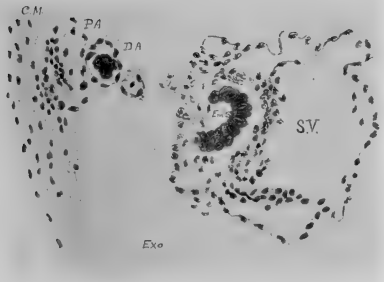

FIG. 14.

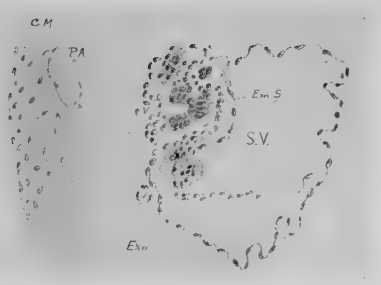

FIG. 15

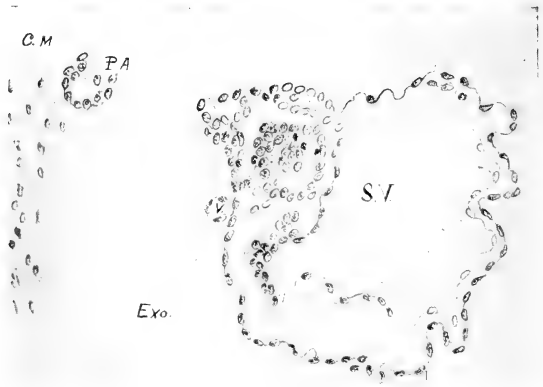

FIG. 16.

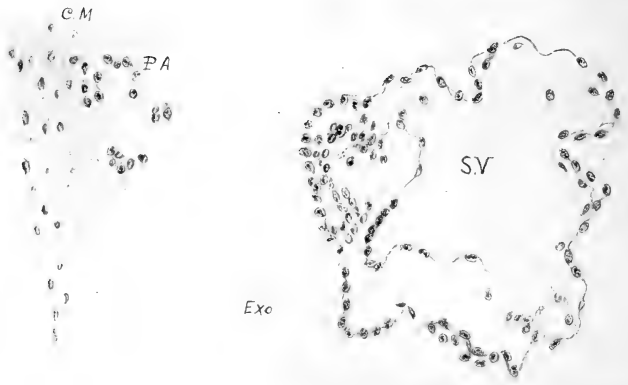

FIG. 18.

FIG. 17.]

FIG. 19.

FIG. 20.

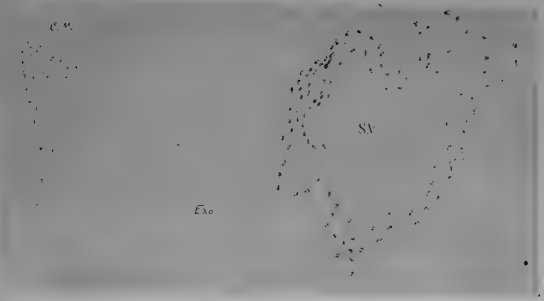

FIG. 21.

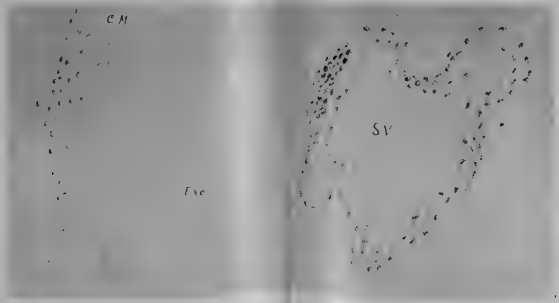

FIG. 22.

Fig. 24.—Photomicrograph from section No. 125. Zeiss, planar, × 15 diam. (approximately). This photomicrograph shows the relation of the ovum to the parts where it has implanted itself. It is separated from the surface by a very thin capsularis and in the middle of the upper margin of the photograph may be seen a place where the chorion mesoderm is adherent in the form of a teat-like projection to the capsularis, which is defective at this place. The open ring seen a little to the right of the center of the chorion cavity (exocœlom) is a transverse section of the yolk sac which hangs down beyond the end of the embryonic shield. The chorionic mesoderm is surrounded by the trophoblast. The ovum rides as it were on a wedge-shaped group of enormously, cystically, enlarged gland spaces, and lacunæ, filled densely with maternal blood. To the right and to the left are seen the enlarged glands of the uterine mucosa. Differentiation into a decidua spongiosa and compacta is not well seen in this section. At the left lower corner there is a strip of the muscularis.

Fig. 25.—Photomicrograph from section No. 153. Zeiss, planar, × 29 diam. Showing the chorionic cavity filled with a coagulated granular material. To the right is seen the embryo anchored by the allantoid stalk to the chorion mesoderm. In the upper corner a thin strip of degenerated decidua capsularis.

Fig. 26.—Photomicrograph from a section outside of the implanted ovum (from first series of sections, unnumbered), Zeiss, planar, × 15 diam. Showing large cystic gland spaces partly filled with blood. To the left a differentiation into a decidua compacta and spongiosa is well marked. The lower margin of the photomicrograph shows the muscularis uteri.

Fig. 27.—Photomicrograph from section No. 83. Zeiss Apochromat. 8 mm., proj. occ. No. 4, × 210 diam. Gland spaces near the ovum filled with spherical hyaline masses.

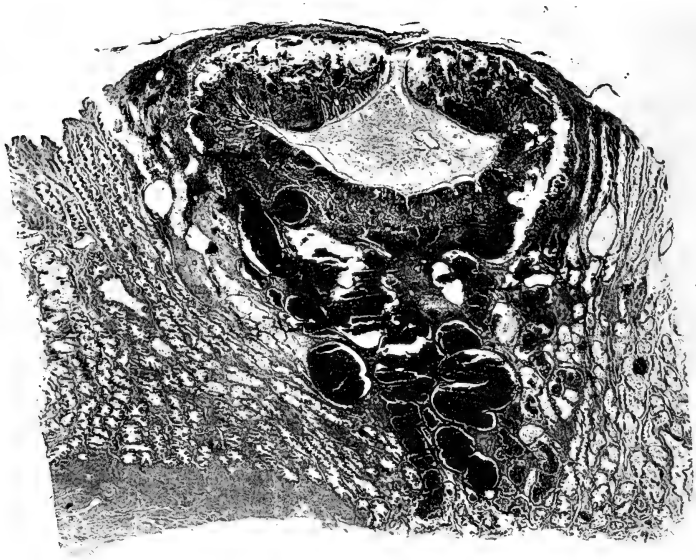

FIG. 24.

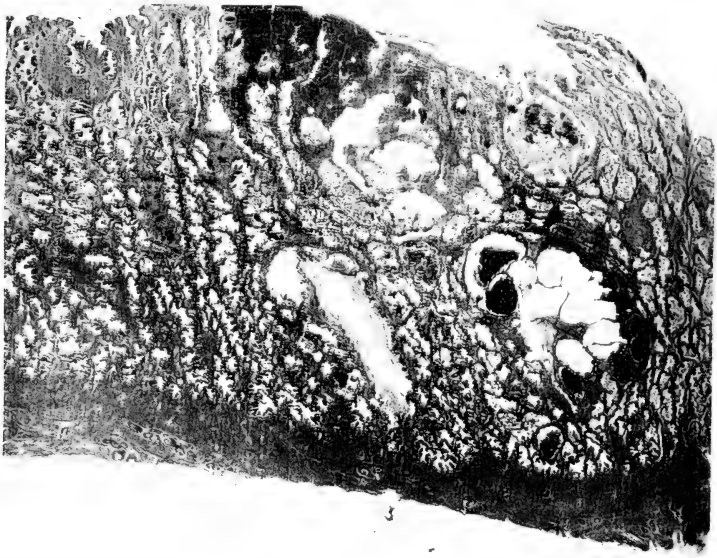

FIG. 26.

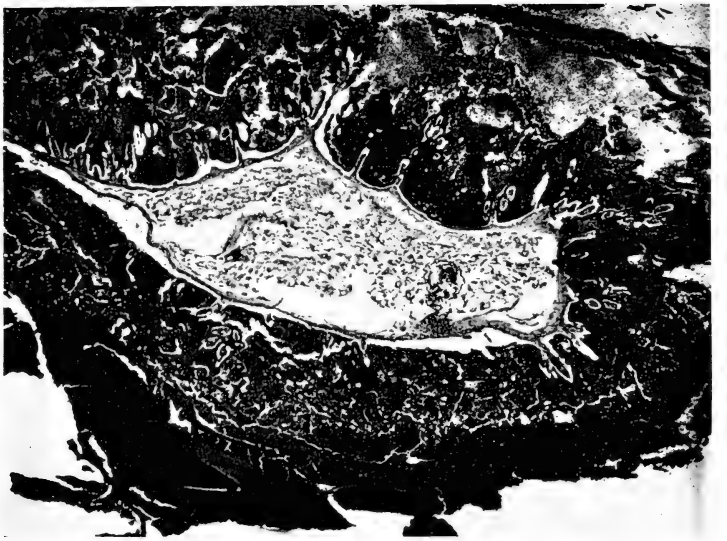

FIG. 25.

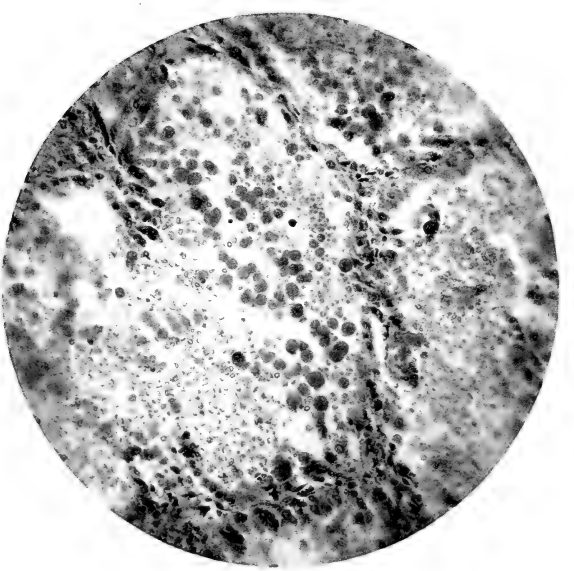

FIG. 27.

FIG. 28.—Photomicrograph from section No. 101. Zeiss Apochromat. 16 mm., proj. occ., No. 4, × 100 diam. A coagulated crescentic mass containing a coarse network of parallel fibrin threads stretches across the field.

FIG. 29.—Photomicrograph from section No. 210. Zeiss Apochromat. hom. oil. imm., 2 mm., proj. occ., No. 4, × 1000 diam. A group of trophoblast cells, in the center one with karyokinetic figure.

FIG. 30.—Photomicrograph from section No. 186. Zeiss Apochromat., hom. oil. imm., 2 mm., proj. occ., No. 4, × 1000 diam. Cuticle and rods of the syncytium shown on the free order running like a polar axis from A to B. (The photomicrographs were prepared under the author's direction by Mr. Frank T. Harmon.)

FIG. 28.

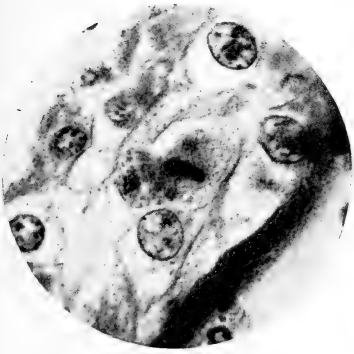

FIG. 29.

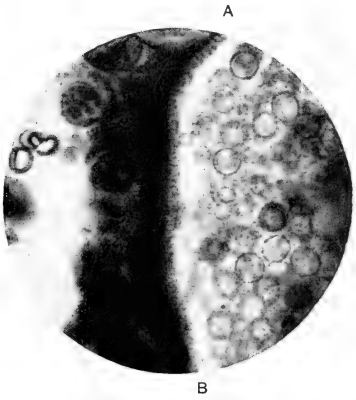

FIG. 30.

I. ON THE OCCURRENCE OF FAT IN THE EPITHELIUM, CARTILAGE, AND MUSCLE FIBERS OF THE OX.

II. ON THE HISTOGENESIS OF THE ADIPOSE TISSUE OF THE OX.

BY

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From the Anatomical Laboratory.

I. On the Occurrence of Fat in the Epithelium, Cartilage, and Muscle Fibers of the Ox.

An extensive study of the process of fattening in the ox is being made by the Agricultural Experiment Station at the University of Missouri. This first paper deals with the histogenesis of the adipose tissue and the occurrence of fat in the other tissues. Other papers will follow later. The department of anatomy is co-operating with the experiment station on the histological part of this investigation. The entire experiment is under the direction of Dean H. J. Waters, to whom I am indebted for many special favors.

It is to be borne in mind that fat occurs not only in adipose tissue cells but also in many other tissues. In the adult small fat droplets are present in cartilage cells and in several kinds of epithelial cells. In the young fœtus small fat droplets are found inside some of the muscle fibers as well as in the cartilage and epithelium. The fat in these tissues bears no special relation to the fat in the adipose tissue cells. No true adipose tissue cells are found until about the 20 cm. stage or later, but long before this stage fat droplets are found in abundance in cartilage cells, muscle fibers, and hepatic cells. During starvation the fat cells are emptied, but the quantity of fat in the other tissues is unchanged. In its development the adipose tissue

is apparently not influenced by the fat contained in the other tissues.

Material and Methods. The material used was largely calf fœtuses ranging in length from 3 cm. to 105 cm.[1]—the latter size being about full term. No data have been obtained as to the relation between the age of the fœtus and its length. All descriptions apply to the ox unless otherwise stated. Considerable material from steers in different stages of fattening has been made use of.

For the study of the fat in the epithelial and muscular tissues the material was usually fixed in 20 per cent formalin.[2] This fixative makes the tissue very firm so that free-hand sections may be made sufficiently thin for some purposes. Usually, however, frozen sections were made with a Bardeen freezing microtome. The sections were stained in a saturated solution of Scarlet red or Sudan in 70 per cent alcohol. In using Scarlet red it was found advantageous to adopt Traina's suggestion (36, pg. 10) of keeping the solution with excess of Scarlet red on the top of an ordinary paraffin oven (this gives about the temperature recommended) for about two weeks before using. Precipitates are formed less readily from a solution prepared in this way. The stain was filtered each time before it was used. The sections were covered while staining as a slight evaporation of the alcohol is sufficient to cause the formation of a precipitate. After having been stained, the sections were washed in 70 per cent alcohol and then put in water. If they are washed in water directly, a precipitate may be formed by dilution of the alcohol. Of course, the presence of a precipitate makes the detection of fine fat droplets difficult or impossible. By taking the above-mentioned precautions I was able to exclude precipitates as a source of error.

There are only a few minor differences in the staining powers of Scarlet red and Sudan. When only large droplets of fat are to be stained, Sudan has the advantage in that it acts more rapidly; but

[1]The crown-rump measurement is meant.

[2]Frozen sections were also made occasionally from material fixed in Zenker's fluid, Gilson's fluid, 80 per cent. alcohol, and 10 per cent. formalin.

in studying fine droplets Scarlet red is somewhat better since it leaves the protoplasm colorless. Sudan sometimes stains protoplasm to a considerable degree. Sudan as well as Scarlet red will stain fat droplets of any size an intense red when allowed to act an hour or more.

Distribution of fat in the tissues of the fœtus.

In the calf there are no rounded fat cells present until about the 20 cm. stage or later; but fat droplets are found in the various tissues long before this period.

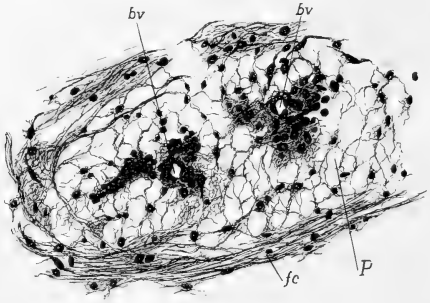
Keinath (18) studied some of the musculature and cartilage of calf fœtuses. In a 3.5 cm. fœtus, a longitudinal section through the musculature of the anterior extremity shows fine fat droplets in masses or in rows between the fibrillæ. Individual fine fat droplets occur in the cartilage cells. In a 4.5 cm. fœtus, fat droplets are also present inside the muscle fibers and in the cartilage cells. A 9 cm. and a 12.5 cm. fœtus each showed fat in the fibers of the pectoral muscles, but none was found in the heart muscle. Two fœtuses, 20 cm. and 40 cm. long respectively, contained no fat in the pectoral muscles. The same observer found no fat in the musculature of a 1.5 cm. pig.

My own observations are as follows: 3 cm. fœtus (two specimens). No fat was found in muscle, liver, skin, or cartilage. This finding does not necessarily contradict the observations of Keinath—the fat content of tissues both fœtal and adult is subject to considerable variations.

4.7 cm. fœtus. The liver shows fat droplets in the cells near the larger veins. No fat was found in the cartilage of the vertebræ or that of the femur. The musculature of the thigh and back contained no fat.

7 cm. fœtus. The hepatic cells are loaded with large and small fat droplets (Fig. 1). The fat is more abundant near the veins. The genital gland contains a large amount of fat in the interstitial cells. The muscles of the neck, thigh, and back were studied for the presence of fat. Many muscle fibers in each situation contain fine fat droplets in between the fibrillæ. There is usually one fat

droplet seen in a section of a muscle fiber. The sections were 30 microns to 60 microns thick. In a section through the neck a group of muscle fibers, in paired muscles on either side of the cartilaginous neural arch, is loaded with fat droplets. The group is symmetrically situated, occupying exactly the same part of each muscle. This may mean that the presence of fat is closely associated with the growth of the fibers, as these two zones of muscle must be in about the same developmental stage. A few cells of the cricoid cartilage contain one or more fine droplets of fat. The cartilage cells of the centra of the vertebræ contain in some sections no fat at all, in other sec-



Text Figure 1. Preadipose tissue around two small blood vessels. From the subcutaneous tissue of the brisket of a 24 cm. fœtus. *bv*, blood vessel; *fc*, ordinary fibrillar connective tissue; *P*, preadipose tissue. Fixation, Gilson's fluid. Iron-hæm. stain. × 200.

tions nearly every cell contains one or more fat droplets. Fat is also present in the cartilage cells of the femur. No fat is present in the kidney or in the renal preadipose tissue.

7 cm. fœtus (Zenker fixation). Fine fat droplets are present in the cartilage of the femur. No fat was found in the muscles of the thigh. No other tissues were examined.

10.1 cm. fœtus. A great many hepatic cells contain one or more fat droplets. A considerable number of the droplets are annular or crescentric in shape. The cells around the large veins are more densely packed with fat droplets than those elsewhere. No fat is

present in the kidney. The interstitial cells of the testis are loaded with small fat droplets. Most of the fibers of the psoas muscles and the lumbar portion of the erector spinæ contain a few fine droplets. No fat was found in the flexor muscles of the thigh. The cartilage cells of the centra and neural arches contain fat droplets (Fig. 2, Pl. I). On the edge of the ossification center the swollen cells contain a large amount of fat. This may be the normal process of degeneration for these cells.

14 cm. fœtus. The cells of the convoluted tubules of the deepest part of the renal cortex are full of fat droplets. A few fine fat droplets are present in the branched cells of the renal preadipose tissue. The cartilage of the innominate bone contains fat—one or two droplets to a cell. A few fine fat droplets are present in the fibers of the psoas muscles.

14 cm. fœtus. A few fine droplets are present in the fibers of the adductor muscles of the thigh. Fine droplets are found in the cartilage cells of the vertebræ. No fat is found in the trachea, œsophagus, or muscles of the neck. The liver contains a large amount of fat. There is a large amount of fat in the ovary—nearly all of it in the cortex. The spleen contains no fat. In the inguinal region some exceedingly fine fat droplets are present in the branched cells of the preadipose tissue adjacent to a large blood vessel.

17 cm. fœtus. No fat is found in the kidney. Some fine fat droplets are seen in the cells of the renal preadipose tissue.[3] Fat droplets are found inside the fibers of the psoas muscles (Figs. 3 and 4, Plate I). The cells of the innominate cartilage contain fat droplets (Fig. 5, Plate I).

20 cm. fœtus. A few fine fat droplets are present in the renal epithelium—mainly in that of the convoluted tubules. A few fine droplets are also found in the cells of the renal preadipose tissue. The cartilage cells of the vertebræ nearly all contain fat droplets. The muscle fibers of the erector spinæ and psoas muscles nearly all contain one or more fat droplets in each section.

[3]These droplets can be seen clearly only with the oil-immersion lens. Their identification as fat droplets in this specimen is not absolutely certain.

24.7 cm. fœtus. In the renal epithelium of the columns of Bertini fat droplets are present, elsewhere in the kidney they are absent. The renal preadipose tissue contains no groups of fat cells at this stage—it is all in the branched-cell condition (Fig. 6, Plate II). In certain lobules nearly every cell contains fine fat droplets (Fig. 7, Plate II). The fat is more abundant near the nucleus, but occurs far out in the processes of the cells. These cells have no special relation to the blood vessels such as is found when groups of true (rounded) fat cells are forming. Such spindle-shaped cells, containing fat droplets, are common on the edge of a growing fat lobule; but it seems striking to find all the cells of the lobule containing fat before any true fat cells are formed. The cells of the cartilage of the vertebræ contain fat. The erector spinæ muscle contains considerable fat in the fibers next to the laminæ of the vertebræ, but very little is found elsewhere. No fat is found in the fibers of the psoas muscles.

After about the 22 cm. stage, true fat cells begin to appear first in the preadipose tissue of the brisket[1] and renal region and later in other situations. Only scattered observations have been made as to the fat content of the tissues after this period.

28 cm. fœtus. The liver contains a considerable amount of fat. Exceedingly fine fat droplets are found in the muscle fibers.

37 cm. fœtus. The liver, kidney, and psoas muscles were examined. No fat was found.

40 cm. fœtus. A large amount of fat was found in the liver. No fat was found in the kidney. The fibers of the psoas muscles contain no fat, but a clump of fat cells was found near a blood vessel in the intermuscular connective tissue.

43 cm. fœtus. The hepatic cells contain a considerable amount of fat, mostly in the form of fine droplets. Coarse irregular droplets are scattered through the cells of the renal tubules.

45 cm. fœtus. The liver shows a fat content similar to the 43 cm. specimen. There is a large amount of fat in both the cortical

[1]The part of the brisket between the sternum and the pectoral muscles in the median plane develops into a large mass of fat. It is this part of the brisket that is referred to when that term is used.

and the medullary renal tubules. No fat was found in the psoas muscles.

51 cm. fœtus. The liver contains a large amount of fat. Some of the droplets seem to be in the endothelial cells, but this point could not be determined satisfactorily. No fat was found in the fibers of psoas muscles.

85 cm. fœtus. The liver contains many fine fat droplets. Most of the droplets seem to be between the hepatic cells as in the 51 cm. specimen.

105 cm. fœtus. The muscle fibers of the psoas muscle contain no fat, but fat cells are present around a few large blood vessels in the coarse intermuscular septa.

The above observations, though not as complete as might be desired, show clearly that fat occurs in the protoplasm of many of the fœtal tissues both before and after the formation of true adipose tissue. It is present in some tissues at least before any preadipose tissue has been formed. It may be present in the liver at any fœtal stage. The greatest fluctuations in quantity are found in the liver. Some of the observed variations in the quantity of fat present are surely due to variations in the distribution of fat in the organ. One cannot examine every part of a large organ, and it is very improbable that the fat is always uniformly distributed.

The fat seems to disappear from the muscle fibers about the time of the first formation of true adipose tissue. But, notwithstanding this apparent connection, it seems to me that the fat in the muscle fibers is not merely stored fat. As pointed out in the 7 cm. fœtus described above, the presence of the fat seems to be intimately connected with the growth of the muscle fibers. It is probably required in the metabolism of the fiber in the early stages of its development.

The occurrence of fat in the tissues of cattle nine months or more old.

Fifteen animals have been slaughtered thus far in our experiment. In these the liver, kidneys, salivary glands, pancreas, and thymus have been carefully examined for the presence of fat. Por-

tions of muscle from six or eight different parts of the body have also been examined for the presence of fat.

The muscle samples have been examined in this laboratory by Mr. H. H. Bullard, who is making a special study of the musculature of the ox. His observations on the fat content of the muscle fibers are given below.

Three of the animals slaughtered were very fat. One of these, a well-known prize-winner in the show-ring, was three years old, and had been excessively fat for over a year. The subcutaneous fat was in some places over 8 cm. thick. None of these animals had any fat in the epithelium of the glands examined or inside the muscle fibers. Fat droplets were present in the cells of the articular cartilage.

The second group of animals, three in number, were moderately fat. In two of these fat droplets were found in the hepatic cells. The cartilage cells of all three contained fat droplets.

A third group of six animals were comparatively thin—the subcutaneous layer of fat being only a few millimeters thick. In one of these some of the cortical renal tubules were full of fat droplets. The cartilage cells of all contained fat. Two animals showed a considerable amount of fat in the cortical cells of the adrenal.

A fourth group of animals were exceedingly thin, having been kept on submaintenance several months. They were fed so that they were made to lose weight at the rate of about half a pound a day. The connective tissue fat deposits were nearly exhausted. One of the animals showed a large amount of fat in the cortex of the adrenal. All three show fat inside many of the muscle fibers. The cartilage cells of one animal were examined and found to contain the usual amount of fat.

It appears from the above observations that in cattle nine months or more old no fat is found in the salivary glands, pancreas, or spleen. In the thymus fat was often found in the Hassall corpuscles.

Fat was found in the hepatic cells in two moderately fat animals, but was absent in all the others. In one of the submaintenance animals the cells had shrunken to about half their normal diameter and their outlines were no longer visible. It is clear that the presence

of fat in the liver cells has no relation to the fatness of the animal. Fat was found in the renal epithelium in only one of the fifteen animals slaughtered, and this animal was comparatively thin. (I examined the kidneys of two 1400-pound fat steers slaughtered by Swift & Co. at Chicago. In both animals the cells of many cortical renal tubules were full of fat droplets.) Fat may occur in the renal epithelium under normal conditions, but it seems to have no relation to the fatness of the animal.

In two thin animals and in one very thin animal the adrenal gland was found to contain a considerable amount of fat in the cortical cells. The fattest animals had no fat in this situation. This intra-epithelial fat also seems to be independent of the nutritive condition of the individual.

Bullard has found fat droplets inside many of the muscle fibers of the three submaintenance steers. There was only a small amount of connective tissue fat in these animals. The muscle tissue had undergone a marked atrophy. This condition can hardly be interpreted otherwise than as a fatty degeneration or infiltration due to starvation. The absence of fat in the excessively fat animals shows that the muscle fiber of the adult is not used as a place for the storage of fat.

Kemp and Hall (19) examined the muscles of animals fattened for slaughter. They never found fat inside a muscle fiber. Keinath (18) however found fat inside the fibers of pectoral muscle in a fattened ox. He examined only one animal.

The occurrence of fat in the tissues of animals other than the ox.

Fat is found in the glandular tissue and in the muscle fibers of many animals. I have examined the livers of several cats and found in each specimen a large amount of fat. The lachrymal gland is always full of fat, and the kidney usually contains a considerable amount.

Aschoff (2), using Flemming's fixation as the fat stain, finds fat in the new-born child in the kidney, liver, heart muscle, basal epithelium of skin, mast cells, leucocytes, epithelium of intestines,

smooth muscle of intestine, blood vessels, glia cells of central nervous system, ependyma cells, and ganglion cells. He also finds a great amount of fat in the heart muscle of a mouse embryo.

Pfeiffer (26), also using Flemming's fixation as a stain for fat, finds fat in the new-born child in the kidney, liver, some cells of spleen, heart muscle fibers, leucocytes, epithelium and glands of small intestine, and brain. He does not find fat in the tissues mentioned in every case.

Sata (30) fixed human tissues in Flemming's solution, cut with a freezing microtome, and stained again in one per cent osmic or in Flemming's solution. He finds the lachrymal and submaxillary glands full of fat. He finds fat also in the parotid gland and in the pancreas.

Erdheim (7) studied the thyroid gland with Sudan. He always finds fat in the human thyroid in the part of the cells next the lumen. He finds fat absent from the thyroid of the fœtus and about half the newborn. The droplets increase in size with age. They occur in adenoma and in carcinoma. Fat is present also in the parathyroid and the hypophysis. The amount of fat is not dependent on the nutritive condition of the individual.

Walbaum (37) finds fat inside the muscle fibers in normal children in about two-thirds of the cases examined, and in about the same per cent of rachitic children. He believes that the presence of fat in this situation is not directly connected with the nutritive condition of the individual.

Hansemann (15) finds fat often in the epithelium of the human kidney.[5] He regards it usually as a pathological condition, but thinks that it may possibly occur under normal conditions. He finds the renal cells full of fat in a woman weighing over 300 lbs. He states that in swine the renal epithelium contains almost no fat when the animal is poor, but a large amount when the animal is fat. Cats and dogs have fat in the renal epithelium.

Traina (36) has contributed a very valuable piece of work on intraepithelial fat. His observations were made on marasmic cad-

[5]This paper contains a good survey of the literature on this point.

avers and on rabbits killed by starvation. Fat is present in the epithelium of the human lachrymal glands, pancreas, suprarenal, thyroid, and testis.[6] Fat is present in the rabbit in the organs just mentioned and in the liver and kidney. The marasmic condition in man does not affect the intraglandular fat, though the connective tissue fat may be greatly reduced.

In rabbits which have died of starvation, the fat in the epithelium is not lessened in quantity, though the cells be reduced to half their normal volume, and the connective tissue fat depots almost completely emptied. Traina concludes that there is no connection between the nutritive condition of the individual and the intraepithelial fat. He regards this fat as a constant and integral constituent of the cell protoplasm and considers it analogous to cell pigment. It is not stored energy to protect the body from starvation as is the fat deposited in the connective tissues.

Significance of fat deposited in epithelium, cartilage, and muscle.

Walbaum (37) showed that fat in the muscle fibers in children is not directly connected with the nutritive condition of the individual. Erdheim showed that the same is true of the fat in the cells of the thyroid. Traina showed that practically all intraepithelial fat is unaffected by malnutrition and starvation.[7]

My conclusions are essentially in accord with those of Traina. The fat occurring in the epithelial tissues and in the cartilage seems to be in no way connected with the nutritive condition of the animal. It is neither removed by starvation nor increased by excessive fattening. It is certainly not food stored to protect the body against starvation as is the fat in the adipose tissues. It is not even used by the cell in which it is deposited, since the cell may undergo great atrophy without appreciable diminution of the fat content.

Fat is not present in the muscle fibers of adult cattle under normal conditions, but fine fat droplets appear in the later stages of atrophy caused by a prolonged submaintenance ration. There is no evidence

[6]The liver and kidney were not normal in his cases.

[7]Traina believes, however, that a part of the fat in the liver is temporarily deposited. He did not work on the kidney.

that the fat deposits in epithelium, cartilage, and muscle take any part in the normal metabolism of the cell.

An apparent exception to the conclusions stated above is found in Hansemann's observation that in swine the renal epithelium contains almost no fat when the animal is poor, but a large amount when the animal is very fat. Hansemann regarded the fattened condition in swine as abnormal and analagous to obesity in man. More extended observations will be necessary to settle this point.

SUMMARY OF PART I.

Fat droplets were found in the cells of the liver in nearly all the fœtuses examined—the youngest being 4.7 cm. long. Fat was also found in the hepatic cells of two moderately fat steers.

Fat droplets were found inside the muscle fibers from the 7 cm. to the 28 cm. stage. No fat was found in this situation in older fœtuses, but in three very thin steers, about one year old, fat droplets were found inside some of the muscle fibers of several muscles. This is, however, due to atrophy.

The cells of hyaline cartilage were found to contain fat droplets throughout fœtal life (from the 7 cm. stage) and also in the adult.

The renal cells of some fœtuses contain fat droplets. In certain parts of the kidneys of two large fat steers, the cells of the renal tubules were loaded with fat droplets.

The fat droplets found inside epithelium, cartilage, and muscle, unlike the fat in the fat cells, is independent of the nutritive condition of the animal.

II. On the Histogenesis of the Adipose Tissue of the Ox.

The development of adipose tissue has been the subject of numerous investigations. The work of Flemming stands out preeminently. The last comprehensive paper was published by Hammar in 1895. Since the appearance of Hammar's paper, our histological technique has been greatly improved by the introduction of Mallory's connective tissue stains, Scarlet red, and Sudan. It is hoped that a careful study of one species with the advantages of the newer methods has brought out enough new facts to justify a revival of the question.

The structure of the tissue will be described from the earliest fœtal stages until the fully-formed condition is attained. Some of the fat cells at birth differ only in size from those of the adult animal; but the final stages of differentiation nearly always take place after birth.

Material and Methods. The material employed was almost exclusively calf fœtuses from the 3 cm. stage until about full term. A number of observations have been made on the histological changes occurring in adipose tissue during fattening; but this phase of the subject has not been completely worked out and will only be referred to incidentally.

A large part of the material was fixed in Zenker's fluid or in Gilson's fluid and embedded in paraffin. The stains used on the sections thus prepared were mainly Mallory's anilin blue and iron-hæmatoxylin. Iron-hæmatoxylin is especially valuable for this study. When the differentiation in iron-alum is omitted, leaving the hæmatoxylin intense, the finest connective tissue fibers are brought out, though not differentiated from the nuclei and protoplasm. The results obtained by this method are readily interpreted by comparing with a similar section stained with Mallory's anilin blue. Some sections were overstained with iron-hæmatoxylin and then moderately differentiated to bring out the Altmann granules and the nuclei. Eosin or Congo red were sometimes used as counterstains after iron-hæmatoxylin.

Considerable material was fixed in 20 per cent formalin. Formalin material cannot be stained well with Mallory's anilin blue, but the Altmann granules are brought out well by iron-hæmatoxylin after this fixation. Frozen sections were made at practically all stages and stained with Sudan or Scarlet red as controls for the embedded material. The same precautions were taken with Scarlet red and Sudan, as described in Part I.

A discussion of the older literature of adipose tissue may be found in the papers of Czajewicz and Flemming. The older papers will not be referred to here. It is sufficient to give only the general conclusions to which the more recent investigations have led.

As is well known, two theories of the development of adipose tissue

became prominent in the seventies. One of these views, first clearly formulated and developed by Flemming (8, 9, 10, 11, 12), is that adipose tissue is only fibrillar connective tissue in which the cells have become filled with fat. Adipose tissue is not a special kind of tissue. Czajewicz (6) and Virchow had come to conclusions similar to this before the appearance of Flemming's work. Jakowski (17) agreed with this conception. These investigators gave almost no attention to the primitive fat organs.[8]

The other theory advanced by Toldt, is that adipose tissue is a special kind of tissue. It develops from special organs (Toldt's Fettkeimlager, Kölliker's Primitivorgane der Fettläppchen). All the adipose tissue of the body is formed by outgrowths of these primitive organs. This was the view of Ranvier (29), Klein (20), Bobritzky (3 *a*), Metzner (23), Altmann (1) and others.[9] Essentially the same view was held by Löwe (22).

Waldeyer's view (38) is a compromise between Flemming and Toldt. He states that fat cells develop both from special cells (those of the primitive organs) and ordinary connective tissue cells. He also states that wandering cells may enter a fat lobule and become fat cells. Löwe also assigns an important role to the wandering cells in the formation of fat cells. Gage (13 *a*) held that fat cells develop mainly from ordinary connective tissue cells, but that wandering cells may also become fat cells.

Kölliker (21) took the position that the fat cells are all derived from connective tissue cells. Some cells begin to accumulate fat as ordinary branched connective tissue cells; others (those of the primi-

[8]Flemming gave a little attention to the primitive organs in his last paper.

[9]Toldt (35) in a later discussion admits that many fat cells are developed not from the primitive organs but from connective-tissue cells. But he does not regard these connective-tissue fat cells as true fat cells. He seems to think that they are analogous to cells like those of the liver, which may accumulate fat but which are not regarded as true fat cells. As an argument for this contention that the cells of the primitive organs are not connective-tissue cells he points out that the cells of the primitive organs do not revert to a branched condition when deprived of their fat as do the cells of ordinary connective tissue. Toldt did not study the type of fat organ found in the calf fœtus.

tive organs) lose their processes and become rounded or polygonal before the deposit of fat begins. This distinction was based on the study of animals like the cat in which the primitive organs are composed of polygonal cells rich in protoplasm.

Hammar (14) made a comprehensive and thorough study of adipose tissue and acquired a deep insight into the subject. He agrees with Kölliker that all adipose cells are ultimately derived from fixed connective tissue cells. The primitive organs are formed from branched mesenchymatous cells. He distinguishes primary and secondary adipose tissue-formation. By the former he means those instances where the tissue is formed into well defined lobules (primitive fat organs) before fat impletion begins; by the latter he means the formation of adipose tissue without primitive organs. In primary adipose tissue-formation the cells may develop a large amount of protoplasm before the deposit of fat begins (primitive organs of rat, rabbit, cat, etc.); or they may begin to accumulate fat without showing any marked increase of protoplasm (primitive organs of calf, man, dog). Hammar's work reconciled the views of Flemming and Toldt in a very satisfactory and convincing way.

Hammar found that the primitive organs of some animals, as the rat and rabbit, develop at first as compact masses of cells rich in protoplasm. These polygonal cells next accumulate many small fat droplets—the droplets being always separated by protoplasm. In some animals as the rabbit, the small droplets increase in size and finally flow together to form a single droplet, thus producing an ordinary fat cell. In other animals, however, as the rat, the cells persist in the early multiglobular form throughout life. This latter tissue Hammar calls brown adipose tissue. The so-called hibernating gland of some animals has the same structure.

In man and in the ox Hammar finds primitive fat organs only adjacent to the kidneys. Subcutaneous fat is formed without any preformed lobules. Hammar did not attach any great significance to his classification of primary and secondary adipose tissue-formation. He recognized the possibility of the occurrence of transition forms and even mentions one instance of such in the scalp of a human fœtus. As will appear later, my observations show that

this classification does not apply very well in the calf. A peculiar open-meshed tissue, which I shall call preadipose tissue (Text Figs. 1 and 3, and Fig. 6, Plate II), precedes in every case the formation of adipose tissue in the embryo. The preadipose tissue varies in different regions in the quantity developed before the true fat cells begin to form, and it remains in the preadipose condition longer in some situations than in others; but the differences are not marked enough to warrant Hammar's classification. The term, primitive fat organ, may however be retained, if desired, to designate the renal preadipose tissue, since this tissue is more sharply marked off than preadipose tissue in other situations and persists longer in the preadipose condition.

The primitive fat organs.

These were first mentioned by Kölliker in the mesentery of the cat in 1856. Toldt first gave a description of them and brought them to the attention of anatomists. His study of these structures, sharply defined as they are in the animals he studied, led him to the belief that adipose tissue is a special kind of tissue derived from these organs only. He thought that all adipose tissue is derived from outgrowths of these organs. He believed that the subcutaneous adipose tissue is developed principally from the primitive organs of the axilla and inguinal region.

In the new-born rat Auerbach (3) describes masses of brown adipose tissue (these are presumably primitive organs) in the interscapular and interrenal region, in the axilla, neck, and thoracic cavity. These organs have been described in the guinea-pig, mouse, rat, cat, etc. In this paper it will not be desirable to give the detailed distribution of the primitive fat organs in these animals, since, as Hammar has pointed out, they have an entirely different structure here to that which one finds in the calf.

Hammar describes a primitive fat organ in the renal region in man, the dog, and the calf. As far as I know this structure has not been described by any other observer. The primitive organs of the cat, etc., consist of well-defined masses of closely-packed cells

rich in protoplasm; but in the calf, man, and dog these structures are composed of a reticular tissue with branched cells.

Preadipose tissue of the calf.

I have not used the term "primitive organ" in my descriptions in the calf since, as will appear later, it seems to make a distinction of no fundamental importance.

Renal preadipose tissue. Hammar finds this tissue first in a 11.5 cm. fœtus. He found no trace of it in a 5 cm. fœtus. He describes it as appearing first as a sheet of tissue parallel to the surface of the kidney. Later it branches to form lobules. The fat cells begin to appear in masses around the blood vessels (30.5 cm.-41 cm.).

My own observations agree essentially with those of Hammar. A 4.7 cm. fœtus showed a very early stage of the renal preadipose close to the kidney on its ventral surface. The preadipose tissue has at all times a characteristic reticular appearance in sections which enables one to distinguish it readily from the adjacent tissue in which the general direction of the fibers is parallel to the surface of the kidney (Text Figs. 2 and 3). In the 4.7 cm. specimen it forms a very thin layer (3 or 4 meshes deep), extends only a short distance on the kidney, and passes gradually into the adjacent tissue.

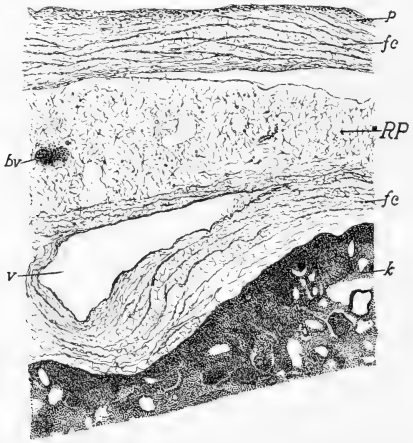
In a 9 cm. fœtus there is a continuous sheet of the preadipose tissue along the ventral surface of the kidney. Its greatest thickness is about 225 microns.

In a 12 cm. fœtus the greatest thickness of the sheet of preadipose tissue is about 375 microns. It tapers out on the edges.

Text Fig. 2 shows a transverse section of the body vertical to the surface of the kidney (k) of an 18.5 cm. fœtus. The preadipose tissue ($R\ P$) is in the form of a sheet, 450 microns thick at its thickest portion, and parallel to the surface of the kidney. It is yet unbranched. No true (rounded) fat cells are present. Blood vessels ($b\ v$) of considerable size are to be seen. It is fairly well marked off from the adjacent tissue except at the extremities where it seems to be growing rapidly.

Text Fig. 3 represents a section vertical to the surface of the kidney of a 25 cm. fœtus. The section does not extend entirely to the peritoneum. The preadipose tissue has branched out and become subdivided into lobules (*L*). Numerous small blood vessels are to be seen.

Text Fig. 4, from a 40 cm. fœtus, shows a later stage than the preceding. Groups of rounded fat cells have begun to appear around the blood vessels. These small adipose lobules (*a l*) increase in size



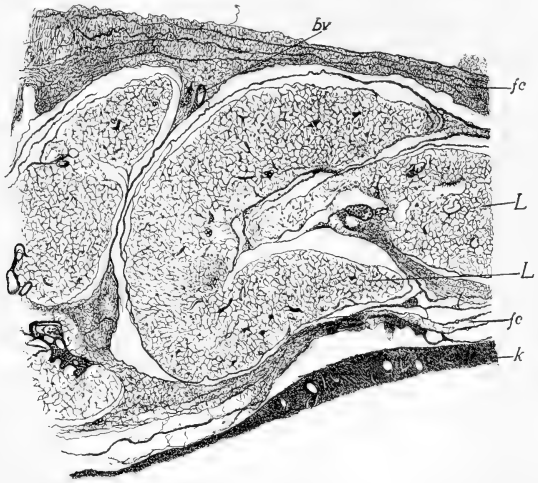
Text Figure 2. Transverse section through the renal preadipose tissue of a 16 cm. fœtus. *br*, small blood vessel; *fc*, fibrillar connective tissue; *k*, kidney; *p*, peritoneum; *RP*, renal preadipose tissue; *v*, vein. Fixation, Zenker's fluid. Iron-hæm. stain. × 50.

at the expense of the preadipose tissue around them, becoming closely crowded, and finally forming a solid mass of young adipose tissue. In fœtuses over 40 cm. long the renal adipose tissue is usually a solid mass as just described. One preadipose lobule forms a number of adipose lobules.

Structure of preadipose tissue.

Before describing the preadipose tissue of other regions, I shall explain its structure here. Under moderate magnification the tissue

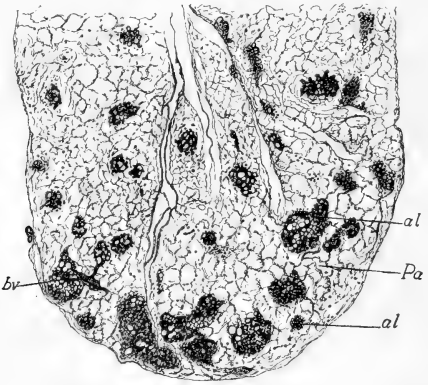
has a characteristic reticular structure not unlike the framework of a lymph gland (Text Figs. 1 and 3). By means of special stains and strong magnification its true structure may be seen. It was mentioned by Hammar that connective fibrillæ were present in the renal preadipose tissue (primitive organ). For this reason he regarded it as a fibrillar connective tissue before the formation of fat cells began. Hammar seems not to have had very convincing dem-



Text Figure 3. Transverse section through the renal preadipose tissue of a 25 cm. fœtus. *bv*, small blood vessel; *fc*, fibrillar connective tissue; *k*, kidney; *L*, lobule of preadipose tissue. Fixation, Zenker's fluid. Iron-hæm. stain. × 50.

onstrations of the presence of the fibrillæ. This phase of the question has not received the attention which it deserves. Fig. 6 (Plate II), and Fig. 8 (Plate I) were drawn from the renal preadipose of an 18.5 cm. fœtus. The section, from which Fig. 6 was drawn, was stained by Mallory's anilin blue method—the blue having been allowed to act about one hour. The connective tissue fibrillæ (*f*) stain with difficulty and then stain much less intensely blue than

collagenous fibrillæ out in the surrounding tissue. The coarse orange-colored fibers (*pr*) are the processes of the cells. The cells seem for the most part to be bipolar and often are spindle-shaped. Some are provided with very long processes. It is easily possible that some have more than two processes—I could not determine this very well in the thin sections used. The open spaces of the tissue, which give it the reticular appearance, are clearly brought out. The cell processes were followed in some cases for a considerable distance



Text Figure 4. Section through a lobule of renal preadipose tissue from a 40 ccm. fœtus when the true fat cells are just beginning to form. *al*, lobule of adipose tissue; *bv*, blood vessel; *Pa*, preadipose tissue. Fixation, Zenker's fluid. Iron-hæm. stain. × 58.

among the collagenous fibrillæ, but their exact method of termination was not determined.

Fig. 8, Plate I was drawn from a section stained deeply with iron-hæmatoxylin and not decolorized. This is an excellent stain to use as a control for Mallory's anilin blue. It stains the fine fibrillæ black. The processes (*pr*) of the cells are coarser and darker than the fibrillæ (*f*) so that their recognition is easy. The nuclei of the cells can hardly be distinguished however on account of the intensity of the stain.

These figures show beyond doubt that the renal preadipose tissue is a fibrillar connective tissue. It cannot, therefore, be regarded as a special tissue in Toldt's sense distinctly different from ordinary fibrillar connective tissue.[10]

The renal preadipose tissue is not developed from the original protoplasmic mesenchyme. Before any trace of the renal preadipose tissue is to be seen the tissue around the kidney is already differentiated into a fibrillar tissue similar to that shown in Textfigure 2 (*fc*). The structure and development of this tissue has been described by Mall (22 *a*). The preadipose tissue develops from this fibrillar tissue and the lobule seems to grow to a large extent by the transformation of this tissue on its periphery. In the early stages the preadipose lobule is not sharply marked off but passes gradually into the adjacent fibrillar tissue.

One very interesting fact, which has not been called attention to by other observers, is that the branched cells of the preadipose lobule may contain fat droplets long before any true adipose tissue is present. The development of groups of rounded fat cells, such as shown in Text Fig. 4, seldom begins in the renal preadipose before the 30 cm. stage; but fine fat droplets were observed in the cells of the renal preadipose as early as the 14 cm. stage. By staining frozen sections with Scarlet red I have found considerable numbers of fine fat droplets in the renal preadipose cells of three specimens 14 cm., 17 cm., and 20 cm. long respectively. In a 24.7 cm. specimen whole lobules were found where practically every cell contains one or more coarse or fine fat droplets (Plate II, Fig. 7). The cells containing fat occur in all parts of the lobule and have no special relation to the blood vessels. In none of the four specimens just mentioned was there any adipose tissue present. They were all in stages such as shown in Text Figs. 2 and 3. The preadipose cells are evidently well along in their differentiation toward true adipose cells.

[10]Of course Toldt did not examine this kind of preadispose tissue. He examined only the solid primitive organs of the rabbit, cat, etc. It would be interesting to know if connective-tissue fibrillæ are present in the mesenchyme out of which these organs are developed.

Small fat droplets were also noted in the subcutaneous preadipose tissue of the inguinal region and of the brisket. But the preadipose tissue of these regions persists only a short time before the cells are filled with fat, so this condition is hardly comparable to that in the renal preadipose where the branched cells contain fat droplets a long time.

Preadipose tissue of the omentum.

The time of the first appearance of this tissue varies considerably. The youngest fœtus in which it was noted was 33 cm. long. This specimen showed a very early stage. The preadipose tissue can be distinguished from the adjacent mesenchyme only by its being a sheet of tissue in which the nuclei are decidedly closer together than elsewhere. The cells are similar to those of the preadipose. The mass of tissue is not at all sharply marked off from the adjacent mesenchyme, and it does not show a marked reticular structure.

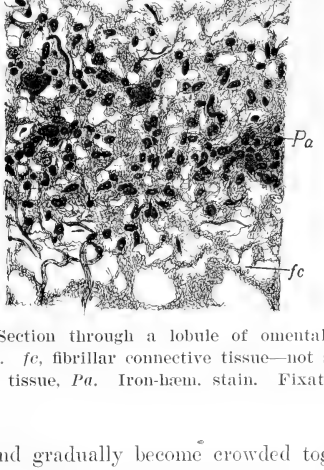
Text Fig. 5 shows a later stage—a section through the preadipose tissue of a 40 cm. fœtus. The tissue here forms a well-defined flattened mass. Its finer structure is shown in Fig. 9, Plate II. It will be noted that it is not essentially different from renal preadipose tissue. The cells are of the same type. The fine collagenous fibers stain a little stronger and are somewhat coarser than those of the renal tissue. Large collagenous fibers (*cf*) occur in the omental tissue. This tissue does not have a pronounced reticular structure such as seen in the renal region.

In many places in the omentum the preadipose is not nearly so compact as that shown in the figures. The same sheet of tissue shown in the figure has a very diffuse arrangement in another part of the same section. The finer structure of the tissue remains the same, but the nuclei become bunched in small groups (5 or 6 to a group) separated by intervening mesenchyme. This diffuse arrangement does not seem to correspond to the blood vessels. No true fat cells are yet formed in this specimen.

The omental preadipose tissue is by no means so sharply defined as the renal even in its most compact places; but it is a mass of tissue formed some time in advance of the development of true adipose

tissue and having a relation to the blood vessels scarcely more intimate than that seen in the renal. It is not a mere differentiation around a blood vessel such as occurs in the subcutaneous tissue (Text Fig. 1). If we use Hammar's classification, it is difficult to decide whether we are dealing with primary or secondary adipose tissue-formation.

The later stages in the development of the omental adipose tissue are not very different from those in the renal. Nests of fat cells form around the small blood vessels making the fat lobule. These



Text Figure 5. Section through a lobule of omental preadipose tissue from a 40 cm. fœtus. *fc*, fibrillar connective tissue—not sharply marked off from the preadipose tissue, *Pa*. Iron-hæm. stain. Fixation, Zenker's fluid. × 335.

increase in size and gradually become crowded together to form a mass of adipose tissue. In many parts of the omentum the preadipose tissue seems to develop along the course of the blood vessels as in the subcutaneous tissue.

Preadipose tissue of the brisket.

The part of the brisket studied was the region between the ventral surface of the sternum and the pectoral muscles. In this region in the adult we find lobules of adipose tissue separated by heavy

cords of fibrous tissue. This adipose tissue begins to develop early. In an 11 cm. fœtus a small mass of mesenchyme is present between the ventral surface of the sternum and the overlying pectoral muscles. This tissue is present in somewhat greater quantity in a 13 cm. fœtus, but no true adipose cells are yet present. The tissue is somewhat reticular in structure.

A 22 cm. fœtus shows a considerable mass of tissue in the situation above described. Little clumps of fat cells are present around the blood vessels. The coarse bundles of fibrous tissue are partially differentiated. In some places there is a considerable amount of preadipose tissue around the nests of fat cells. None of the preadipose tissue is sharply marked off from the developing fibrous tissue.

The tissue which forms the fat of the brisket is not then formed into preadipose tissue until a short time before it is transformed into adipose; but a somewhat reticular connective tissue is present in this region for some time previous.[11]

In its further growth the small masses of adipose tissue formed around the blood vessels increase in size and become crowded together, except where they are separated by the cords of fibrous tissue which characterize this region.

Subcutaneous preadipose tissue.

Text Fig. 1 shows the preadipose tissue around the blood vessels of the subcutaneous tissue over the sternum of a 24 cm. fœtus. The preadipose tissue is developed to a considerable extent in advance of the formation of true fat cells. It seems to be formed only a short time before fat impletion begins. Its structure is similar to that of renal preadipose tissue except that the processes of the cells are somewhat thinner (Text Fig. 6). In the inguinal region, the preadipose tissue forms around the blood vessels as above described, but does not follow them so closely. Larger areas of pre-

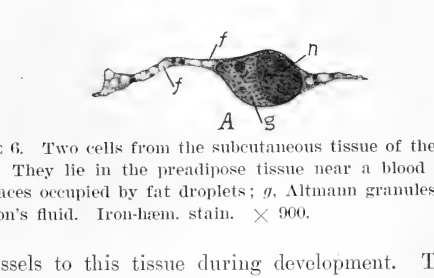
[11]In the section of the brisket a mass of preadipose tissue was noticed on the internal surface of the sternum in the thoracic cavity. This mass is fairly well-defined and contained no fat cells at 13 cm. It was not studied further.

adipose tissue were found. I have not examined the subcutaneous fat-formation in any other part of the body.

The manner of growth of adipose tissue in fattening will be taken up later, but I wish to mention in this connection the development of intramuscular adipose tissue. Fat cells are formed around the blood vessels as in subcutaneous tissue,—the process extending out on the smaller vessels as the animal fattens. Fat cells were noted in the psoas muscles of a 51 cm. fœtus and in several older specimens.

The relation of the deposit of fat to the blood vessels.

The earliest investigators of adipose tissue noted its great vascularity. Flemming emphasized strongly the intimate relation of



Text Figure 6. Two cells from the subcutaneous tissue of the brisket of a 24 cm. fœtus. They lie in the preadipose tissue near a blood vessel (Text Fig. 1). *f*, spaces occupied by fat droplets; *g*, Altmann granules; *n*, nucleus. Fixation, Gilson's fluid. Iron-hæm. stain. × 900.

the blood vessels to this tissue during development. The relation of the blood vessels to the preadipose tissue of the renal region does not impress me as being especially close; but no one can fail to notice such a close relation as shown in Text Figs. 1 and 4, where the true adipose tissue is beginning to form. Equally impressive are sections of the liver in many specimens. In several fœtal livers the cells adjacent to the veins were full of fat when little or none was found elsewhere (Fig. 1, Plate 1). In two young steers fat droplets were found only in the cells adjacent to some of the medium sized veins. When we compare the deposit of fat in the liver with its first appearance in connective tissue there is seen to be a striking

resemblance. The fat passes out of the blood stream and is taken up by the adjacent cells. Possibly the fat itself (in some soluble form[12]) acts directly upon the relatively undifferentiated connective tissue cells causing them to pass into the preadipose and later into the adipose condition.[13]

Detailed changes in the fat cell during development.

As was said above, the preadipose cell is a branched cell, in sections appearing usually bipolar and frequently spindle-shaped. Its long coarse processes lie among the collagenous fibrillæ (Figs. 6 and 9, Plate II; and Fig. 8, Plate I). It may accumulate small fat droplets at this stage long before any further changes occur. A 24.7 cm. fœtus showed whole lobules of renal preadipose tissue in which nearly every cell contains fat droplets (Fig. 7, Plate II). Usually it seems that no fat is deposited in the cell until about the time its transformation into a true rounded fat cell begins. In the first formation of a fat lobule the deposition of fat begins in the preadipose cells adjacent to a blood vessel and extends outwards. The blood vessel is the center of the lobule. As the lobule grows the preadipose cells around its periphery are gradually converted into fat cells. Some fat cells are formed inside the lobule. Most of the drawings of individual fat cells were made from cells on the periphery of the lobule, since the cells are not crowded there and can be more easily studied.

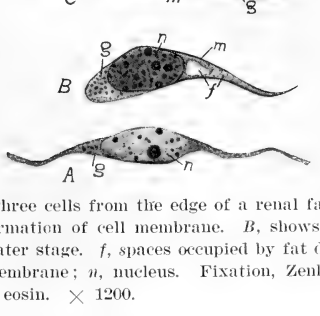
Text Fig. 7 shows some early stages in the formation of the fat cells. The cells were drawn from the edge of a renal fat lobule of a 42cm. fœtus. Cell A shows the first appearance of Altmann granules (g), but has not yet developed a cell membrane or fat droplets. It is spindle-shaped and has long processes. Cell B is not all included in the section. It has fat droplets and Altmann granules. A thin cell membrane (m) is present. In cell C a number of fat droplets

[12]The physiologists have shown that fat is moved through the tissues in some soluble form and not as small droplets.

[13]This assumption does not, however, explain why the fat passes out of the vessels at any particular place.

are present separated by thin layers of protoplasm. The cell membrane (m) is now clearly shown. The cell is still pointed at the ends.

Text Fig. 6 shows two cells from the subcutaneous tissue of the brisket of a 24 cm. fœtus. They lie near a blood vessel around which a few fat cells have appeared. The processes of the preadipose cells of this region are somewhat thinner than those of the renal tissue. Both cells, especially cell A, show fat droplets in their processes. I have not determined the exact way the cell process disappears, but several cells like cell A have been observed. It seems that in these



Text Figure 7. Three cells from the edge of a renal fat lobule of a 42 cm. fœtus. *A*, before formation of cell membrane. *B*, shows first appearance of cell membrane. *C*, later stage. *f*, spaces occupied by fat droplets; *g*, Altmann granules; *m*, cell membrane; *n*, nucleus. Fixation, Zenker's fluid. Stained with iron-hæm. and eosin. × 1200.

cells the cell process is transformed into fat droplets which are moved up into the body of the cell.

Text Fig. 8 represents two cells well into the edge of a fat lobule, They were drawn from the same section as those shown in Text Fig. 7. The cells have increased greatly in size, though the processes have not yet disappeared. The protoplasm is thickly studded with Altmann granules. The cell membrane is sharply marked except on the processes.

Text Fig. 9 was drawn from the same specimen from which Text Figs. 7 and 8 were taken. The cells lie well into the edge

of a fat lobule. These cells have lost their processes and become rounded. Cell B has a large amount of protoplasm, but only a little fat. This multiglobular cell is somewhat similar to the cells of brown adipose tissue. In both cells the cell membrane is readily seen.

Figure 10, Plate 11, was drawn from the inguinal adipose tissue of a 32 cm. fœtus. Fat cells of different stages of development are shown. Most of the smaller cells are of the type shown in Text figure 9—rounded cells with considerable granular protoplasm and

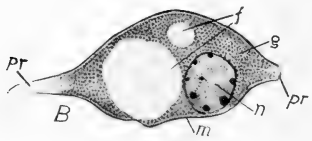
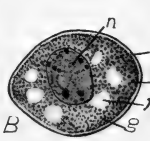
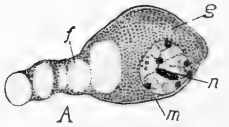


Fig. 8.





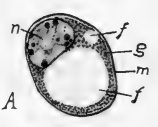


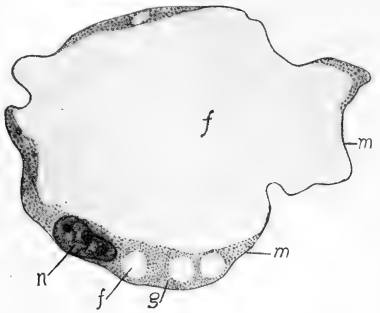
Fig. 9.

Text Figure 8. Two young fat cells from the renal adipose tissue of a 42 cm. fœtus. The cells lie well into the edge of the lobule. *f*, spaces occupied by fat droplets; *g*, Altmann granules; *m*, cell membranes; *n*, nucleus; *pr*, processes of cell. Fixation, Zenker's fluid. Stained with iron-hæm. and eosin. × 1200.

Text Figure 9. Two young fat cells from the renal adipose tissue of a 42 cm. fœtus. The cells lie well into the edge of the lobule. *f*, spaces occupied by fat droples; *g*, Altmann granules; *m*, cell membrane; *n*, nucleus. Fixation, Zenker's fluid. Stained with iron-hæm. and eosin. × 1200.

one or more fat droplets. Most of these cells were not derived directly from the original branched cells of the preadipose tissue, but from the division of small cells in the interior of the lobule. As will be described later, the fat lobule grows, in its early stages at least, by division of small fat-free cells inside the lobule as well as by the addition of cells on its periphery. The cells formed inside the lobule are not branched; they become rounded directly by increasing their protoplasm and accumulating fat. The larger cells (about 25 microns

in diameter) show a well developed peripheral zone of protoplasm containing Altmann granules. It will be noted that many collagenous fibers (cf. stained blue) occur crowded between the fat cells. These fibers become included in the adipose tissue during its formation. The preadipose tissue often contains a number of coarse collagenous fibers (note lower right-hand corner of figure) and these are crowded together in thin layers as the fat cells increase in size. These fibers do not occur in renal adipose tissue. The fibers of the renal preadipose seem to be completely absorbed.

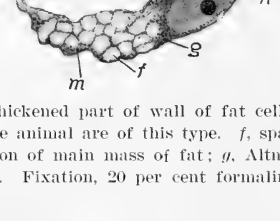


Text Figure 10. Cell from the renal adipose tissue of a 105 cm. fœtus (about full term). *f*, spaces occupied by fat; *g*, Altmann granules; *m*, cell membrane; *n*, nucleus. Fixation, Zenker's fluid. Stained with iron-hæm. × 1200.

Text Fig. 10 represents a fat cell from the renal adipose tissue of a fœtus about at full term (105 cm.). Nearly all the cells of the tissue at this stage are similar to this cell. The border of the cell is wavy because of shrinkage from fixation and embedding. The cell membrane is readily seen. In some parts of the circumference, particularly around the nucleus, a thick zone of granular protoplasm is present. The zone of protoplasm contains Altmann granules and often small fat droplets.

Text Fig. 11 is from the scrotal adipose tissue of a steer about 3½ years old. The animal was fairly well fattened and weighed 1,260 pounds. The drawing shows only a part of the wall of a fairly large

fat cell. A mass of protoplasm containing small fat droplets is shown. No other thickening is present in the wall of this cell. A great many cells of this type are present in this animal in the scrotal fat and in various portions of the subcutaneous fat. Many of the cells show more than one thickening in the wall caused by small accumulations of protoplasm. Similar small masses of protoplasm containing small fat droplets were found in cells of all sizes up to 125 microns, but the protoplasm is present less frequently and in smaller amount in the larger cells. The same kind of fat cells were found in large numbers in corresponding portions of the adipose tissues of several other fat steers. These cells are clearly similar to the cells of the full-term fœtus (Text Fig. 10). They are probably merely young fat cells. High magnification reveals the presence of some very small



Text Figure 11. Thickened part of wall of fat cell from a fat steer. A great many cells in the animal are of this type. *f*, spaces occupied by small fat droplets; *F*, position of main mass of fat; *g*, Altmann granules; *m*, cell membrane; *n*, nucleus. Fixation, 20 per cent formalin. Stained with iron-hæm. × 900.

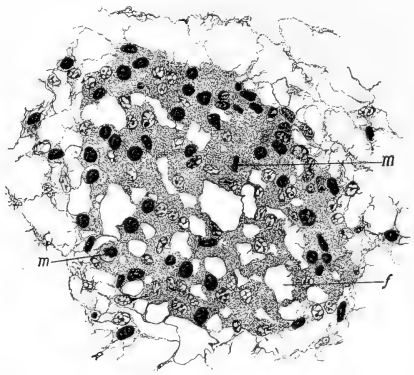
masses of protoplasm immediately under the cell membrane in many large fat cells, but the protoplasm does not form a continuous layer.

The cell membrane is differentiated from the peripheral protoplasmic layer of the cell. It begins to develop when the cell is yet branched (Text Fig. 7, B and C; Text Fig. 8, A and B). It develops only a short distance on the cell processes. The membrane increases in thickness as the cell grows. It has long been known that the cell membrane is different chemically from the protoplasm and connective tissue fibers. It is not stained by Mallory's anilin blue[14]

[14]If the tissue be fixed in formalin, anilin blue will stain the membrane of the fat cell.

nor by Weigert's elastic tissue stain. It may be stained by fuchsin, eosin, Congo red, etc.

Altmann's granules are present in the protoplasm of the fat cell from the time it begins to assume the rounded form. There is no conclusive evidence, however, that they stand in any intimate relation to the formation of fat, since protoplasmic granules which stain similarly are of such widespread occurrence. Metzner (23) has attempted to show that these granules are directly transformed into fat droplets, but his observations are far from convincing.



Text Figure 12. Fat lobule from renal adipose tissue of a 40 cm. fœtus. The dark nuclei are in some stage of division. *f*, spaces occupied by fat; *m*, mitotic figures. Fixation, Zenker's fluid. Stained heavily with iron-hæm. × 400.

Growth of adipose tissue.

It has been pointed out that the blood vessel is the center around which the fat lobule develops. Whether in a mass of preadipose tissue, or in ordinary connective tissue, the first fat cells appear immediately around the blood vessels. The lobules thus established increase in size to a large extent by the addition of cells adjacent to the periphery. The increase in the number of fat cells is, however, to a considerable extent due to the division of fat-free cells

inside the lobule. Text Fig. 12 represents a lobule of renal adipose tissue from a 40 cm. fœtus, under a low magnification. A number of nuclei in the lobule stain intensely with hæmatoxylin and are probably in some stage of division. Two mitotic figures (m) are shown. Fig. 10, Plate II, shows clearly these young fat cells among the older cells. Probably only a few of these young cells were developed directly from the original branched cells of the preadipose tissue. They arise by the division of fat-free cells inside the lobule.

In almost any section of adipose tissue a few nuclei may be seen crowded in the angles between the fat cells. The protoplasm around these nuclei is so small in amount that it can hardly be demonstrated. From a study of fattening animals I am convinced that these interstitial cells may form many new fat cells when the animal fattens. I have not, however, found mitoses in the interstitial nuclei. It is generally believed that a cell does not divide after any considerable amount of fat has been deposited in it.

A great part of the growth of the fat lobule is certainly due to the increase in size of the individual fat cells. A cell increasing its diameter from 15 to 150 microns (the latter being a common size in fat cattle) increases its cross-sectional area 100 times and its volume 1,000 times. The subcutaneous fat cells of thin cattle are markedly smaller than those of fat cattle. The increase in the volume of the fat cells already present is sufficient to account for a large part of the increase in the mass of the adipose tissue during fattening, but a numerical increase of the fat-holding cells also occurs.

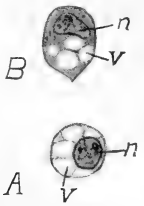
As pointed out above, fat cells with a peripheral protoplasmic margin containing small fat droplets (Text Fig. 11) were found in large numbers in the subcutaneous adipose tissue of several fattening steers. This type of cell is usually comparatively small, and is essentially identical with the fat cells of the fœtus. (Text Fig. 10 and Fig. 10, Plate II.) They are very probably newly-formed fat cells developed from the interstitial cells referred to above.

During the fattening of the animal it is evident that some new fat lobules are formed around old or newly-formed blood vessels.

It can no longer be maintained that the fat cell is a special kind of cell in the sense used by Toldt, distinctly different from the ordi-

nary connective tissue cells. In the ox it seems that in general the blood vessels are important factors determining the development of adipose tissue. The connective tissue cells which happen to lie adjacent to a blood vessel at the time a fat lobule is forming are the ones which become fat cells. In certain situations, however, as on the kidney and in certain parts of the omentum, so-called primitive organs are developed which bear no close relation to the blood vessels. The blood vessels cannot be regarded as the main determining factors here, though they may take part.

There is not, however, a random formation of fat cells in the connective tissues. The lobules of adipose tissue, in which nearly all new fat cells appear, are well-defined structures. It is only in



Text Figure 13. Two leucocytes from the omentum of a 16 cm. fœtus. *n*, nucleus; *v*, vacuole. Stained with iron-hæm. Fixation, Gilson fluid. × 1200.

the early stages of their formation that cells are added to the lobule from the connective tissue adjacent to the periphery. I intend to treat this phase of the subject more fully in a later paper.

Text Fig. 13 shows two leucocytes from the omentum of a 16 cm. fœtus. A great many such cells are present at this stage. They usually contain several vacuoles. By staining with Scarlet red it is seen that these are not fat droplets. It is possible that such cells have lent support to the erroneous conception that leucocytes may form fat cells.

In conclusion I wish to thank Dr. R. R. Bensley for sending me some necessary material. Most of the drawings accompanying this paper are the work of Mr. G. T. Kline; a few were made by Miss McGill.

SUMMARY OF PART II.

A peculiar open-meshed tissue (preadipose) precedes the formation of true adipose tissue. The renal preadipose tissue is formed into well-defined lobules a long time before the true fat cells appear. The masses of preadipose tissue in the omentum are much less sharply marked off than those of the kidney and persist only a short time before the formation of the fat cells begins. The subcutaneous preadipose tissue is found as masses around the blood vessels (Text Fig. 1) and persists only a short time in the preadipose condition.

The preadipose tissue consists of loosely-arranged cells with two or more long coarse processes. A great many fine connective tissue fibers occupy the ground substance of the tissue (Fig. 8, Plate I; Fig. 6, Plate II). The branched cells may contain fat droplets a long time before they begin to assume the rounded form.

The preadipose tissue is clearly a fibrillar connective tissue. My results support Flemming's view that adipose tissue is a modified fibrillar connective tissue.

In the formation of a fat lobule the cells adjacent to the blood vessel are filled with fat first. The filling of the cells with fat extends from the blood vessel outwards in all directions. This process is closely similar to the deposition of fat in the liver where it is deposited first in the cells immediately adjacent to a vein and later into those lying farther out.

The branched preadipose cell becomes rounded by the accumulation of fat in its interior. Its processes are absorbed.

The cell membrane is differentiated from the peripheral protoplasmic layer of the cell. It begins to form when the cell is yet branched.

The Altmann granules are found in the protoplasm of all fat cells. They are first observed when the cell is yet branched and before the first fat droplets are formed.

The mass of adipose tissue increases in amount in fattening (a) by the increase in the size of its cells, (b) by the formation and filling of new cells in the interior of the lobule, (c) by the formation of new lobules.

Appendix.

Since receiving the page proof of this article I have discovered that after formalin fixation, usually only a part and often none at all of the fat-content of muscle fibers and epithelium can be stained with Scarlet red, Sudan, or osmic acid. If the tissue be stained fresh in the stains mentioned above, a considerable amount of fat can be demonstrated in the muscle fibers of a number of animals including the ox; but if the tissue be fixed in formalin a few days it is often impossible to demonstrate any fat in this situation. I have found that after a few days' fixation in formalin little or no fat can be stained in the muscle fibers of the frog or chicken, and with the exception of a few samples the same is true of the ox muscle fibers.

Mr. H. H. Bullard has recently prepared special concentrated solutions of Scarlet red and Sudan which stain a great many more droplets in muscle fibers and epithelium than are brought out by the ordinary stains. We have not yet decided whether all the droplets brought out by Bullard's stain are neutral fat, but it seems probable that they are all of a fatty nature.

The use of Bullard's stains on fresh material gives such splendid pictures that it seems certain there must be a revision of all the work that has been done on the fat-content of tissues both physiological and pathological.

In a short time papers will appear setting forth the results mentioned above.

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EXPLANATION OF PLATES.

PLATE I.

FIG. 1. From a section of the liver of a 7 cm. fœtus. The fat droplets in this and the succeeding figures are colored red. *an*, annular fat droplet; *h*, hepatic cell—those near the vein (*V*) are filled with fat droplets. Fixation, 20 per cent formalin. Frozen section stained with Scarlet red and hæm. × 800.

FIG. 2. Cartilage cells from the centrum of a vertebra of a 10.1 cm. fœtus. Fixation, 20 per cent formalin. Frozen section stained with Scarlet red. × 1300.

FIGS. 3 and 4. Cross-section of muscle fibers of psoas muscle of a 17 cm. fœtus, showing fat droplets in the interior. Fixation, 20 per cent formalin. Frozen section stained with Scarlet red. × 1300.

FIG. 5. Cartilage cell from the innominate cartilage of a 17 cm. fœtus, showing many fat droplets. Fixation, 20 per cent formalin. Stained with Scarlet red and hæm. × 1300.

FIG. 8. Renal preadipose tissue from an 18.5 cm. fœtus. *f*, fine fibrillæ; *n*, nucleus; *pr*, process of cell; *s*, space. Fixation, Zenker's fluid. Stained heavily with iron-hæm. and not decolorized. × 800.

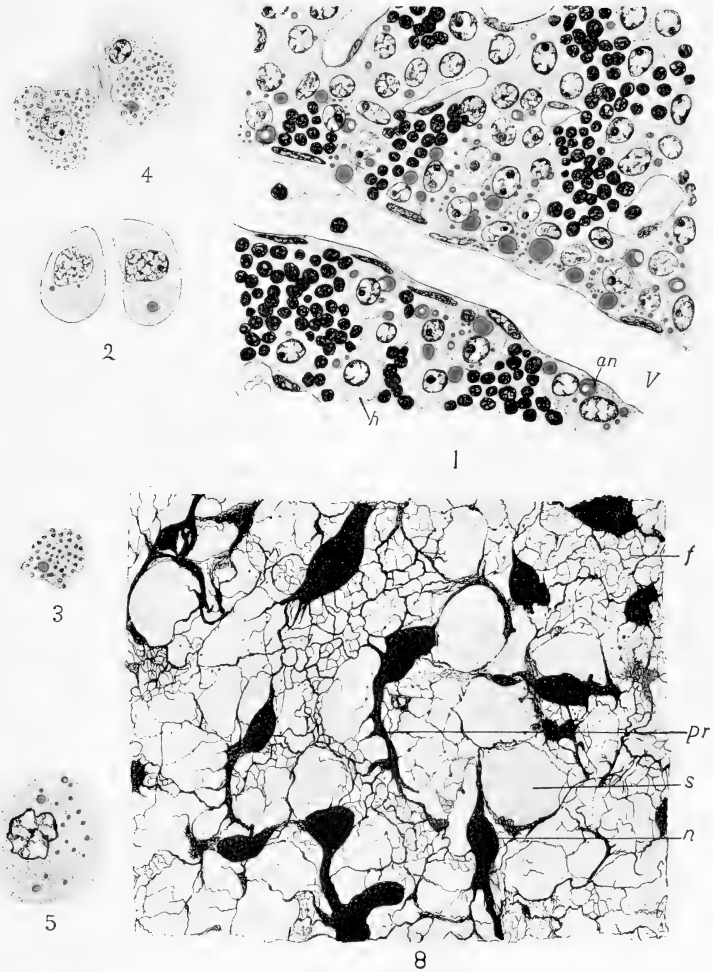
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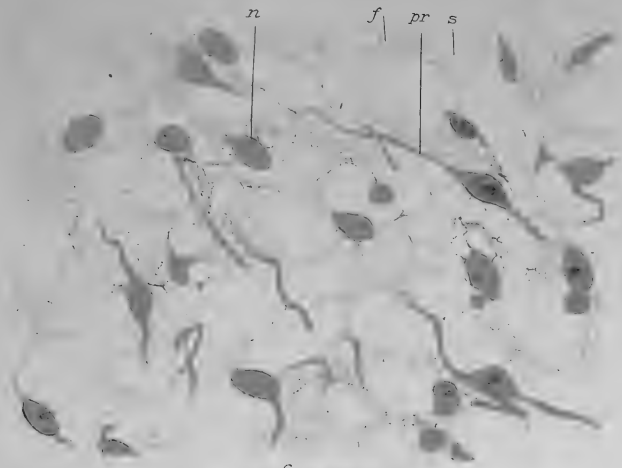
Plate II.

Fig. 6. Renal preadipose tissue from an 18.5 cm. fœtus. *f*, fine fibrillæ; *n*, nucleus; *pr*, cell process; *s*, space. Fixation, Zenker's fluid. Mallory's anilin blue. × 1200.

Fig. 7. *a*, *b*, *c*, *d*. Cells of the renal preadipose tissue of a 24.7 cm. fœtus, showing fat droplets. Fixation, 20 per cent formalin. Frozen section stained with scarlet red. × 1700.

Fig. 9. Preadipose tissue of the omentum of a 40 cm. fœtus. *f*, fine fibrillæ; *bc*, blood capillary; *cf*, coarse collagenous fiber; *n*, nucleus; *pr*, process of cell; *s*, space. Fixation, Zenker's fluid. Mallory's anilin blue. × 1300.

Fig. 10. Inguinal adipose tissue from a 32 cm. fœtus. *cf*. collagenous fibrillæ; *f*, spaces occupied by fat; *n*, nucleus. Fixation, Gilson's fluid. Stained with Mallory's anilin blue. × 1200.



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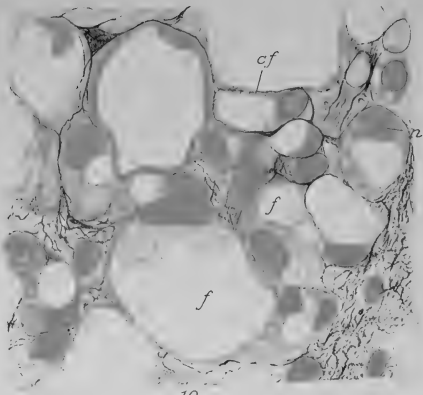

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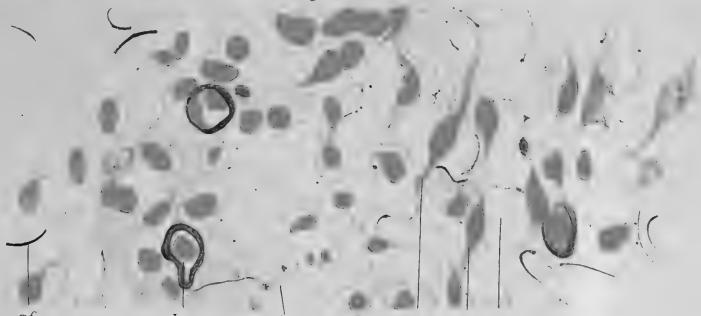

ON THE DEVELOPMENT OF THE SUPERFICIAL VEINS OF THE BODY WALL IN THE PIG.

BY

HELEN WILLISTON SMITH.

From the Anatomical Laboratory of the Johns Hopkins University.

WITH 11 FIGURES.

Satisfactory studies of the vascular system of young embryos have been impossible until quite recently, for the embryologist has been unable to see much more than mere fragments of the growing ends of this system in embryos treated by the usual methods of preparation. During the past few years the method of injection of the vascular system of young embryos has been perfected more and more in this laboratory, so that now we are able to procure complete injections in the very youngest stages. In order to make proper headway in the study of the morphology of the vascular system it is necessary to study the development of the primitive vessels in the embryos. For instance, more fundamental conditions can be obtained for the study of development in the umbilical vein, than in the vessels of an organ; in the former case there is but one vessel to be followed, while in the latter there are millions, and it is practically impossible to find the same terminal twig from stage to stage.

The key to the situation is obtained when the main trunks and all their branches are brought out sharply by means of injection in their very earliest and in subsequent stages. This has now been accomplished by a number of investigators in this laboratory, most successfully, probably, by Dr. Evans, who injected many of the embryos I have studied.

In order to obtain perfect injections it is necessary to inject live embryos, and, in addition to tadpoles and chicks, an abundance of

pigs of various stages of development are available every day. In the latter case the injection of India ink is made into the umbilical artery towards the heart which, while beating, enables one to obtain perfect injections.

Studies in this laboratory have shown conclusively that the entire vascular system is developed from a common plexus of capillaries which gradually extends over the whole body, a part of which is transformed into arteries and a part into veins. Throughout development the vessels are functioning, and the formation of arteries and veins is only an expression of the law of functional adaptation of the extensive capillary plexus peripheral to a beating heart. How could it be otherwise, for the arteries and veins are not formed step by step by sprouts, or by the union of independent anlages, but they are functioning from the time of their simplest beginning until the animal dies.

The present study was undertaken, at the request of Dr. Mall, in order to follow, in a relatively simple field, the gradual evolution of the vascular system from its first appearance until its adult form is reached. This study is one of a series, two of which by Dr. Evans are now in press, and others are in preparation. If circumstance will permit I hope to follow this with an account of the development of the deeper vessels of the body wall.[1]

We find in the earliest stages considered in this paper a relatively simple circulation in the body wall, one in which the posterior cardinal and the umbilical veins are both formed, but have comparatively few ramifications. As the embryo grows the limb buds appear and the membrana reuniens closes in around the umbilical

[1]The literature on the standpoint taken in this study is as follows: Aeby, Der Bau des menschl. Körpers, 1871; Baader, Inaug. Diss., Bern, 1866; Thoma, Untersuch. über die Histogenese und Histomechanik des Gefässsystems, 1893; Flint, Johns Hopkins Hospital Reports, IX, 1900, Amer. Jour. Anat., II, 1903, and VI, 1907; Mall, Amer. Jour. Anat., IV., 1905, and V, 1906; Rabl, Arch. f. m. Anat., LXIX, 1907; Evans, Anatomical Record, II, 1908, and Amer. Jour. Anat., IX, 1909.

The specimens on which the present article is based were demonstrated at the last meeting of the Association of American Anatomists, Baltimore, December, 1908.

cord. The posterior cardinal and the umbilical veins share in draining the limb buds, one dorsally, the other ventrally, and the umbilical drains as well a plexus which forms in the membrana reuniens. As the posterior cardinal vein sinks into the depth, though it continues to partly drain the limb buds and to receive its segmentals from the myotomes, the relatively vast area of the membrana reuniens drains entirely into the umbilical vein. Then, as the plexus in the membrana increases in complexity and the muscle layer shifts into it ventrally, larger longitudinal anastomoses form along the body wall draining up under the anterior limb bud to the vessels there, which connect in turn with the cardinal veins. Soon a definite vessel is formed, the thoraco-epigastric, which increases rapidly in size until it collects to itself almost all the tributaries of the umbilical vein. The plexus in the membrana reuniens, in consequence, gradually dies out until only a few vessels in the median line and in the lower ventral region remain. Meanwhile on the mesial side of the muscle layer and ribs, other vessels, namely, the internal mammary and the deep epigastric veins and arteries have been formed from a longitudinal plexus, and the intercostal vessels have been spun out like strands of cobweb in the intercostal spaces. All these vessels not only anastomose among themselves, but also have very numerous communications with the superficial vessels. Finally, it comes about, by changes given in detail later, that the thoraco epigastric loses its axillary connections, and drains into the internal mammary. After this the vessels of the body wall are not changed in kind, but in degree only, and the condition of the adult is practically achieved.

Turning to the literature concerning the development of the superficial blood vessels of the body wall, we find a number of observations upon the subject, but these are given, in great part, either as isolated or unexplained steps, or in an attempt, more or less satisfactory, to explain the condition in the adult. These latter observations, for the most part, are made upon fœtuses, and so only a clue can be gleaned here and there concerning the early development. Among observations upon very young embryos are those of Coste, Kölliker, His, and Mall.

His describes a human embryo in which the membrana reuniens contains vessels emptying into the sinus reuniens above, and into the umbilical vein below. This is a condition found in pig embryos of about 7 mm. and one that is easily interpreted by a study of younger and older stages. This observation is confirmed and extended by Mall, who says, "It appears, then, that during the third week of development, while the umbilical veins still empty into the sinus reuniens, an extensive plexus is formed throughout the greater extent of the membrana reuniens, which receives blood from the aorta on its dorsal side, and empties into the umbilical vein on its ventral side. As the umbilical vein changes its position to enter the liver, this circulation through the membrana reuniens is broken up as a much earlier circulation through the umbilical vesicle was broken up."

Kölliker and Coste give a description of a somewhat older type. Kölliker pictures a cow's embryo in which the membrana reuniens is filled with a minute plexus radiating from the myotomes to the umbilical vein, and Wertheimer quotes Coste as follows, speaking of the vessels in the abdominal wall of the adult: "Ces vaisseaux sont les restes du riche appareil veineux transitoire, qui des parois abdominales sur lesquelles ils étaient repandus, se portent vers la veine ombilicale ou allantoïdienne droit dans laquelle ils pénètrent par une foule de troncs, placé les uns à côté des autres. Ils suivent la destinée de la veine qui les reçoit et s'éteignent complètement avec elle."

That I am correct in naming the vessel described above as the thoraco epigastric is evident upon comparison with the thoraco epigastric in the human adult. This is a vein subject to considerable variation, and described somewhat differently in the text-books. Toldt and Spalteholz and Piersol describe it as extending subcutaneously from the superficial epigastric vein, on the anterior and lateral surface of the trunk, to enter the long thoracic. Sabotta says it may either enter the long thoracic or the axillary vessel directly. In this laboratory I have examined a number of cadavers which show variations, agreeing for the most part with the two types given by Sabotta. The thoraco epigastric vein is unques-

tionably the same as that described by F. T. Lewis in rabbit embryos, and called by him the external mammary vein. (Amer. Jour. Anat., 1906.)*

In general we may say that the thoraco epigastric is a vessel connecting the veins of the epigastric plexus with the axillary veins. This is exactly the condition of the vein in the pig embryo of about 20 mm., and though in the adult we find it has undergone further change, embryologically it must be regarded as the same vessel.

Joris, after speaking of this same stage, says, "Il est donc un moment où les veins umbilicales représentent les seul troncs collecteurs des vaisseaux pariétaux, enfin, les veines pariétales perdent leur troncs collecteurs par l'atrophie de la partie supérieure des veins ombilicales, et finissent par se rattacher au système veineux cave." There is never a time in the embryo pig in which the umbilical vein drains the whole body wall, taken in a broad sense to include the limb buds, but it is certainly the chief collecting vessel for a considerable period. Finally the vessels emptying into it do atrophy, as the blood flowing from the body wall is directed into the cardinal veins. The method by which this is effected is, as I have said, chiefly through the development of the thoraco epigastric vein, the description of the origin, growth and permanent condition of which gives the connecting links between stages in the embryo and those of the adult.

Concerning the adult condition in man, especially in the ventral body wall, much has been written, and though an account of that literature does not come strictly within the bounds of this paper, it may be of interest to give a brief résumé of it here. It deals chiefly with the question whether or not the umbilical vein remains patent, and the various points of communication between the portal and systemic circulation. There is considerable difference of opinion, due perhaps to the different methods of attack upon the problem, and to the great amount of anatomical variation which unquestionably must exist.

*Since this article went to press Dr. Lewis has also adopted the term thoraco epigastric. Amer. Jour. Anat., Vol. IX, No. 1, Feb., 1909.

The names chiefly associated with this work are those of Robin, Baumgarten, Wertheimer, Sappey, Burow, Pfeifer, His, and recently Joris.

I have not seen Robin's papers, but he is quoted by most of the other authors, generally with more or less indignant sorrow because of the incorrectness of his views, namely, that the umbilical vein never receives vessels from the abdominal wall in the fœtus, and is completely obliterated after birth.

Baumgarten, on the other hand, according to Pfeifer and Joris, admits the existence of collateral veins in the adult, describes their arrangement as constant and normal and says that the part of the umbilical vein remaining patent is connected with the deep epigastric veins.

Wertheimer comes to the conclusion, from injections of thirteen fœtuses and young infants in the hepatic extremity of the umbilical vein, that ordinarily the umbilical vein becomes absolutely occluded. However, there is a venule of later formation running in the obliterated cord in the adult, and he admits that cases may exist in which the umbilical vein remains open permanently. This possibility he explains by reference to comparative anatomy, for in amphibians the umbilical vein persists as the anterior abdominal vein.

Sappey is somewhat more positive than Wertheimer in stating that the umbilical vein is absolutely occluded in the adult, but he says accessory veins exist, which he divides into a superior and an inferior group.

"Le groupe supérieur est constitué par des veinules que descendent de la partie médiane du diaphragme vers la face convexe du foie et qui viennent se distribuer sur les lobules auxquels adhère le ligament suspenseur. Par une de leurs extrémités, ces veinules communiquent avec les veines diaphragmatiques, et par l'autre avec les divisions sus-lobulaires de la veine porte.

"Le groupe inférieur comprend toute une série de veinules qui se portent de la partie sus-ombilicale de la paroi abdominale antérieure vers le sillon longitudinal du foie. Ces dernières, comprises dans la partie du ligament suspenseur qui renferme le cordon de la

veine ombilicale, se trouvent en communication, à leur origine, avec les veines épigastriques et les veines tégumenteuses de l'abdomen."

Burow describes a vein formed from the union of branches from the right and left epigastric. This vein passes up towards the liver and enters the upper part of the umbilical vein. Joris maintains that this vein is the same one as that described by Sappey, with the difference that in the one case the vein enters the liver, and in the other enters the unobliterated portion of the umbilical vein just before it reaches the liver. He says, moreover, that the right as well as the left umbilical vein may persist in the adult as a communication between the liver and the right epigastric vein. His work was done by injecting the portal vein after ligature of the vena cava above and below the liver. These injections were made on fœtuses two months old and older, and on a few infants.

His has made a classification of these veins as follows:

"(1) VV parumbilicales (Sappeyi) welche von der Nabelgegend aus zur Leber emporsteigen und in deren Substanz sich einsenken.

"(2) V supra umbilicalis (Baumgarten's Burowsche Vene) welche in das obere, offen gebliebene Ende der V. umbilicalis einmündet.

"(3) VV umbilicovesicales. (Braunes' Burowsche Venen.)

"(4.) VV umbilicoepigastricae, welche beiderseits in die VV epigastricae inferiores profundae einmünden."

Summing up these observations it seems evident that ordinarily there are some small venules running from the region of the diaphragm to the umbilicus, and that these connect the portal system with small branches of the deep epigastrics.

In pigs about three centimeters, in which all the vessels of the membrana reuniens appear to have atrophied, sections show that small vessels pass from the liver in the median line to the umbilical vein, and that there are also some twigs connecting the umbilical vein with the plexus in the region of the epigastric veins. However, from a study of the vessels of the membrana reuniens in younger embryos it is evident that the probability of variation is great which naturally complicates studies of this kind very much.

The smallest injected embryo considered here is one about 6 mm. (Fig. 1). It may be noted that this specimen is twisted upon

itself through a considerable angle. This twisting is probably not normal, but it permits of a very good view of the blood vessels, and this embryo has therefore been selected for illustration. The strik-

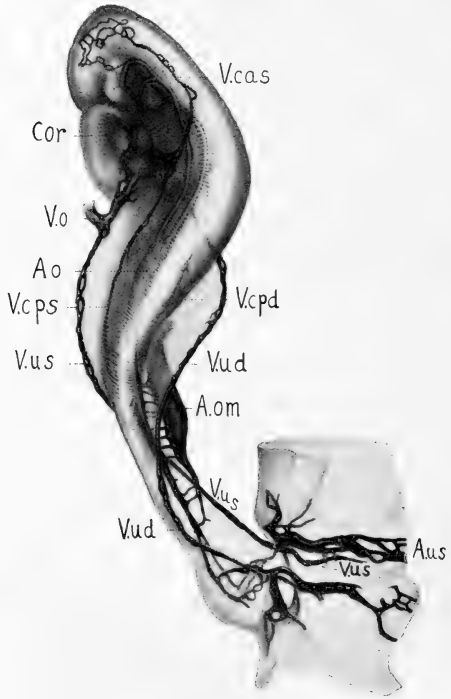

Fig. 1.—Embryo 6 mm. long. Enlarged 17 times.

Cor, heart; *V.o*, omphalomesenteric vein; *Ao*, aorta; *V.cps*, left posterior cardinal vein; *V.us*, left umbilical vein; *V.ud*, right umbilical vein; *V.cas*, left anterior cardinal vein; *V.cpd*, right posterior cardinal vein; *A.om*, omphalomesenteric artery; *A.us*, left umbilical artery.

ing thing about it is the extreme simplicity of its vascular system. The aorta is large. It gives off a number of spicule-like vessels, many of which on the ventral side unite to form the omphalo-

mesenteric artery, a conspicuous, bulky vessel that increases in size as it passes anteriorly. Just below the omphalomesenteric artery the aorta divides into two vessels which in turn break up into two groups of capillaries, each of which reunites to form an umbilical artery. In the head region there are only two branchial arches formed. From the anterior of these a branch is given off which passes into the capillaries that unite to form the anterior cardinal vein (V. cas.). The anterior cardinal vein runs back to meet the posterior cardinal (V. cps.) and the two form a plexiform union before they enter the heart together. The posterior cardinal cannot be traced below the mesonephros, but it is possible that the injection is incomplete as in embryos a very little older than this it extends dorsally to the posterior limb bud. The umbilical veins are plainly visible, running from the allantois along the edge of the membrana reuniens to the sinus reuniens which they enter together with the omphalomesenteric veins. (Vv. om.) These vessels are not clean cut, but show clearly their plexiform origin. Along the left umbilical particularly, we see many loops and openings. There are also some spicule-like projections here and there along the course of the umbilical veins that form the anlage of the future plexus of the membrana reuniens.

The second embryo, 7 mm., pictured here in Fig. 2, corresponds roughly, as I have said, with that described by His. Probably the most striking thing about it is the size of the umbilical veins (Vvu). In the figure the right vein is seen to run from the cord in a long curve for the whole length of the mesonephros to the liver. At a point very little below the liver, a relatively small vein is seen looping up superficially and emptying at the anterior portion of the liver into the sinus reuniens (SR) above. This vessel is a part of the umbilical that does not sink into the depth, and receives numerous tributaries from the arm bud. The arm bud, however, drains also into the posterior cardinal vein (Vcp) above, and below by five good connections, directly into the large umbilical vein (Vud). Below these connections, until the posterior limb bud is reached, there is, as yet, only a very narrow strip of body wall to drain. Such few vessels as there are here run into the

posterior cardinal (Vcp), but lower down there is a very rich plexus, lying ventral to the anlage of the posterior limb bud, which communicates freely with a similar plexus on the opposite side of the

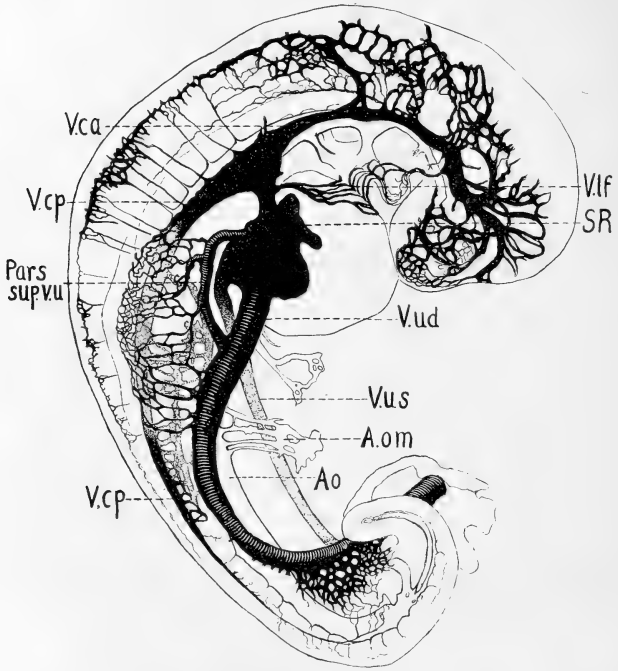

Fig. 2.—Embryo 7 mm. long, in which the injection is complete. The vessels in the head are semi-diagrammatic. Enlarged 21 times.

V.lf, linguo facial vein; *SR*, sinus reuniens; *V.ud*, right umbilical vein; vein; *V.us*, left umbilical vein; *A.om*, omphalomesenteric artery; *Ao*, aorta; *V.ca*, anterior cardinal vein; *V.cp*, posterior cardinal vein; *Pars sup. V.u*, superficial part of umbilical vein.

body, and as the figure shows, with its respective umbilical vein. Dorsal to the posterior limb bud the posterior cardinal (Vep) arises from numerous capillaries which unite to form a vein running along

the body wall. This receives some small veins and finally sinks into the depth under the anterior limb bud, receives two or three twigs from it, and uniting with the anterior cardinal (Vca) enters the sinus reuniens in a graceful, sweeping curve. The sinus reuniens also receives a vein, the inferior jugular, formed by the union of a number of capillaries which rise in gill arches, where they anastomose with twigs to the anterior cardinal. The veins that form the anterior cardinal (Vca) are very large and striking, particularly the one which curves in a half circle above the anastomosing tips of the cervical segmental arteries to enter the anterior cardinal. A chain of anastomoses along the spinal cord, formed from the tips of the segmental arteries passes from the region of the head to a point below the anterior limb bud. This plexus drains back into the posterior cardinal vein through its segmentals. The aorta is enormous. Three aortic arches are shown. The most anterior of which is not well injected. The omphalomesenteric artery (Aom) is represented by four vessels which unite at some little distance from their origin. In the tail the aorta divides into two vessels which anastomose at the tip.

The next embryo, Fig. 3, is a very little larger than that just described, the chief point of difference being the presence of a thick capillary mesh in the membrana reuniens.

As before, there remains a superficial part of the umbilical vein (Vud) draining the posterior limb bud, which also drains largely into the posterior cardinal vein. The limb bud contains a fine plexus of veins which tend to form a border vein, while dorsal to it the well-developed mesh runs out upon the body wall to unite with the general plexus of the membrana reuniens. The plexus of the membrana reuniens is evidently the same as that described by Coste. It is very characteristic of the membrana, being made up of comparatively large vessels anastomosing among themselves, but for the most part passing very directly to the umbilical vein which they enter by parallel veins.

The next embryo in the series, Fig. 4, shows these vessels even better developed, since the membrana has progressed farther. It represents most completely the phase in the body wall when it drains

chiefly into the umbilical vein. The lower part of the membrana reuniens (MR) as high as the point where the umbilical vein enters the liver, is seen to be full of veins. These rise from a fine network of capillaries which is fed by the segmental arteries and extends

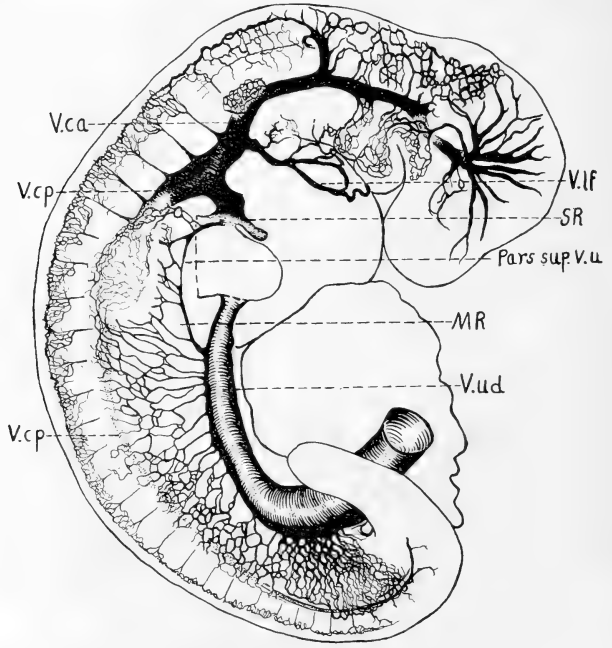

Fig. 3.—Embryo 8 mm. long. Enlarged 14 times.
Only the superficial vessels are shown.
Vlf. linguo-facial vein; *SR.* sinus reuniens; *Pars sup. V.u,* superficial portion of umbilical vein; *MR.* membrana reuniens; *V.ud,* right umbilical vein; *V.ca,* anterior cardinal vein; *V.cp,* posterior cardinal vein.

in a crescent shape, with the concavity directed ventrally between the two limb buds. In the lower portion, this network is entirely irregular, but higher up it forms a more or less connected plexus which passes partly into another plexus extending ventrally to the

anterior limb bud out towards the region over the liver, and partly, into two distinct, though very fine ropes of capillaries running up under the anterior limb bud, the more dorsal of which is the anlage of the thoraco epigastric vein (Vte). Over most of the

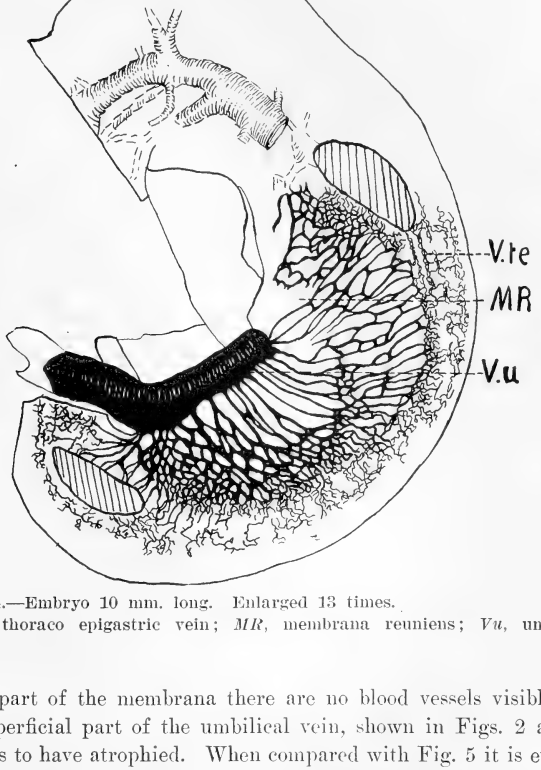

Fig. 4.—Embryo 10 mm. long. Enlarged 13 times.
V.te, thoraco epigastric vein; *MR*, membrana reuniens; *Vu*, umbilical vein.

upper part of the membrana there are no blood vessels visible and the superficial part of the umbilical vein, shown in Figs. 2 and 3, appears to have atrophied. When compared with Fig. 5 it is evident that the system draining into the umbilical vein is receding and giving place to one draining longitudinally between the limb buds.

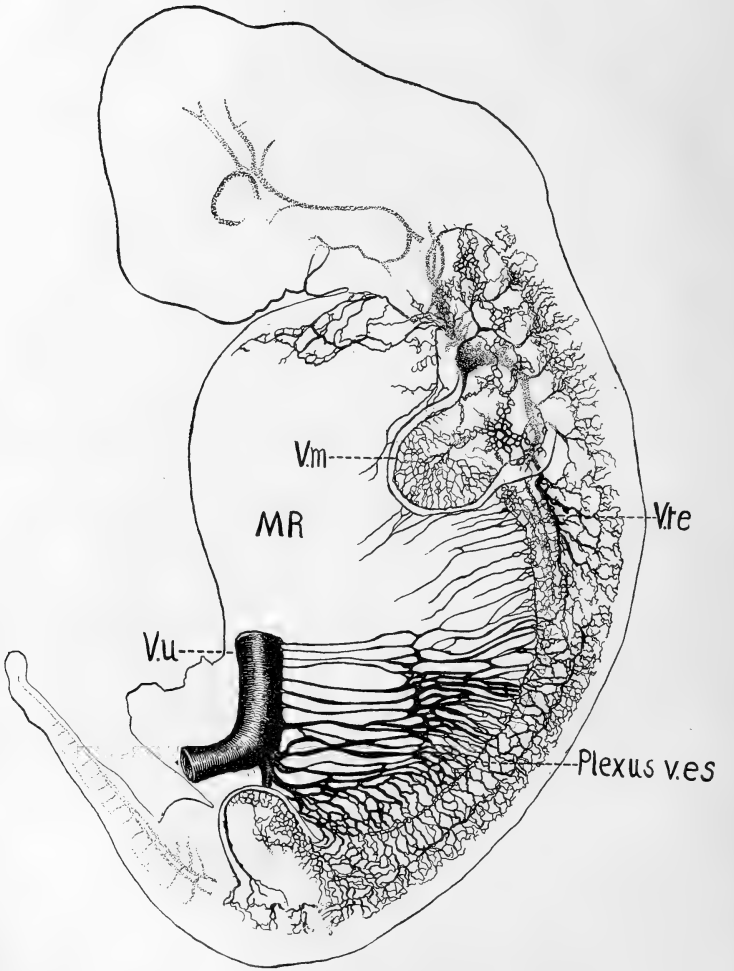

Fig. 5.—Embryo 15½ mm. long. Enlarged 10 times.
A few vessels in the depth are dotted.
V.m, border vein; *MR*, membrana reuniens; *V.te*, thoraco epigastric vein; *Ves*, superficial epigastric vein; *V.u*, umbilical vein.

There are still many blood vessels in the membrana (MR), but they show a tendency towards atrophy. The thoraco epigastric (Vte), on the other hand, which in the previous figure (Fig. 4) was nothing but a thread of capillaries, in Fig. 5 shows as a very distinct vessel passing up under the limb bud, receiving the primitive ulna, and running on up to enter the posterior cardinal at its junction with the anterior cardinal. The central connections of the thoraco epigastric, in this figure, show very well, but the picture is not always so uncomplicated. It will be noticed that the vein runs dorsal to the artery while in the adult it is normally ventral. The change is effected as follows. There is at the root of the arm bud a capillary mesh surrounding the artery connected above with the cardinals at their junction and below with the primitive ulnar. In young stages the dorsal portion of this capillary mesh or loop is often the more prominent, but, later, the blood tends to take the ventral short cut and the dorsal part astrophies. The thoraco epigastric may be looked upon as a continuation of this mesh extending out upon the body wall. It drains a small area, below and dorsal to the limb bud and has numerous connections with the capillaries supplying the lower part of the membrana reuniens. These latter capillaries, it will be noticed, show a tendency to form into a long rope-like plexus, running from the region ventral to the posterior limb bud up towards the anterior limb bud. This rope of capillaries later forms the superficial epigastric (Ves) and may therefore be considered as part of the permanent type.

That the injection over the heart of this embryo is not complete is very probable because sections of this same stage show injection all over the upper heart region. This is shown in Fig. 6. Here, on either side of the U-shaped aortic arches, we find good-sized veins that empty into the internal jugulars (Vji). These veins drain the upper part of the membrana over a bib-shaped area, somewhat greater than is shown in the section, and anastomose on the sides with veins that enter the posterior cardinal at its junction with the anterior cardinal. Fig. 7 is from a section of this same embryo at the level of the omphalomesenteric artery and shows the way in which the blood is supplied to the muscle layer and to the body wall. The

paired segmental arteries are seen passing out at a wide angle from each other, and each breaking up into a tuft of vessels, twigs of which run into the inner and outer side of the muscle layer and to the anterior spinal artery and cord. These twigs pass into fine capillaries which may then be traced into the veins. The venous blood is car-

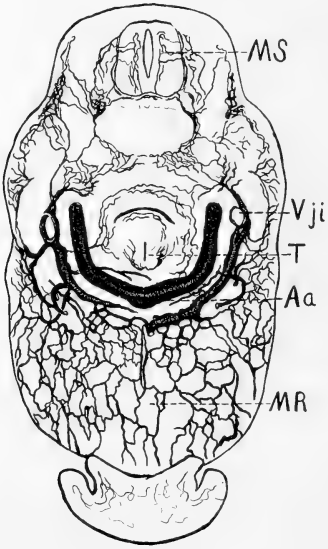

Fig. 6.—Section one-half millimeter thick of an embryo 15½ millimeters long.

MS, spinal cord; *V.ji*, internal jugular vein; *T*, trachea; *Aa*, fourth aortic arches; *MR*, membrana reuniens.

ried off in one of three ways. Either it may run through the thoraco epigastric to the posterior cardinal, or it may run into the membrana to the umbilical vein, or it may run back through the dorsal segmental veins into the mesonephros to the posterior cardinal veins (Vcp). The sections of this embryo also show that at this stage the internal mammary vein and artery are not present, as such, but that

the capillaries on the mesial side of the muscle layer have a tendency to unite and knot together to form a chain, the upper end of which runs into the posterior cardinal. The artery is represented by a few fine capillaries which run from the subclavian artery out under the arch of the thoraco epigastric.

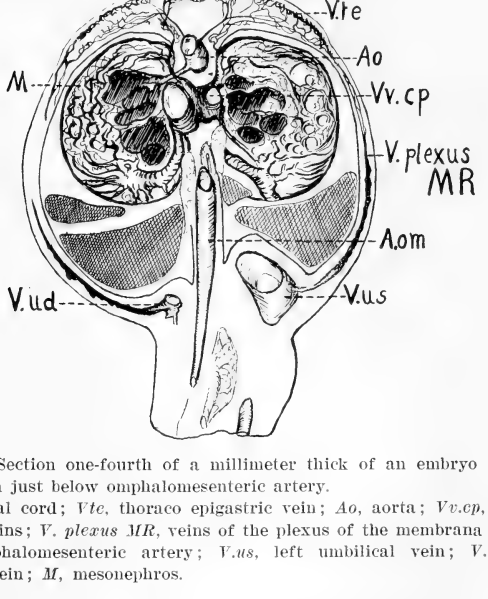

FIG. 7.—Section one-fourth of a millimeter thick of an embryo 15½ mm. long; taken just below omphalomesenteric artery.

MS, spinal cord; *Vte*, thoraco epigastric vein; *Ao*, aorta; *Vv.cp*, posterior cardinal veins; *V. plexus MR*, veins of the plexus of the membrana reuniens; *A.om*, omphalomesenteric artery; *V.us*, left umbilical vein; *V.ud*, right umbilical vein; *M*, mesonephros.

From this stage on, however, the internal mammary vein and artery as well as the thoraco epigastric and the plexus of the superficial epigastric developed rapidly. This further development is seen in Fig. 8. The thoraco epigastric (Vte) and superficial epigastric plexus (Ves) are now draining practically all of the dorsal side of the muscle layer of the body wall which has grown considerably farther

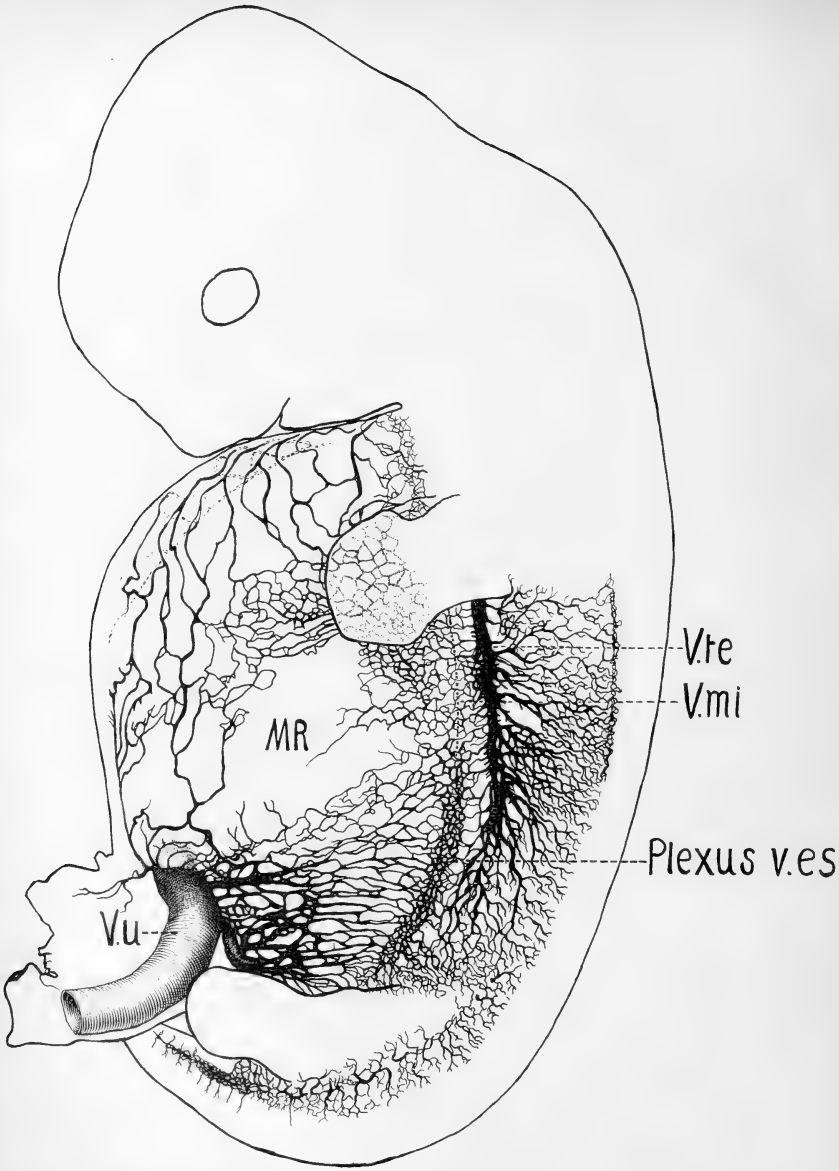

FIG. 8.—Embryo 18 mm. long. Enlarged 12 times.
Only most superficial vessels are shown.
V.te, thoraco epigastric vein; *V.mi*, internal mammary vein; *MR*, membrana reuniens; *Plexus V.es*, plexus of superficial epigastric vein; *V.u*, umbilical vein.

forward carrying the blood vessels with it. The internal mammary (Vmi) is represented in the figure by a straight line ventral to the thoraco epigastric (Vte). It lies on the mesial side of the muscle layer and has very numerous capillary connections with the thoraco epigastric. Posteriorly it anastomoses with the plexus of the deep epigastric, which runs beneath the superficial epigastric and is not shown in the drawing. The membrana reuniens is still large and well supplied with blood vessels, though it is evident that less blood runs from the body wall to the unbilical vein than formerly. As the internal mammary and thoraco epigastric veins now lie, their paths to the heart are about equal in length, and it is natural therefore that blood supplied to the outer side of the muscle layer should pass back through the thoraco epigastric and that to the inner, through the internal mammary. It is evident, however, that, as the muscle layer grows forward, it will carry with it the internal mammary. The thoraco epigastric being an axillary vessel must still continue to empty into the axilla and therefore were the lower part of the vessel carried forward, the course of the blood through it, on the outer side of the muscle layer, would become more round about than that through the internal mammary. It is therefore to be expected that the blood, following the path of least resistance, will tend to flow from the lower part of the thoraco epigastric through the numerous connections into the internal mammary and that, these vessels enlarging in consequence, the path to the internal mammary will become so easy that practically all the blood from the lower outer body wall will pass that way. This is what proves to be the case. In embryos about 18 or 19 mm. long the thoraco epigastric, as such, reaches its maximal development. Then it drains practically all the outer body wall between the limb buds, back as far as the circulation connected with the spinal cord, while ventrally it receives vessels from the membrana and anastomoses very frequently with the superficial epigastric and with the internal mammary. These anastomoses grow larger so that while the more posterior part of the thoraco epigastric becomes practically continuous with the superficial epigastric, anteriorly it begins to drain largely into the internal mammary. At this latter point a characteristic elbow is usually formed from which, as is

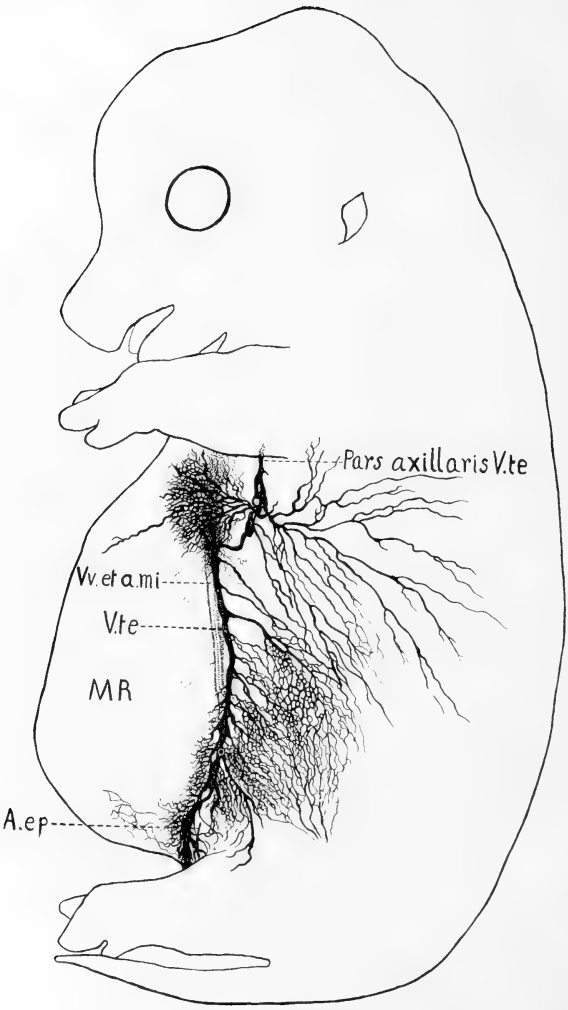

FIG. 9.—Embryo about 3 cm. long. Enlarged 6 times.

All vessels except those immediately connected with the thoraco epigastric vein are omitted.

Pars axillaris V.te, axillary portion of thoraco epigastric vein; *Vv. et a.mi*, internal mammary veins and artery; *V.te*, thoraco epigastric vein; *MR*, membrana reuniens; *A.ep*, deep epigastric artery.

shown in Fig. 9, vessels pass to the internal mammary. Only one of these persists in older stages. There are all manner of variations as to the proportion of the thoraco epigastric that is left draining into the axilla. Sometimes almost the whole vessel is taken over completely and sometimes, as in Figure 9, a considerable portion is left. This figure shows very well the transition stage of the thoraco epigastric. The vessel is seen passing on along the edge of the muscle layer to a point where a fan-shaped plexus of vessels conceals its anastomoses with the internal mammary. Here it swings in a long loop back to its axillary connection. This loop is, however, noticably smaller than the vessel below the turn, and it is evident that a good deal of the blood has already been deflected into the internal mammary. This connection with the internal mammary is plainly shown in the next figure. (Fig. 10). It also shows how far the internal mammary has developed (AmiVvmi), that it is now a double vein with the artery running between, receiving anterior intercostal veins, which anastomose with the intercostals proper. It also receives a great many fine, anastomosing vessels, which have been dissected away from the upper part of the membrana reuniens. The membrana is still of considerable size but the blood vessels are of a very feeble type compared with the earlier ones, and drain almost entirely back to the body wall through the superficial and deep vessels. As the membrana grows smaller and smaller, the blood vessels on the surface atrophy until only those along the edge of the advancing muscle layer are left visible. This is shown in figure 11, which is from an embryo 3 cm. long. This embryo illustrates chiefly, however, a case where almost all the thoraco epigastric along with the superficial epigastric with which it forms a continuous vessel, drains into the internal mammary. The internal mammary has been carried forward, while the stump of the thoraco epigastric has been left well back in the axilla. This is a very typical case. The chief variation being, as I said before, in the proportion of the thoraco epigastric taken over to the internal mammary. As the membrana continues to recede, and the muscle layer advance, the internal mammary vessels are carried nearer together and along with them the superficial veins, so that ultimately we find the transferred portion of the

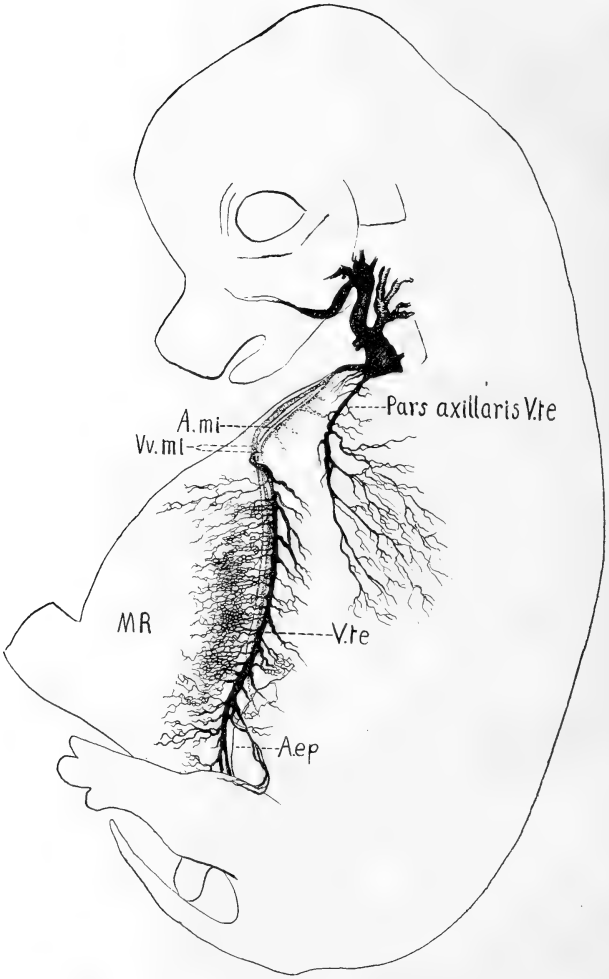

FIG. 10.—Embryo 3 cm. long. Enlarged about 5 times.
The anterior limb is removed.
Pars axillaris V.te, axillary portion of thoraco epigastric vein; *Vv. et a.mi*, internal mammary artery; *Vv.mi*, internal mammary veins; *MR*, membrana reuniens; *V.te*, thoraco epigastric vein; *A.ep*, deep epigastric artery.

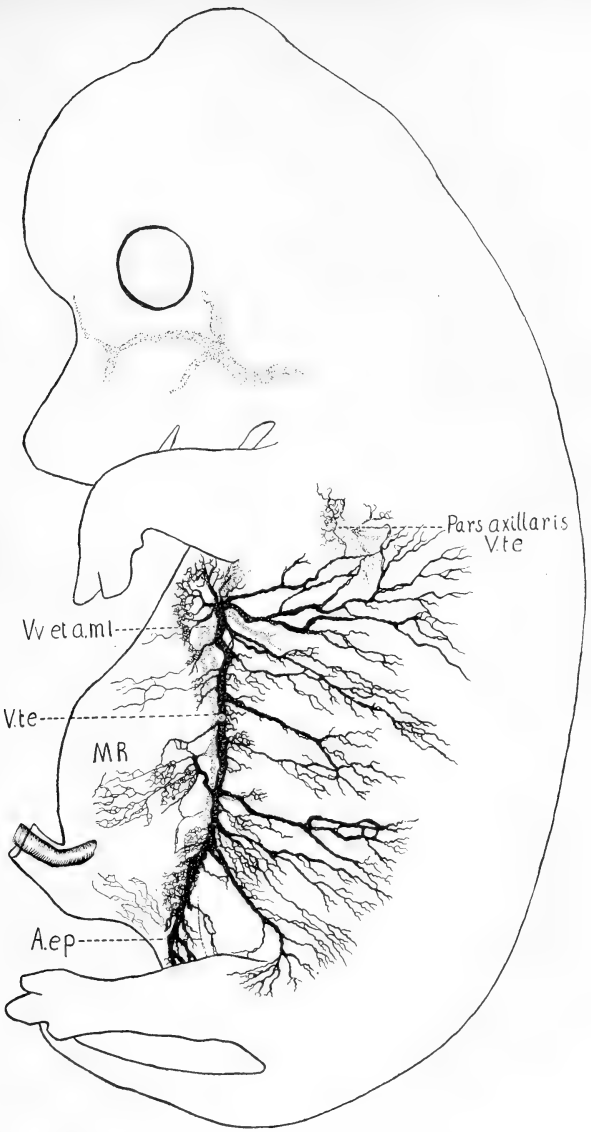

FIG. 11.—Embryo 3 cm. long. Enlarged 6 times.

Pars axillaris V.te, axillary portion of thoraco epigastric vein; *Vv. et a.mi*, internal mammary artery and veins; *V.te*, thoraco epigastric vein; *MR*, membrana reuniens; *Aep*, deep epigastric artery.

thoraco epigastric as a very superficial vessel that may be seen plainly in uninjected specimens, running from its forked origin near the hind leg, along the milk ridge up to the manubrium at which point it dips sharply down to enter the internal mammary vein. This completes the history of the changes in the superficial blood vessels of the body wall.

During this development, four circulations obtained in the superficial layers. First, the circulation in which the posterior cardinal plays a part. Second, the circulation across the membrana reuniens to the umbilical vein; then, thirdly, the formation and growth of the thoraco epigastric and superficial epigastric on the outer side of the muscle layer; and somewhat later, that of the internal mammary veins and artery on the mesial side. The thoraco epigastric grows with the superficial epigastric until they drain the whole superficial body wall dorsalwards as far as the spinal cord. Meanwhile the internal mammary vessels have grown. The intercostal veins and arteries also have been spun out of the plexuses of the segmental vessels, as the muscles and ribs invade the membrana reuniens, and they anastomose with the anterior intercostals from the internal mammary vessels. The internal mammary vein increases in size. Its connections with the thoraco epigastric increase also, and gradually the end of the thoraco epigastric is switched off, and we reach the fourth and final stage in which the superficial body wall drains largely into the internal mammary vein. After this condition is reached further change is not so much in kind as in degree.

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THE VASCULARIZATION OF THE HUMAN TESTIS.

BY

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From the Anatomical Laboratory of the Johns Hopkins University.

WITH 9 FIGURES.

The literature of investigations on the vascularization of the testis is very meagre, the absence of recent studies of the blood supply of the testis being evident in studying text books on anatomy. The descriptions in these books are taken from Arnold,[1] who in 1847 published the first serious attempt at a solution of the blood supply to this gland.

Shortly after Arnold's publication Huschke and Kölliker added to a small degree to his description, but practically all anatomical text books and atlases reproduce the original Arnold illustrations and present his original description.

The cause of this is manifest and can be readily assigned to the necessity of awaiting the invention of the newer, simpler and more perfect methods of injection which have recently been furnished. Arnold had at his disposal only the most difficult means for injections and it is surprising that he was able to carry his studies as far as he did. He was aided only slightly by these injections, and like von Baer before the days of the microtome, had to depend principally upon careful dissections. He succeeded, however, in tracing the course of the spermatic artery and its branches through the cord to the testis.

In 1904, under the guidance and at the suggestion of Professor Mall, I undertook an investigation of the vascularization of the testis of the pig. This study was begun with the idea that a knowledge of the blood supply of the male sex gland of this mammal

[1]Arnold. Handbuch der Anatomie des Menschen, 1847. Surgeon-General's Library, Washington, D. C. The original article could not be located.

would greatly simplify the later research on this gland in man. Such, however, was not the case and results obtained in late years by various investigators tend to disprove the theory that the general structure and blood supply of the organs of lower mammals is always similar in detail to that in man.

Text books have too frequently adopted the results obtained from investigations on dogs, pigs or other mammals and have incorporated the illustrations and descriptions without specifying that such results have not as yet been verified for man.

So very marked are the differences of the gross structure and blood supply of the human testis as compared with that of the pig that the study of the human testis was practically an entirely new research. The methods used were similar and will be briefly detailed, but in no other way did a study of the testis of the pig aid in solving the problem of the blood supply of the male sex gland of man.

Professor MacCallum, of the pathological department, very kindly placed at my disposal the testes from autopsy subjects, and for this courtesy I desire to express my thanks. These specimens were usually brought to the anatomical laboratory within twenty-four hours after death and were in excellent condition. Professor Mall and Professor Brödel also aided me in obtaining several valuable specimens of human embryonic testes, and to them I wish to express my appreciation. Embryonic material in fit condition for injection is most difficult to obtain, and I regret that a series of human embryonic testes could not be injected similar to that of the pig.[2]

My injections were made with either India ink or celloidin. The detailed technique of these injections is given in my separate publication[3] on clearing methods, but the following improvements should also be noted: The gland or organ under investigation must be fresh and must contain no clotted blood. Warm, normal saline

[2]Hill, E. C. On the Gross Development and Vascularization of the Testis. Am. Jour. Anat., Vol. 6, No. 4.

[3]Hill, E. C. On the Schultze Clearing Method as Used in the Anat. Lab. of the Johns Hopkins University. J. H. H. Bull., Vol. 17, No. 181.

solution is forced into the artery under a pressure of 60-80 mg. of mercury, and is allowed to pass through the vascular tree and issue from the returning veins. After a short interval, depending upon the size of the organ, the fluid pouring from the vein will contain little blood and later will be clear. The gland, however, has become firm from the passage of fluid from between the endothelial cells of the capillaries. It seems odd that a pressure below the normal systolic blood pressure should cause this extravasation, but this may perhaps be due to the rapid death of the endothelial cells. Air is then forced into the artery and in an incredibly short time bubbles of air are seen at the openings of the veins, the organ becomes soft and no sign of tension is manifest in the vessels, which are now thoroughly emptied. Then follows a similar injection of equal parts of absolute alcohol and ether, and after the appearance of this mixture at the openings of the veins a second "blowing out" is resorted to. In this instance, however, the cells lining the vessel walls are more or less fixed with absolute alcohol and ether and their lumen is open and ready for the celloidin injection mass.

The celloidin, usually 7 per cent., colored with vermillion or finely powdered lamp black, readily enters the vessels against no backward pressure and flows into and completely fills the smallest vessels.

By using a thin celloidin for the arterial and capillary vessels, and a thicker celloidin for the veins, a very instructive and beautiful double injection is obtained with comparative ease. Such specimens are valuable for microscopic work, for clearing in 1 per cent. potassium hydroxide and glycerine and for corrosion work. The vermillion and lamp black withstand the ordinary laboratory reagents even in concentrated forms. The method of corrosion used is simply that of peptic digestion as devised for such studies by Dr. Mall.[4] In digesting the testis it was found advisable to place the gland in concentrated hydrochloric acid for 6 or 8 hours in order to soften the tough fibrous albuginea. This was followed by a digestion at 38 degrees C. in the thermostat with pepsin and

[4]Mall, F. P. A Study of the Structural Unit of the Liver. Am. Jour. Anat., Vol. 5, No. 3.

.3 per cent. hydrochloric acid. These specimens in pure glycerine can be permanently kept cleansed of any small particles of undigested gland. They are not at all friable, and one perfect double injection clearly defines the entire blood supply of the gland. Other thick blocks of the testis were cleared without digestion in 1 per cent. potassium hydroxide after hardening in 95 per cent. alcohol. These specimens showed the capillary network around the lobules and careful dissection revealed the lobular arrangement with its blood supply.

Water macerations similar to those used by Dr. Mall[5] in studying the structure of the spleen were attempted without success. This was rather puzzling until investigations of the reticulum surrounding the lobules was begun. So dense and firm are the bands of reticulum encasing these tubules that the entire gland will macerate in water before any of the cells can be shaken out. Also the trabeculae, which figure so conspicuously in all illustrations of the human testis, disappeared under maceration. The causes of this will be taken up in discussing the blood supply.

In studying the reticular structure entering into the gland, Mall's method[6] used so successfully by Flint[7] in his work on the adrenal was followed.

Arnold, in 1847, described the course of the spermatic artery and its branches. His beautiful illustrations show quite accurately and clearly the anterior and posterior scrotal arteries and the external and internal spermatic arteries. He traced the latter in the cord where it gives off two or three branches to the epididymis, one of which anastomoses with another small branch of the internal spermatic artery which follows down the vas deferens. His diagram and account show these branches to the gland proper passing under the albuginea, but not penetrating the testis at the mediastinum. Arnold noted in his article the observations made by

[5]Mall, F. P. The Structure of the Spleen. Johns Hop. Hosp. Rept.

[6]Mall, F. P. Reticulated Tissue and Its Relation to the Connective Tissue Fibres. Johns Hop. Hosp. Rept., Vol. 1.

[7]Flint, J. M. The Blood Vessels, Angiogenesis, Organogenesis, Reticulum and Histology of the Adrenal. Johns Hop. Hosp. Rept., Vol. 9.

Huschke as to the comparative size of the renal artery and spermatic vessels. Huschke states that the calibre of the renal artery is 15 times that of the internal spermatic artery, although the weight of the testis is only one-eighth as great as that of the kidney. This is a most interesting observation and explains the difficulty met with in attempting injections of the testis. Not only is the spermatic artery very small, but it is long and near the human sex gland becomes tortuous, although this is hardly appreciable as compared to the great tortuosity met with in the spermatic artery of the pig. Arnold in describing the veins states that these veins "entsprechen in ihrer Anordnung im Allgemeinen den Arterien." He traces the blood from the testis through the pampiniform plexus and its subsequent branches back into the renal vein or aorta. Thus it is seen that he does not trace the blood farther than to the gland itself, and makes no attempt at interpreting its course after reaching the mediastinum or albuginea.

That he should have been in doubt about the distribution of the arterial supply near the mediastinum is easily understood because only the most perfect double injection made by methods but recently developed, could aid in unravelling the profusion of veins and arteries at this portion of the gland. Astley Cooper has investigated a capillary plexus covering the inner surface of the tunica albuginea, which he has termed the tunica vasculosa.

Aside from these valuable contributions, practically no work has been done on the blood supply of the testis; hence the following results seem worthy of record.

The spermatic artery arises from the abnormal aorta as a long slender branch passing into the abdominal rings, and from here follows in the spermatic cord to the testis. The vessel which directly supplies the testis proper and which is a direct continuation of the spermatic artery has been named by Arnold the internal spermatic artery. The spermatic artery, before giving off its terminal branch or branches to the testis, gives off ordinarily a branch, the external spermatic artery, high in the cord, just below the external abdominal ring which in time divides in two or more branches to supply the membranes of the testis. Until the sper-

matic artery ends in the one or more terminal branches to the testis, Arnold's original description is excellent. These terminal branches (A and B, Fig. 1) become tortuous just before reaching the mediastinum of the testis and near the globus major of the epididymis send one rather large vessel to supply the tunica albuginea. This branch, one of the capsular branches, encircles the gland on the inner side of the albuginea and sends deep branches into the glandular substance which anastomose with the ascending arteries given off at the mediastinum. The terminal arteries, after giving off the capsular branch, break up near the mediastinum and send a great number of small arteries into the gland. A small branch from the terminal arteries descends to the globus minor and passing under the tunica albuginea (C A, Fig. 1) runs under this capsule and anastomoses with the capsular branches given off at the level of the globus major. These capsular branches send out many small arteries, most of which are rather tortuous and encircle the gland on the inner side of the albuginea. These vessels and their branches supply a capillary plexus on the inner side of the tunica albuginea which has been called the tunica vasculose by Astley Cooper. Branches from these penetrate the glandular substance and anastomose with the arteries given off at the mediastinum from the spermatic artery.

Also the capsular artery sends small branches to the tunica parietalis visceralis. In most of the specimens examined a large branch (F, Fig 1) passes from the capsular artery to the mediastinum and anastomoses with the arteries in this portion of the gland.

Generally at the level of the globus minor of the epididymis a

Fig. 1.—Arterial supply of human adult testis. A portion of the gland has been removed so as to show the penetration of the arteries through the mediastinum into the glandular tissue.

A. B., main terminal branches to testicle; C., branch following spermatic cord and encircling and supplying vas deferens; C. A., capsular artery—a branch from B.; C. E., caput epididymis—shown in outline; D., branch of capsular artery lying on innermost side of albuginea; E., outline of epididymis; F., central artery connecting vessels of mediastinum with capsular branches; M., mediastinum. × 3½.

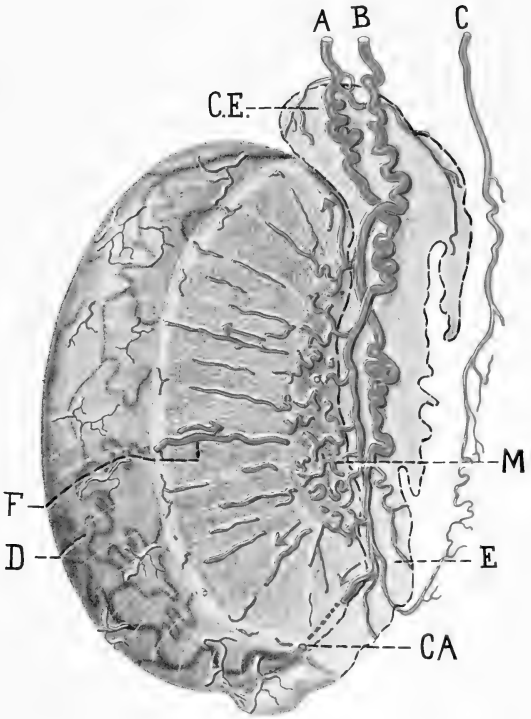

FIG. 1.

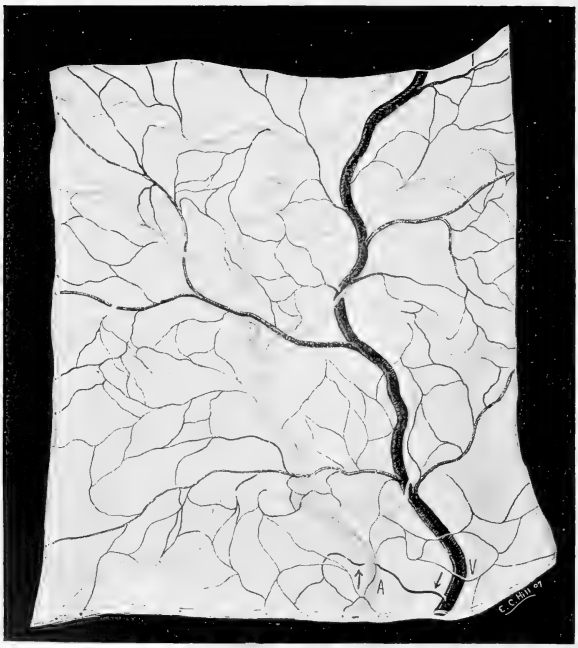

FIG 2.

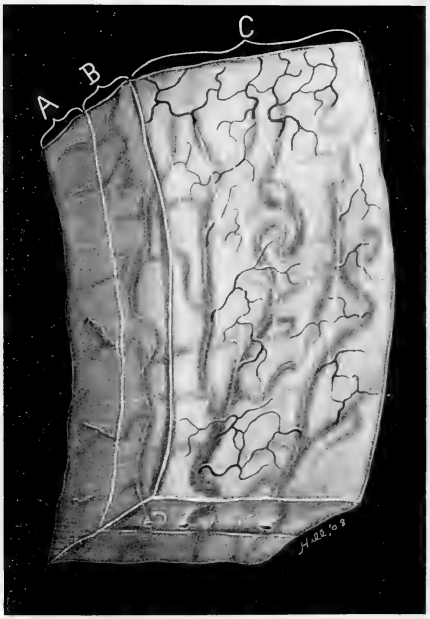

FIG. 3.

branch is given off from the spermatic artery which anastomoses with the artery supplying the vas deferens (C, Fig. 1).

The blood supply of the tunica vaginalis parietalis (Fig. 2) is uninteresting. The arteries pass to this tunic near the epididymis, are slightly tortuous, give out small branches, which ending in capillaries, form a plexus. The blood from this plexus is collected into veins which in turn empty near the epididymis.

The blood supply of the tunica albuginea and the branches given off from the capsular arteries is shown in Fig. 3. A thick section, including the albuginea and a portion of the glandular substance, taken from an injected gland and cleared, shows three general groupings of vessels:—

First (A), those dipping perpendicularly into the glandular substance and following the trabeculae to supply the lobules.

Second (B), capsular arteries and their branches on the inner side of the tunica albuginea, and

Third (C), the small branches given off from the capsular arteries which pass outward and penetrate the albuginea supplying it and the tunica vaginalis visceralis.

Corrosion specimens of the human testis show very clearly the arrangement of the vessels, and these, when placed in glycerine, may be isolated in order to study their characteristics. (Fig. 4.)

In this connection it may be of interest from the standpoint of comparative anatomy to note the marked differences between the blood supply of the testis of the pig and that of man. This is especially well shown by isolating arteries from corrosion specimens of each. In man the distribution of these vessels and their course

Fig. 2—Blood supply of tunica parietalis of the human testis. Injected with India ink under 20 mm. Hg. pressure. Drawn with camera lucida. × 26⅔. A., artery; V., vein.

Fig. 3.—Celloidin arterial injection of capsule and outermost glandular tissue of human adult testis. Specimen cleared in 1 per cent KOH and glycerin. Three arrangements of vessels are shown. 1st. Surface vessels on outer side of tunica albuginea. (c) These arise from branches of capsular arteries (b) which lie on inner side of albuginea. From these latter vessels branches (a) penetrate the glandular tissue and anastomose with the ascending arteries coming from the mediastinum. × 10.

is "rational." If a student knew that the vessels entered the mediastinum and also penetrated the substance of the gland through branches from the capsular artery, he could readily picture the distribution of these vessels. In the pig testis the vessels enter from the capsule, but form most unusual loops. Reference to my former article on the pig's testis[2] will serve to bring out the contrast with the arrangement in man; both in the penetrating vessels and their branches and in the supply to the albuginea, etc.

Practically the whole blood supply of the testis of the pig comes from one large capsular artery which encircles the gland sending tortuous rib-like branches around it. These branches send other branches deep into the gland to the mediastinum without giv-

Fig. 4.—Isolated arteries of human adult testis. These vessels were injected with celloidin and the testis was then digested in HCl and pepsin. × 2½.

ing off any branches, then loop back and supply the tubules through the return loops. This unique arrangement may be occasioned by the central location of the mediastinum (Fig. 5) which is so essentially different from the lateral location of this collecting portion of the human testis.

A cross section of the fresh testis gives an impression that very thick, strong trabeculae pass from the mediastinum to the albuginea giving the appearance of a fan. That these are trabeculae

Fig. 5.—Sagittal section through testis of adult pig to show central location of mediastinum. E., epididymis; M., mediastinum; S. C., spermatic cord.

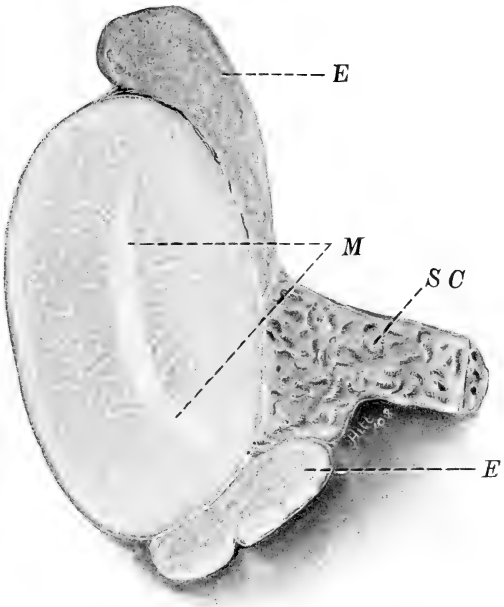

FIG. 5.

is true, but their apparent thickness is due to the fact that arteries and veins follow in radiating lines in the very fine connective tissue bands, and it is principally the presence of these vessels that has given rise to the erroneous idea of heavy trabeculae.

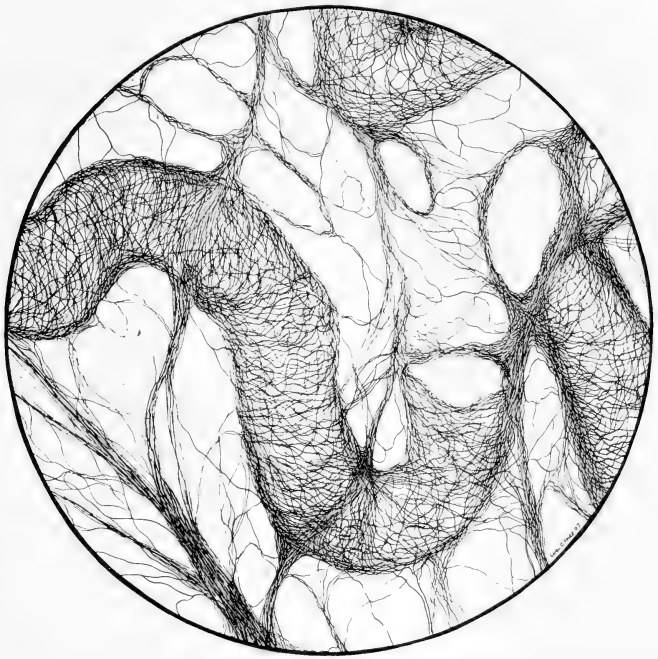

Fig. 6.—Reticulum of human testis, showing reticular formation around tubules. Digested in HCl and pepsin, stained with picric acid and acid fuchsin. × 10. Drawn with camera lucida.

Whitehead[8] has published some very instructive results accompanied by illustrations of the reticulum of the testes of various

[8]Whitehead, R. H. Studies of the Interstitial Cells of Leydig, No. 6. Anatomical Record, No. 8, 1908.

mammals including man. On one point he has not laid especial emphasis, and that is the great strength of the reticulum of the testes of man and its resistant powers.

Testes treated with weak KOH do not macerate readily, and even after the capsule has become disintegrated the tubules are firm and resistant. They are elastic and can be teased out of their full length without breaking. This is due to the great amount of reticulum surrounding them (Fig. 6). A similar reticular structure is found in the tubules of the kidney (Mall), only the reticulum of the testis is denser. It is the presence of this large amount of reticulum that prevents the preparation of specimens of the gross structure of the lobular arrangement of the spleen which Professor Mall found of such value in studying that organ.

Turning again to the description of vessels we find that the comparatively large branches given off at the mediastinum divide into many small branches which radiate toward the albuginea like spokes in a wheel. These ascending arteries (A A, Fig. 7) pass between the lobules and give off capillary branches to the tubules. An anastomosis may be noted between the ascending arteries and the descending arteries (D. A., Fig. 7) given off from the capsular arteries and their branches. The veins follow the general course of the arteries. There are several large capsular veins which encircle the gland, lying on the inner side of the capsule and emptying into the pampiniform plexus. These capsular veins receive blood from the capillaries and veins on the inner surface of the tunica albuginea, from the tunica vaginalis visceralis and from anastomoses with the ascending veins which enclose the lobule (A. V., Fig. 7). The blood is also returned to the pampiniform plexus by descending veins which follow the course of the arteries and empty into the venous plexus at the mediastinum (D. V., Fig. 7).

Fig. 7.—Sagittal section of human testis; to show blood supply. Injected with red and blue celloidin, cleared in 1 per cent KOH and 20 per cent glycerine. × 4.

A. A., ascending artery; A. V., ascending vein; D. A., descending artery; D. V., descending vein; M., mediastinum; V. D., vas deferens; T. A., tunica albuginea; T. P., tunica parietalis.

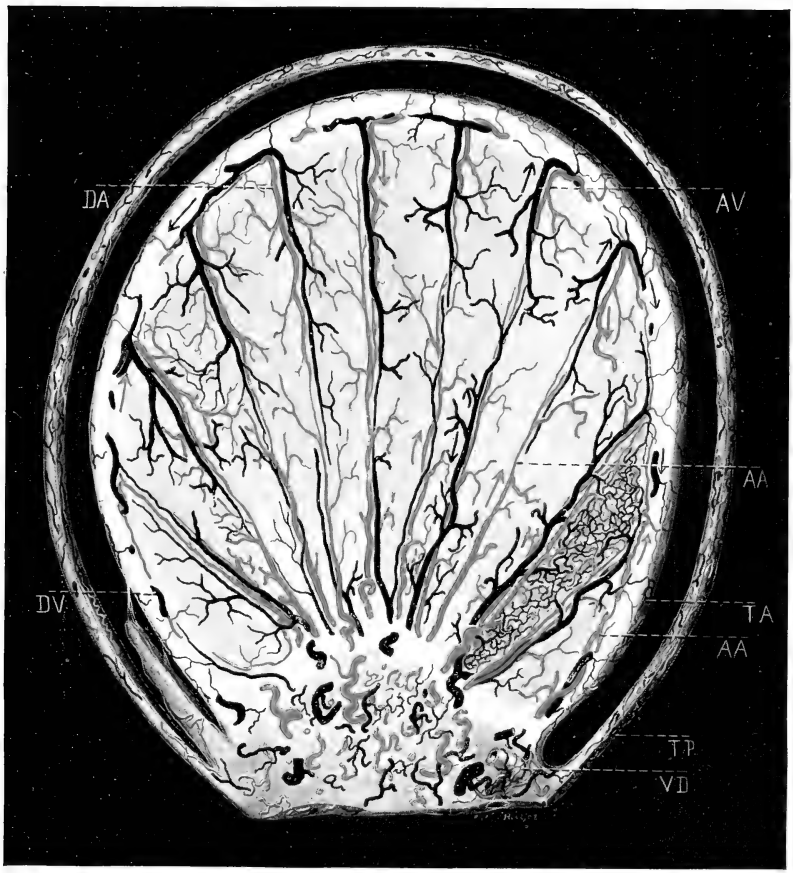

FIG. 7.

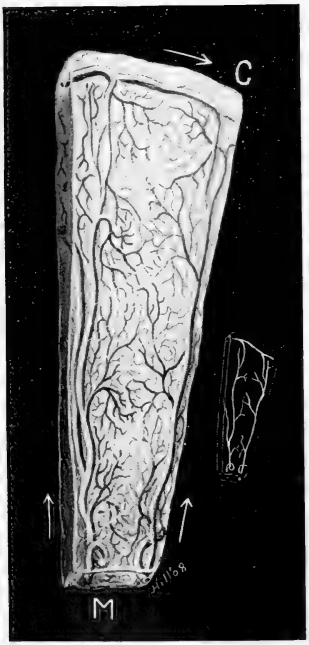

FIG. 8.

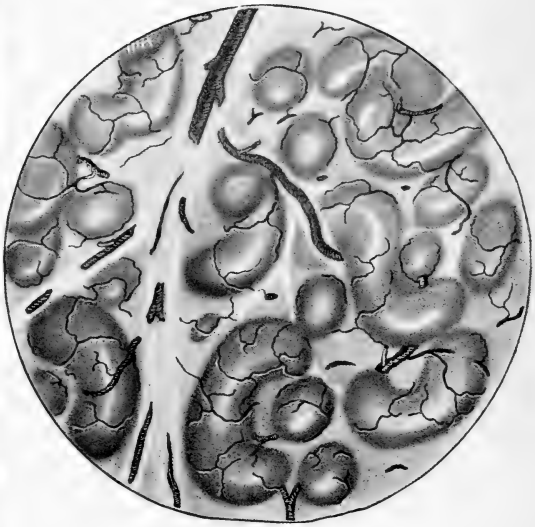

FIG. 9.

Mall several years ago advanced a theory as to the presence of certain units of the blood system which may or may not be peculiar to the organ in which they are found and which correspond to the histological or structural unit of the organ. These units are composed of small branching blood vessels which pass into capillaries, the blood from which is collected into small veins. This theory of vascular units may be briefly summed up in the statement "similar blood supply to similar histological units." These vascular units have since been proved to be present in the liver, spleen and adrenal, but in the testis of the pig I could make out no definite units. In man, however, the lobular arrangement is less complex, and I was enabled to make out vascular units which correspond to units of structure and which repeat themselves similarly throughout the organ, the one bearing a definite and constant anatomical relation to the other.

To determine the units, a testis, after receiving an arterial and venous injection, was placed in 95 per cent alcohol until well hardened, then cut into blocks measuring about .5 cm. in thickness and later cleared in 1 per cent. potassium hydroxide. In this way the tubules remained firm and the lobules could be teased out with their vascular supply. When placed in 20 per cent glycerine the vascular units were clearly defined.

Fig. 8 indicates the arterial supply to a lobule. There is a very profuse anastomosis between the arteries enclosing the lobule and each lobule receives blood from two or more ascending arteries and a like number of descending arteries which are branches of the capsular arteries. From the vessels enclosing the lobules small arterioles are given off which in turn encircle the tubules ending in a plexus around them. Thick microscopic sections of the injected

Fig. 8.—Arterial supply of lobule of human adult testis. Injected with celloidin, cleared in 1 per cent KOH and glycerine. The testis was then cut into large pieces and the lobules teased out. C., capsule showing capsular artery on inner side of albuginea; M., mediastinum.

Fig. 9.—Microscopic section of adult human testis. Injected with 4 per cent celloidin to show arterial and capillary blood supply to tubules. Sections cut to 200 microns in thickness, stained in H. and E. and cleared in creosote. × 50.

testis lightly stained show very beautifully these small capillaries surrounding the tubules (Fig. 9).

The arteries and veins supplying each lobule run in the trabeculæ, and it is principally to their presence that the testis has the appearance of such definite lobular divisions.

STUDIES ON THE VASCULAR SYSTEM OF THE THYROID GLAND.

BY

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With 10 Figures.

It has long been known that the thyroid gland is a very vascular organ. This we would expect both from anatomical and physiological reasons, having such a rich gross blood-supply and exerting such a profound influence through its secretion upon the physical and nervous development of the body. Tschuewsky (1), by a series of carefully performed experiments, has supplied us with data upon the subject. He found that the amount of blood flowing through the thyroid per 100 gram weight of the organ to be 560 ccm. a minute. This same observer, using a like standard of calculation, found the amount of blood flowing through the head to be 20 ccm. per minute, and through the kidney 100 ccm. per minute. Thus the thyroid, according to him and using the blood-flow per gram weight as the standard of comparison, is twenty-eight times as vascular as the head and five and one-half times as vascular as the kidney. Tschuewsky also estimates by a series of calculations that in the dog the entire amount of blood in the body flows through these small glands sixteen times in one day. This enormous blood-supply has led to a great deal of physiological speculation and it has even been suggested that the main function of the tyroid gland consists in acting as a vascular shunt to protect the circulation in the brain (2).

In these studies, begun at the suggestion of Dr. Mall and finished through his constant advice and encouragment, an attempt is made to study a few of the main points of the microscopic blood-supply of this gland. The thyroid glands of the cat, dog and man have been studied principally and the glands were those of the adult ani-

mal. I wish here also to express my thanks to Dr. H. M. Evans for the use of several injected specimens of human thyroids which he had injected in connection with his work upon the parathyroids (3).

The histological structure of the thyroid has been the subject of considerable literature in the past. Baber's (4) classical studies of the minute anatomy of the structure of the thyroid gland was one of the most important of the early contributions to this subject. He described particularly the histology of the follicles and the lymphatics. Langendorff (6), Wölfler (7), Lustig (8), Biondi (9), Hürtle (10), Andersson (11), Kohn (12), Streif (13), Flint (14), Coco (15), and others have given us important contributions upon the subject. These studies show that considerable differences of opinion have existed as regards its histological structure. Wölfler found that the thyroid of the child as well as of the adult contains solid rows of cells at the periphery while the center of the gland is composed mostly of vesicles filled with colloid material. He does not seem, however, to have attempted to divide it definitely into a cortical and medullary portion. Flint does not believe that thyroid gland, of the dog and man at least, can be divided into either lobes or lobules, but that the septa from the capsule penetrate the parenchyma of the gland in an irregular fashion. He has, however, observed pictures that suggest definite lobulation, but thinks that if the original lobulation is present in the embryo it is later lost. Regaut and Pettijean (16) studying the dog, cat, pig and other animals also do not believe that the thyroid gland can be divided into lobules. They maintain that there is no such architecture present, that there is no distribution of blood vessels or lymphatics to warrant the conception of a lobule. Coco (15) gives a description of the structure of the dog's thyroid that summarizes and agrees in general with the conclusions of most observers, and with the descriptions in most text-books. According to him, the thyroid gland is surrounded completely by a thick capsule which gives off numerous prolongations of connective tissue. These septa penetrate the parenchyma of the gland, dividing and sub-dividing it into lobes and lobules. The septa which also support blood-vessels and nerves, become thinner and thinner and finally end by surrounding each follicle in the

form of a delicate membrane which supports the epithelial cells. The sections examined in the course of my own studies indicate a division of the gland by septa into lobes and lobules, and as will be shown in this paper, definite vascular units can be dissected out, which correspond to these structural units. Streif (13), by use of the Born wax model method, has shown that the glandular structure of the thyroid consists of closed follicles which are separated from each other by fine connective tissue. He also showed that a system of canals is not present and that the follicles do not communicate with each other.

There seems to be no extensive literature upon the microscopic blood-supply of the thyroid. Kohlrausch (17) in 1853 drew attention to the fact that the follicles are surrounded by a rich capillary network. This fact, as well as the presence of many lymphatic spaces surrounding the follicles, is mentioned in the textbooks of anatomy and histology. Wölfler devotes some space to the consideration of the blood-vessels. He studied them principally from the embryological standpoint, in their relation to the developing gland. He observed a network of capillaries surrounding each follicle and twigs from the blood-vessels to the follicles, but does not seem to have studied them further and no drawings illustrating these points are found in his monograph. Streckeisen (18) has made a very careful study of the gross distribution of the arteries supplying the thyroid gland. Landström (19) studied the gross distribution of the arteries and paid especial attention to the subject of the arterial anastomoses. His article gives a résumé of the work done upon this subject with a complete bibliography.

In my own studies the injection method was used practically altogether. Specimens were injected with various injection masses—India ink and carmine, ultramarine blue, vermillion (mercuric sulphide) granules in various per cent. gelatine solutions. In some cases single injections were made either into the arteries or veins, in other cases double injections were made, filling both arteries and veins. India ink and carmine both give very good injections of the follicular blood-supply, as the masses on account of the small size of their granules easily penetrate the capillary bed. Some very good

double injections were made by using carmine and ultramarine blue injection masses. The carmine was first injected into the arteries and continued until the capillary bed was filled; then ultramarine blue was injected into the arteries, forcing the carmine out through the capillary bed and over into the veins. The ultramarine blue granules fill the arteries, but fail on account of their large size to pass over into the capillaries, and if we stop at the proper moment we have a double injection, in which the arteries are injected blue, the capillaries and veins red. Partial injections also gave some very instructive specimens. After injection the specimens were hardened in alcohol or formalin, imbedded in paraffine or celloidin, cut and cleared in creosote. It was also possible, by taking a small piece of an injected gland, to dissect out under the binocular microscope, lobules and even single follicles. This, in the case of the human thyroid, gives perhaps the most instructive specimens, as a mounted specimen thick enough to contain a whole lobule, is usually too thick to be studied succesfully under the microscope. Also a lobule or follicle when dissected out, can be turned about and studied from various sides.

The general form and shape of the human thyroid as well as its gross blood-supply is described in almost any text-book of anatomy. Also the variations in shape and position of the thyroid gland in various animals is described in the text-books of comparative anatomy. It will be remembered that the thyroid of the cat and the dog differs from that of the human in consisting of two bean-shaped lobes, one on either side of the trachea, which are connected in the cat by a very thin strip of an isthmus and more completely separated in the dog. In these animals, too, the inferior thyroid artery is of very small size and by far the greater part of the blood reaches the gland through the superior thyroid artery.

There seems to be considerable discussion as to the existence of anastomoses in the human thyroid, between the superior and the inferior thyroid arteries. Landström (19) and also Streckeisen (18) show clearly by their injections that such anastomoses are present upon the surface of the gland, not only between the arteries of the same side, but also between the two sides. In my own injections such sur-

face anastomoses were easily demonstrated. Landström also expresses the conviction that anastomoses also occur within the gland, but did not succeeded in demonstrating them. The method that Landström employed was that of injecting the arteries with Woods' metal, and then taking a Roentgen-ray picture of the gland. In my own studies several glands were injected with celloidin, and then by digesting with artificial gastric juice, corrosion specimens were obtained in which the arterial tree could be followed from its trunk to its termination in the individual follicles. In no case was it possible to find definite arterial anastomoses beneath the surface of the gland. The arterial tree is so exceedingly complex that it is difficult to decide this point absolutely. It is also easy to imagine what a complex picture would be obtained by Landström's method. In many cases when from study under the microscope a definite anastomosis appeared to exist, yet after carefully moving the blood-vessels with a pair of fine needles it was seen that the two arteries did not really anastomose, but ran out each to its termination, entirely independent of the other. In many cases, large branches of the thyroid arteries would turn and twist about in great confusion, without, however, anastomosing. In the cat's thyroid, anastomoses occur as are seen in Fig. 1. Gelatin injections of the dog's thyroid, show a few anastomoses, but they occur between branches of the same artery, and not between branches of the superior and inferior thyroid arteries. At any rate, if anastomoses are present in my specimens of human thyroids they are of small size.

The capsule of the thyroid gland, like similar tissues, has a very scant blood supply. The arteries upon entering the gland give off small branches at various places which join each other to form a network throughout the substance of the capsule dividing it into large diamond-shaped areas. Each artery is accompanied, as a rule, by two veins, which are connected at various places by bar-like veins which run across the artery. The veins anastomose in the same manner as the arteries, and empty at various places into the large veins that are emerging from the interior of the gland. Occasionally a capsule vein empties into a vein within the gland, but such anatomoses are infrequent. The same general scheme of this circulation is ob-

served in the cat, dog and in man. The capsule of the thyroid gland has been divided by some into an internal capsule which can be stripped off only with some difficulty, and an external capsule which strips off readily. The above description refers to the blood-supply of this outer capsule.

Fig. 1.—Corrosion specimen of arterial injection of cat's thyroid, showing numerous anastomoses. × 5.

The manner in which the arteries approach the thyroid gland show some variations in the cat, dog and man. In the dog the superior thyroid artery gives off two main branches, one anterior branch and a posterior branch. Each of these in turn give off four or five smaller

branches which penetrate the gland. In a certain specimen the total number of these branches from both anterior and posterior branches was nine. These branches plunge into the gland and immediately give off branches which run in various directions, some attempting to gain the periphery of the organ, others running still deeper towards

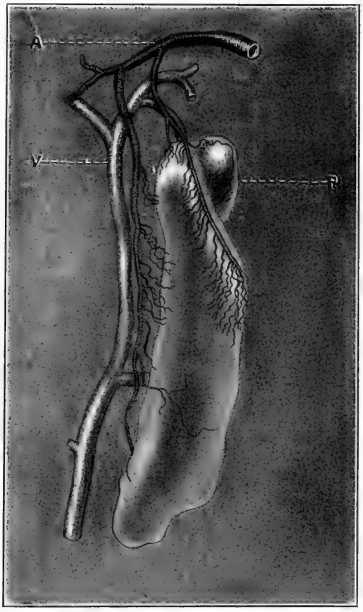

FIG. 2.—Gross blood supply of cat's thyroid. × 5.
A.—Artery. V.—Vein. P.—Parathyroid gland.

the center. These arteries of the second order surround definite divisions of the gland and give off no branches, as a rule, to the follicles. The veins follow more or less the same course, but show frequent anastomoses.

The course of the arteries to the cat's thyroid is somewhat similar to that of the dog. The superior thyroid artery, however, gives off a

much larger number of branches before penetrating the gland. This is illustrated in Fig. 2. The superior thyroid divides into two main branches which course down the sides of the gland, each giving off a large number of branches, some of which are branches of the second order, others of which give off branches of the second order. The total number of the branches given off by the two main divisions of the superior thyroid artery in the specimen drawn is forty-one. The arteries of the second order are distributed as in the dog and pass between lobes of the gland.

The course of the arteries in the human thyroid resembles somewhat that of the dog, but presents differences which are apparent at

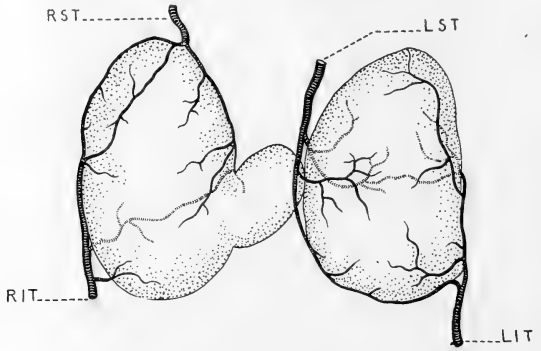

Fig. 3.—Gross arterial blood supply of human thyroid.

R S T—Right superior thyroid artery. R I T—Right inferior thyroid artery. L S T—Left superior thyroid artery. L I T—Left inferior thyroid artery.

first sight. The human thyroid gland differs from that of the dog in shape, in the presence of a well-defined isthmus and in the fact that in man the inferior thyroid artery is as large or larger than the superior thyroid. Variations in the gross blood-supply of the human thyroid are common, but a general scheme seems to be present, with differences in method of anastomoses. Such a general scheme is shown in Fig. 3. Here we see the superior thyroid artery approach-

ing the upper poles of the gland, and the inferior thyroid arteries approaching from beneath. Each artery gives off four or five branches, some of which supply the anterior, some the posterior surface of the gland. The main continuations of these arteries are prone to run along and upon the margins of the gland, and the superior and in-

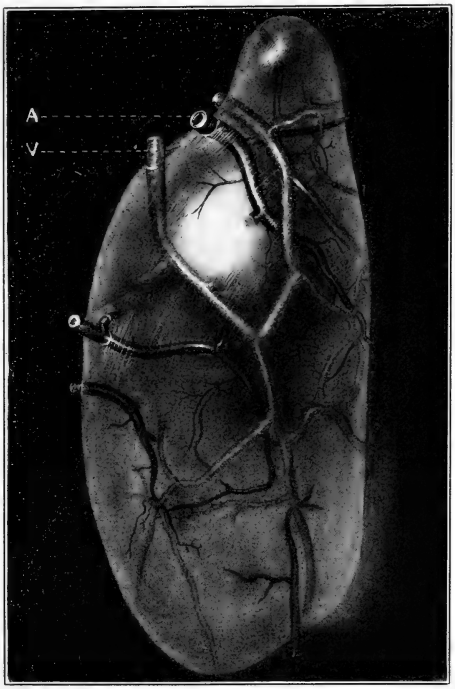

Fig. 4. —Drawing illustrating the venous network upon the surface of human thyroid with accompanying arteries.

A.—Arteries. V.—Veins.

ferior thyroid arteries upon the same sides anastomose, two anastomoses occurring between each artery in the specimen from which

Fig. 3 was drawn. The general scheme of these anatomoses varies considerably in the different human thyroids. Landström, in his article to which reference has previously been made, gives excellent drawings of some of these variations.

In the human thyroid, few large arteries are present in the depths of the gland, and in this respect it differs from the dog. In other words, in the human the branching of the large arteries takes place mostly upon the surface of the gland, and having by their branching obtained their approximate distribution, the smaller branches are sent in.

Fig. 5.—Drawing illustrating arteries of third order passing between lobules and arteries of fourth order supplying the lobules in the human thyroid (partly diagrammatic).

The further distribution of the arteries is essentially the same in both the dog and man. The arteries of the third order, as is shown in Fig. 5, pass between the lobules and give off arteries of the fourth order which supply the lobule. Each lobule is composed of a number of follicles and is supplied usually by from two to five arteries, the number of arteries depending upon the size of the lobule. Fig. 6 and Fig. 7 show two lobules which have been dissected out

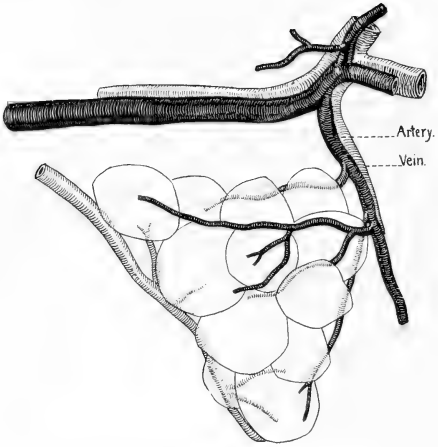

Fig. 6.—Lobule of human thyroid dissected out.

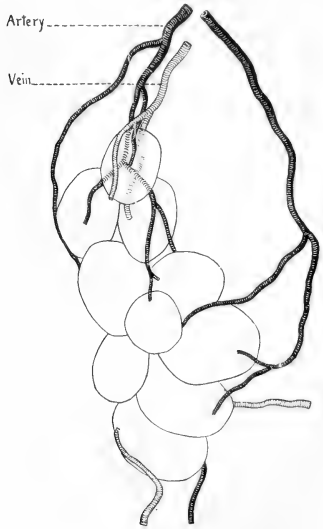

Fig. 7.—Lobule of human thyroid dissected out.

with their blood-supply intact. They resemble, to use the classical Malpighian expression, a cluster of grapes, over which arteries and veins can be seen twining about. These arteries of the fourth order run over the surface of the lobules and give off follicular arteries to each follicle. The follicular arteries end in a rich capillary network which surrounds each follicle. The vein which arises from this capillary network upon the far side to the artery, follows fairly closely the course of the artery. Figs. 8 and 9 show

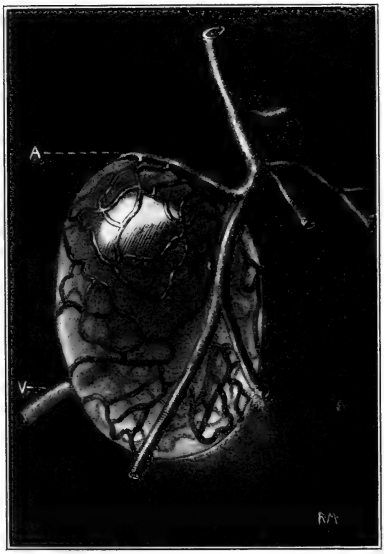

Fig. 8.

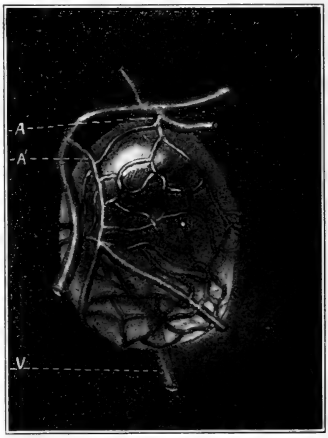

Fig. 9.

Fig. 8.—Single follicle of human thyroid dissected out, showing its follicular artery A, and follicular vein V. × 85.

Fig. 9.—Single follicle of human thyroid dissected out, showing a follicle receiving its blood-supply from its own follicular artery A, and also from the follicular artery supplying an adjacent follicle A'.

V.—Follicular vein. × 85.

the termination of the follicular artery at the follicle, the capillary network and the vein arising from the far side of the follicle.

The relations between the capillaries and the cells of the follicle are seen in Fig. 10. The capillaries lie just outside the cells in the connective tissue that forms a sort of capsule for each fol-

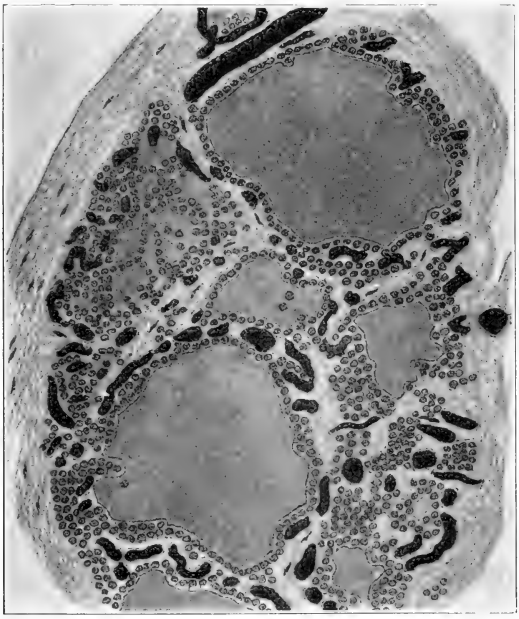

Fig. 10.—Drawing illustrating the relation between the follicular network of capillaries and the cells of the follicle in the human thyroid. Capillaries in solid black. × 200.

licle. It is also noted that compared with the size of the individual cells, the capillaries are very gross structures. This is interesting from the standpoint of the secretory changes in the thyroid. According to Andersson, Hürtle, Biondi and others, the colloid mate-

rial passes from a follicle into the lymph spaces by a gradual obliteration, "melting" or bursting, of some portion of the wall of the follicle. As the meshes in the capillary net are large as compared with the size of the cells, it can be conceived that quite a number of cells can be destroyed without affecting the integrity of the capillary network. Thus an opening sufficient to permit the escape of the colloid material can take place without rupturing a capillary and causing hemorrhage, or at most only a few capillaries need be ruptured. That some capillaries are often ruptured, is shown by the frequent finding of red blood cells in the lymph spaces and in the cavity of the follicle.

The veins that return the blood from the follicles follow closely the path of the arteries, show frequent anastomoses and finally reach the surface of the gland where they anastomose freely.

The average size of the arteries of the first order is .15 mm.; those of the second order .1 mm.; those of the third order .03 mm.; and lastly the follicular arteries are .0125 mm. in size. The capillaries of the follicular network average .008 mm. in size. These measurements are those of the normal human thyroid.

The finer distribution of the blood vessels in the cat's thyroid differs somewhat from that of the dog and man. In the cat the arteries of the second order pass between lobes and the arteries of the third order pass between lobules just as in man. Arteries of the fourth order passing to the lobules are also present, but not so constant. In the cat, however, no follicular arteries are present. Each follicle is not surrounded by a rich capillary network and supplied by its own follicular artery, but the follicles are placed in a loose, wide network, each mesh of which in a cross section appears to surround a single follicle. This network has depth as well as length and breadth and surrounds the follicle in three dimensions. The arteries which supply the lobules, approach the lobules and immediately split up into capillaries without giving off any follicular arteries. The veins collect from the capillaries at a point somewhat removed from the arteries often directly opposite them, but soon approach them and follow the same general course.

This description of the thyroid blood-supply must be taken only as indicating a general scheme. Certain variations will, of course, be noted. The size of the lobules, depending upon the number of follicles composing it, will, of course, vary and with it the number of arterial branches supplying it. Also the blood-supply of the individual follicles is subject to certain variations. In many cases a single follicle, as shown in Fig. 9, besides receiving its blood-supply from what might be termed its own follicular artery, receives small branches from an artery which supplies an adjacent follicle.

The veins while in general following the course of the arteries, also show many variations. Often the vein which springs from the follicular network, instead of passing back side by side with the artery, empties into a vein which follows the course of an artery supplying follicles on the far side of the lobule. Such a picture is seen in Fig. 6. The capillary network surrounding the follicle anastomoses very commonly with that of an adjacent follicle.

Thus it is seen, that in the thyroid, too, we have a definite system of blood-supply, a definite system of vascular units, which repeat themselves with a greater or less constancy throughout the entire organ. These vascular units correspond in most instances very closely with the structural units.

The smallest vascular unit present is the follicular unit, which consists of the follicular network, each in the case of the dog and man, with its own artery and vein. In the cat, as already stated, this network is not so rich and distinctive, and follicular arteries and veins are not present. Yet, the large mesh containing the follicle is the homologue of the network and may be regarded as the smallest unit present. This vascular unit corresponds to the histological unit of the individual follicle.

The next vascular unit in size is the lobular unit. This is composed of (1) the arteries of the fourth order which run over the clumps of follicles having as their direct branches the follicular arteries, and (2) the arteries of the third order which pass between the lobules. This vascular unit corresponds to the structural unit of the lobule.

The next vascular unit which comes into consideration is what might be termed the lobar unit, and is formed by the arteries of the second order, which surround collections of lobules or lobes and give off arteries of the third order. The corresponding structural unit is not so easily determined as are the lobules, but they may be considered as a collection of lobules, which is marked off from a similar collection of lobules by denser septa. The term lobe as used here is the microscopic lobe and does not refer to the lobe of gross anatomy, the term which is applied to a much larger anatomical division of the gland, for in the human the gland is considered as composed of a median and two lateral lobes, and in the dog the term lobe is applied to what is really gross-anatomically considered a right and a left thyroid gland.

As arteries of the first order, for the sake of simplicity, have been grouped together, the branches of the thyroid arteries which ramify over the surface of the gland, supply definite regions and penetrate it giving off branches of the second order.

Finally as the largest unit present, the prime unit, we have the thyroid gland itself, supplied by the thyroid arteries, superior and inferior, which differentiate it from the standpoint of vascularization from surrounding structures such as the thymus, submaxillary gland, etc.

An exhaustive study of the lymphatics of the thyroid does not lie in the scope of these notes. Many observers, among them Baber (5) and more recently Renaut and Petijean (16), have described them and most of the text-books refer to their presence, their extreme richness and their general distribution. Yet a short consideration of their relation to the blood vessels may be of interest. The lymph spaces surround each follicle just outside the capillary network, filling in as it does the interstices left between the follicles. The relation between the capillaries and the lymphatics also indicates how the individual follicles are surrounded by lymphatic spaces. These spaces connect with larger trunks which definitely run in between the different lobules. These trunks in turn run into larger ones between the lobes which follow fairly closely the course of the blood vessels and becoming larger finally unite to form a network of

lymphatics beneath the capsule. From there they empty into lymphatics draining the gland, which usually follow the blood vessels out, one trunk passing upwards towards the submaxillary gland and the other passing to the lower cervical region, as described by Baber and figured by Bartels. In one injection the lower trunk of the left gland was seen to pass directly into the left subclavian vein. These observations were made upon the dog alone.

In connection with the enormous blood supply of the thyroid gland, an anatomical study shows conditions favorable to a rapid and consequently a rich blood supply. The numerous arteries, the fact that each artery does not terminate until it reaches the follicle, the ultimate unit of the gland, would, theoretically considered, aid rather than retard a rapid circulation through the gland. It is rather interesting to note that the thyroid with its follicular artery and vein, resembles to a certain extent the kidney with its glomerulus and vasa afferentes and efferentes, and that the kidney, according to Tschuewsky, shows a blood supply exceeded among the organs he examined, only by the thyroid gland.

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THE STRUCTURE OF SMOOTH MUSCLE IN THE RESTING AND IN THE CONTRACTED CONDITION.

BY

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WITH 7 TEXT-FIGURES AND 7 PLATES.

I. Introduction.

In a preliminary paper, McGill, (3) 1907, were described briefly the finer structural changes which take place during the contraction of the smooth muscle in the intestine of Necturus and some of the mammals. In the present paper a more detailed account of contraction, not only for the intestinal muscle, but for smooth muscle in general, is given. The structure of resting smooth muscle is described, in the main, from the standpoint of its syncytial arrangement. The general histology of resting smooth muscle is discussed at length by Heidenhain, 1900, and will be considered only briefly in this paper.

The structure of contracted smooth muscle was studied in both living and fixed material. The general form of the contraction area is here described, together with a more detailed account of the behavior of the myofibrillæ and of the nuclei. The myofibrillæ have long been considered the contractile elements of smooth muscle. There has, however, been little proof that they are such. It has therefore seemed worth while to study them carefully in an effort to obtain some evidence along this line. Furthermore, the form of the contracted smooth muscle nucleus has recently been much disputed. In this paper an attempt is made to throw some light on this subject also.

This paper is confined to a consideration of vertebrate muscle. Enough work has been done on invertebrate muscle to show that it is

very favorable material, especially for experimental work. The description of contraction in invertebrate smooth muscle along with a review of the literature on the subject is reserved for a later paper.

This work was done in the Anatomical Laboratory of the University of Missouri under the direction of Prof. C. M. Jackson, to whom I am indebted for many valuable suggestions.

II. Literature Review.

1. On the structure of resting smooth muscle.

For the literature on the general structure of smooth muscle, the reader is referred to the excellent review given by M. Heidenhain, 1900. Since 1900 several papers have appeared. Henneberg, 1901, Heiderich, 1902, Forster, 1904, Schlater, 1905, and Soli, 1906, describe smooth muscle as though made up of entirely separate and distinct cells.

The following are the main references to the syncytial structure of smooth muscle: Drasch, 1895, in the skin of the salamander, found around the periphery of the large poison glands a complete layer of smooth muscle fibers, united by wide protoplasmic anastomoses into a syncytium. Schaper, 1902, in Urodela, found the smooth muscle fibers in the mesentery anastomosing more or less and mentions the probability that the tissue forms a syncytium.

Rohde, 1905, in a comparative study of the smooth muscle in both vertebrates and invertebrates, described protoplasmic connections not only between smooth muscle cells, but also between muscle cells on the one hand, and epithelium, ganglion cells and connective tissue cells on the other. Rohde makes the assertion that these intercellular bridges represent the remains of an embryonic syncytium. He gives, however, neither figures nor descriptions of embryonic material to support this view.

McGill, (1), 1907, in the digestive tract of the pig found that smooth muscle arises, in common with the interstitial connective tissue, from the mesenchymal syncytium surrounding the endodermal tube. Some of the mesenchyme cells in the area of muscle formation do not elongate, but persist as the connective tissue cells, connected by

protoplasmic strands with the muscle protoplasm. Often in a single protoplasmic mass connective tissue fibers and myofibrillæ differentiate side by side. In later development most of the connective tissue fibrils are crowded out of the muscle bundles by the rapidly developing myofibrillæ, though some may, even in the adult, retain their primitive relation. As the myofibrillæ form, they tend to run in longitudinal bundles, but always show marked side anastomoses with neighboring bundles. Throughout development, and in many instances in the adult, this syncytium persists. In the adult the syncytial arrangement was demonstrated in the muscle of the digestive tract of Necturus, dog, cat, and pig.

2. On the structure of contracted smooth muscle.

The literature on the structure of contracted smooth muscle is reviewed in detail by Heiderich, 1902, consequently only the more important references dealing directly with the subject will be given in this paper.

The earlier writers on the structure of contracted smooth muscle held that the tissue shortens by a zigzag folding of the fibers. Among the investigators who supported this view may be mentioned Prevost and Dumas, 1823, Remak, 1843, Leydig, 1849, Mazonne, 1851, Meissner, 1858, Schwalbe, 1868, Arnold, 1871, Rouget, 1881, and Marshall, 1887. Shultz, 1895, considered that all zigzag folding and wrinkling of smooth muscle fibers indicate contraction in the absence of tension.

In opposition to the zigzag theory of contraction, Kölliker, 1849, working on the smooth muscle of the ureter, prostate and small intestine of several forms, described knotlike thickenings in the contracted fibers which he considered contraction areas.

Heidenhain, 1861, found two types of contraction in smooth muscle: (1) A peristaltic, where the fibers show knotlike contraction areas with uncontracted areas between; (2) a general or total where there is a shortening and thickening of the entire fiber. The latter type only he held as normal.

Margo, 1862, found, as did Meissner: a cross-striation of the contracted smooth muscle fiber, not due, however, to wrinkles, but to rows of small granules analogous to the sarcous elements of striated muscle.

Van Gehuchten, 1889, described nuclei in the smooth muscle of the frog, which show a distinct spiral form. Regarding their significance he says nothing.

Ranvier, 1889, by injecting lemon juice into the gall-bladder of the guinea-pig and afterwards fixing in osmic acid obtained distinct cross-striations in the smooth muscle fibers which he interpreted as contraction phenomena.

Apathy, 1890, in support of Englemann's inotagmen theory for the contraction of striated muscle, proposed a similar theory for smooth muscle. According to his theory during contraction the chemical composition of the sarcoplasm immediately surrounding the nucleus is so altered that the interfibrillar sarcoplasm draws water from it. In consequence the myofibrillæ embedded in this sarcoplasm swell, becoming at the same time shorter. This in turn causes the shortening and thickening of the entire fiber. Relaxation is the reverse phenomenon.

Klecki, 1891, found that the intercellular bridges are more numerous and longer in contracted than in uncontracted muscle. At times in uńcontracted muscle they are entirely absent. He gives no further description of contraction. However, in his figures he shows some fibers staining lightly, others darkly, in cross section. The dark fibers are the smaller. Which he considers the contracted fibers he does not state.

Roulé, 1890-1891, studying contraction in smooth muscle, came to the conclusion that it is brought about by the myofibrillæ, which decrease in length and increase in thickness.

In his article on intercellular bridges in smooth muscle, Barfurth, 1891, did not discuss contractility. However, his figures taken from cross-sections of the fibers show dark and light fibers, both of equal thickness.

Werner, 1894, found that smooth muscle fibers, left some time in a warm oven, show distinct cross-striations and strongly wrinkled contour.

Drasch, 1895, in the smooth muscle of the poison-glands of Salamander maculosa, in contracted fibers, described cross-striations. He thought they were due to a wrinkling of a membrana propria and

not of the fiber itself. However, in portions of the gland having only scattered muscle cells, he found distinct knot-like enlargements of the fibers, which he attributed to contraction. He also observed that in the contracted fibers, the fibrillar structure is lost, and that such fibers show marked affinity for eosin.

Schaffer, 1899, studying fresh preparations of the intestinal muscle of the horse, found knot-like swellings on some of the fibers which he considered pathological. In these enlargements no myofibrillæ were oberved. Schaffer, in normal contraction, described the entire fiber becoming shorter and thicker, and at the same time losing its fibrillated structure.

Heidenhain, 1900, discusses the behavior of the smooth muscle nucleus. During contraction the nuclei become distinctly shorter and thicker. In certain degrees of contraction this is the only change. In very strong contraction, however, the nuclei are variously folded and twisted. If the resting nucleus is very slender, as in the muscle in the blood vessels, it may, when contracted, wrap up into a spiral. Less elongated nuclei, in firm contraction, show various sorts of folding and wrinkling. Heidenhain considers both the "Grenzfibrillen" and the "Binnenfibrillen" contractile elements, though he gives no direct evidence that they are such.

Henneberg, 1901, studied the smooth muscle of the carotid artery of the ox, both in the resting and in the contracted condition. When the carotid is cut, the proximal end contracts very firmly, the distal end relaxes completely. Small pieces from any part of the artery, if cut out and warmed, slowly contract, if cooled, relax. By quickly removing very small pieces and throwing them into hot water, Henneberg was able to fix the tissue rapidly enough to prevent contraction of the expanded muscle. In material so preserved he found two types of fibers; (a) Long, slender, band-like fibers with no myofibrillæ, with protoplasm staining deep red in eosin and black in iron-hæmatoxylin. In such fibers the nuclei are long and rod-like; (b) spindle-shaped fibers, thicker in cross-section than type (a), with slightly staining, fibrillated protoplasm and short, thick nuclei. Between these two types he found all transitions. The deeply staining, homogeneous fibers, he considered the resting; the lightly staining, fibril-

lated fibers, the contracted fibers. In firmly contracted muscle, fibers of type (b) predominate; in resting muscle, fibers of type (a).

Heiderich, 1902, studied contraction of smooth muscle in the intestine, urino-genital tract and blood-vessels of a number of mammals. He obtained contracted muscle by heating the tissue and by injecting apomorphine. Heiderich's conclusions are almost diametrically opposed to those of Henneberg. He found in contracted muscle, as did Henneberg, two types of fibers: (a) fibers with homogeneous protoplasm, showing marked affinity for eosin, (b) fibers with fibrillated protoplasm, showing little affinity for eosin. The former he considered the contracted, the latter the expanded fibers. In fixed and stained material after certain fixatives the homogeneous fibers have smaller diameter than do the fibrillated fibers. In fresh material this is not the case. Consequently Heiderich concludes that certain reagents must cause more shrinkage in the contracted than in the relaxed fibers. In the homogeneous fibers the nuclei are shorter and thicker than in the fibrillated fibers. The elastic fibers in the neighborhood of the homogeneous fibers run a wavy course, elsewhere they are straight. These facts lend further evidence that the homogeneous are the contracted and the fibrillated the resting fibers. Heiderich describes quite fully the two types of contraction mentioned earlier by Heidenhain, 1861. These types are (a) the peristaltic, occurring in the smooth muscle of digestive and urino-genital tracts and (b) the total, occurring in the blood-vessels. In type (a) the contraction passes over the fiber in a series of waves so that several thickened, homogeneous nodes may appear in each fiber. Between the nodes the myofibrillæ are distinctly seen. In type (b) there is general shortening and thickening of the entire fiber. In explaining the contraction of smooth muscle Heiderich supports the inotagmen theory of Apathy. He found nothing in the structure of the myofibrillæ, however, to indicate their relation to contraction.

Schaper, 1902, in the muscle fibers in the mesentery of Urodela found, at times, spindle-shaped enlargements of the fibers. The myofibrillæ in the mesenteric muscle are frequently segmented, made up of alternate dark and light bands. Schaper mentions the possibility that the segmentation may be due to contraction set up by the fixative.

Benda, 1902, described in smooth muscle two types of myofibrillæ, coarse and fine, corresponding somewhat closely to the "Grenzfibrillen" and "Binnenfibrillen" of Heidenhain. He considered only the fine myofibrillæ contractile elements. The coarse myofibrillæ he believed to be elastic or supportive structures.

Forster, 1904, studied contraction in amphibian and mammalian muscle. According to his description, the muscle cell as a whole contracts in such a way that it is rolled into a spiral. The nucleus follows this spiral winding. From the amount of spiral winding of the nucleus the amount of contraction of the muscle fiber may be determined.

Schlater, 1905, like Forster, described the smooth muscle nucleus as undergoing a spiral winding during contraction. He is of the opinion, however, that the nucleus is entirely passive, but believes that, at the same time, there may be an active decrease in length and increase in diameter of the nucleus.

Soli, 1906, 1907, in the smooth muscle of the stomach of birds, found practically the same conditions described by Heiderich for the peristaltic form of contraction. There is this difference, that in all of his material he found the contraction nodes of greater diameter than the uncontracted internodal segments.

Verzár, 1907, in the smooth muscle cells of the amnion of the chick found with ordinary hæmatoxylin-eosin stains, very distinct boundaries to the cells. When stained with iron-hæmatoxylin, no distinct boundaries were present, and the myofibrillæ, both coarse and fine, apparently run from one cell to another.

McGill, (3) 1907, in a preliminary paper described briefly the fibrillar and nuclear changes which are given in more detail in this present paper.

III. Material and Methods.

1. Material used.

Smooth muscle from the following vertebrate sources was the material used in this investigation: among Amphibia, from the alimentary canal and urinary bladder of the frog and of Necturus; among birds, from the alimentary canal of the chicken; among mammals, from

several regions of the alimentary canal and urino-genital tract and from the blood-vessels of dog, cat, ox, pig and man.

2. Fixation of resting muscle.

To obtain smooth muscle in the resting or relaxed condition several methods were employed. When the tissue was found completely extended, as it frequently is in the various organs of the alimentary canal, pieces could be fixed in that condition. To do this, very small pieces of the resting muscle were clipped out with sharp scissors and thrown quickly into the fixative. The fixative should be one that acts rapidly, such as hot water, hot sublimate solution or hot Zenker's fluid. In using this method the work should be done rapidly or the mere mechanical stimulus of handling the muscle will often cause it to contract. When the fixative used penetrates rapidly, as does hot Zenker's fluid, larger pieces may be fixed without undergoing appreciable contraction. In the large intestine of mammals, where, after stimulation, the contraction proceeds very slowly, whole segments of expanded muscle were removed and fixed in Zenker's fluid without producing contraction. In the small intestine of mammals the smooth muscle is so irritable that even the slightest stimuli set up very rapid contractions. Hence it was found impossible to fix the tissue rapidly enough to prevent some contraction. Even here, however, while small areas were contracted, there were many areas of entirely resting muscle, unless the stimulus was strong and continuous. In the alimentary canal of birds the tissue is so easily stimulated that the pieces could seldom be fixed without showing some contraction. Here the only means of obtaining entirely relaxed muscle was by employing narcotics. In Amphibia, especially during the winter, the irritability of the smooth muscle of the alimentary canal is so low that whole segments were removed and placed in any of the ordinary fixatives without producing contraction.

In the large arteries, upon section, the portion next to the heart contracts firmly, that away from the heart, relaxes completely. Small pieces, taken from the distal end of the cut carotid a few minutes after the artery had been severed, were fixed in hot water or hot Zenker's fluid. They show completely extended muscle. This method was successfully used by Henneberg, 1901, and by Heiderich, 1902.

By the use of narcotics, smooth muscle can be made to relax entirely, and will remain in this condition long enough to be studied fresh or to be fixed and sectioned. Small pieces placed for a short time in 0.5 per cent to 4 per cent cocain solution in normal saline became completely extended. Very weak solutions of atropine sulphate, 0.00005 per cent to 0.001 per cent, had a similar effect. The narcotized tissue was either studied fresh by teasing or by frozen sections, or was fixed and sectioned.

Lastly, living smooth muscle was studied directly under the microscope. Small pieces of the muscularis from the intestine of Necturus, chicken and dog were removed from the recently killed animal and were mounted in either lymph or Ringer's solution on a warm slide, and were examined immediately. At first, due to the stimulation of cutting and mounting, the tissue is firmly contracted. Upon standing a few minutes, however, the fibers competely relax. Though the finer details of structure cannot be made out from living material, yet the general form of the fiber and even of the nucleus and myofibrillæ can be easily studied.

3. Fixation of contracted muscle.

Contraction of smooth muscle tissue was studied from material prepared in a number of ways. In the muscle of the alimentary canal of mammals, especially in the small intestine, the tissue is usually in peristalsis so that pieces taken at random at some point contain contracted fibers. Then the mechanical stimulation of removing the tissue sets up more marked contraction waves. This, together with the stimulating action of the fixative makes it often impossible, without the use of narcotics, to prepare sections of the smooth muscle of the small intestine, which do not show at least a part of the fibers contracted. However, to get very firmly contracted smooth muscle from this region the tissue was specially stimulated, either mechanically or electrically.

In preparing the material the animal was anæsthetized and the abdominal cavity quickly opened; then the stimulus was supplied to one portion of the intestine and continued until an area was completely contracted throughout the whole circumference. The entire segment was then removed and fixed in hot Zenker's fluid. Occasion-

ally small pieces were fixed, but they did not give as good results as did the larger pieces. This was due to the fact that in small isolated pieces of muscle the tension is removed and consequently the fibers in contracting take an abnormal wavy or zigzag course or become otherwise distorted. In every instance when placed in Zenker's solution the larger pieces were perfectly fixed throughout.

When relaxed muscle is placed into fixatives which act slowly the chemical stimulus itself is often sufficient to produce very firm contraction waves.

On opening the abdominal cavity so that the alimentary canal is exposed to the air for even a short time, strong peristalses appear. In the small intestine the waves usually involve only a small part of the entire circumference of the tube. In the large intestine, however, the tube may contract firmly throughout the whole diameter and is easily fixed in this condition. On removing the intestine from a recently killed animal and placing it in warm Ringer's solution (37° C.) contractions, similar to those caused by exposure to air, though stronger, are set up.

The large intestine of dog and cat was found to be one of the most favorable places to obtain both contracted and resting smooth muscle. When this portion of the alimentary canal is stimulated, at the point where the stimulus is applied, it contracts very firmly throughout the entire circumference. Areas between the point of stimulation are just as markedly relaxed. Furthermore, it requires considerable stimulus to start peristalsis and the tissue contracts very slowly. Hence whole segments were removed and fixed with portions remaining entirely relaxed, others firmly contracted. This was consequently excellent material for a comparative study of resting and contracted muscle.

When the large intestine of the dog is filled with feces, the muscle normally contracts in rings around the feces. At other points it is relaxed. From such areas with no special stimulus entirely contracted and entirely relaxed muscle was obtained.

In the chicken the muscle of the alimentary canal is highly irritable so that the slightest stimulus induces contraction. The fixative alone is sufficient to produce strong peristaltic waves. Contracted muscle from the stomach, small intestine and cæcum was studied.

In Amphibia, as previously mentioned, the muscle of the digestive tract is very inactive. In order to obtain contracted muscle from these forms the tissue was stimulated for some time with a rather strong electric current. It was often possible on the side of the intestine where the stimulus was applied, to obtain a very firmly contracted area while the remainder of the muscle in the circumference of the tube was entirely relaxed.

For contracted arterial muscle, the proximal end of the previously severed carotid was taken and rapidly fixed in hot Zenker's fluid. Only mammalian blood-vessels were studied.

In the urino-genital tract no special effort was made to get contracted muscle, but usually in fixed material contraction waves were seen.

The morphological changes in smooth muscle produced by a number of drugs which cause contraction were studied. The tissue was placed in a physiological solution of the drug until contracted, and then examined, either living or after fixation in sections. The drugs used were pilocarpine, apomorphine and strong solutions of atropine (0.01 per cent, or stronger). The drug effects were tested on intestinal muscle of Necturus, cat and dog.

Contraction in smooth muscle was also examined directly in fresh muscle. Small pieces of muscle from the intestine of Necturus, chicken, dog and cat, and from the carotid artery of ox were clipped out with sharp scissors and mounted in normal saline, Ringer's solution, or blood serum, upon a warm slide. The slide was fitted with foil electrodes for electrical stimulation. Thin longitudinal strands of the muscle were placed between the electrodes, then a cover-glass supported by thin strips of filter paper was adjusted. The tissue was examined directly under the microscope. Low power lenses were usually adequate, but for finer structures a water-immersion (Zeiss H lens) was used. After mounting, the tissue was allowed to stand for a few minutes, or until it relaxed completely, then a weak electric current was passed through. As the stimulus was applied the muscle fibers contracted. The course of the contraction waves and some of the finer structural changes accompanying them could then be observed.

In the peristaltic type of contraction there passes simultaneously over neighboring fibers a very characteristic sort of contraction wave. It has seemed important to determine whether the simultaneous occurrence of contraction in contiguous fibers is due to nerve control or to some morphological connection between the fibers themselves. To determine this it was necessary to exclude stimulation of the muscle fibers through the nerves. This was done by immersing the tissue for some time in very weak solutions of atropine sulphate. After such treatment the tissue could still be mechanically stimulated to contraction. Atropine is supposed to cause relaxation of smooth muscle cells by paralyzing the motor nerve endings in the fibers. This point has not been entirely proved, but most recent work seems to confirm the statement. Unger, 1907, reviews the literature on this subject and gives additional data. In testing the atropine effect the methods of Unger were used.

The development of the power of contraction in smooth muscle was studied in a series of pig and chick embryos, together with a few observations on an eight months human fœtus. In most instances the fixative was relied upon to furnish the necessary stimulus. However, some experimental work was done. The muscle from the small intestine of pig embryos ranging in length from two centimeters to thirty centimeters was stimulated in solutions of pilocarpine and apomorphine, then fixed and sectioned. Tissue from the muscular stomach and small intestine of the chick embryos from five days to twenty days old, was studied both living and after fixation. The fresh muscle was studied directly under the microscope. The fixed muscle was stimulated mechanically before fixation.

4. Methods of fixation, embedding and staining.

The muscle was fixed in several different reagents, including alcohol, alcohol-formalin, saturated aqueous solution of corrosive sublimate, hot water, Gilson's, Flemming's and Zenker's fluids. Zenker's fluid proved most satisfactory both for general structure as well as for bringing out cytological details. The rapidity with which it penetrates even large pieces of tissue makes it especially valuable in this work. With it, the muscle fibers could be fixed in any stage of contraction desired, the reagent itself not acting as a stimulus.

For demonstrating the gross form of the muscle fiber, macerating fluids were occasionally used. Those giving the best results were 30 per cent ethyl alcohol, 30 per cent methyl alcohol, 20 per cent nitric acid and various strengths of potassium hydroxid solutions. They of course served as poor fixatives of the cell protoplasm as well as macerating solutions.

Most of the material was embedded in paraffin and cut in sections from three micra to ten micra thick. To bring out the general form of the smooth muscle fiber the thicker sections were often most useful. Some tissue was embedded in celluloid, but only for comparison. Fresh material cut on a freezing microtome immediately after removal from the body gave for some purposes quite favorable results.

Fresh material unstained was found to show much of the finer structure of the tissue; however, for the more detailed work stains were indispensable. Fresh muscle stained *intra vitam* by methylene blue or neutral red gave good results in some respects.

Fixed material sectioned and stained was used for most of the work. For all general staining, as well as for the demonstration of contraction waves and myofibrillæ, Heidenhain's iron-hæmatoxylin with a counter-stain of eosin or Congo red was found to give excellent results. With this method, by staining and decolorizing for various lengths of time, very different effects may be produced. Moreover, structures which are not shown by the ordinary method may be demonstrated by repeated immersion in the hæmatoxylin, followed in each case by the extraction of the hæmatoxylin in iron-alum. Iron-hæmatoxylin was especially valuable for staining areas of contraction and for tracing the myofibrillæ through such areas. When the hæmatoxylin is only partially extracted by the iron-alum the contraction waves are stained uniformly black throughout. Further extraction brings out the continuity of the myofibrillæ through such areas. When the sections are further decolorized the hæmatoxylin is entirely removed from the contraction nodes before it leaves the nuclei. Such sections counterstained in either eosin or Congo red show the contraction waves intensely red.

Delafield's hæmatoxylin, Hansen's hæmatoxylin, and Flemming's triple stain were also used as general stains.

For differentiating the connective tissue in which the smooth muscle cells are embedded, Mallory's anilin-blue connective tissue stain, Van Gieson's picrofuchsin stain, Weigert's elastic tissue stain and various iron-hæmatoxylin mixtures gave good results. Mallory's stain, at the same time, was found to be a good differential for muscle. With this stain the myofibrillæ of relaxed muscle stain intensely red. In contracted muscle the entire contraction node stains yellow. The nuclei are yellow, all collagenous fibrils blue. Van Gieson's mixture stains contraction nodes intensely yellow, collagenous fibrils bright red. Van Gieson's stain at the same time is an excellent nuclear stain. Elastic fibers were brought out best by Weigert's resorcin stain, though iron-hæmatoxylin often showed them distinctly.

IV. Structure of Resting Smooth Muscle.

1. General structure of smooth muscle.

No attempt will be made here to go into the details of the structure of resting smooth muscle. In this paper the subject will be considered chiefly from the standpoint of the histogenesis of the tissue, which was not considered by Heidenhain. Hence before discussing the histology of the adult tissue it has seemed advisable to give a short resumé of the process of development. For a review of the literature on this subject, together with a detailed description of the histogenesis of smooth muscle, the reader is referred to a paper by the author, McGill (1), 1907.

In the digestive and respiratory tracts of the pig, smooth muscle arises in common with the interstitial connective tissue, from the mesenchymal syncytium surrounding the endodermal tube. The differentiation of smooth muscle begins in the mid-œsophagus of the 5 mm. pig embryo. A condensation of the mesenchyme, with the elongation of the mesenchymal cells initiates the process. As the nuclei elongate, the myofibrillæ appear in the surrounding protoplasm. They arise as coarse, varicose, deeply staining fibrils, which grow rapidly and soon run for long distances through the syncytium without regard to cell boundaries. In later development these coarse myofibrillæ break up, in large part, to form finer myofibrillæ, but some may persist as the coarse myofibrillæ found in some adult smooth muscle.

The interstitial connective tissue arises *in situ*. Some of the mesenchyme cells in the area of muscle formation do not elongate but persist as the connective tissue cells. These are connected, at least until a very late stage, and at times in the adult with the protoplasmic syncytium of the muscle tissue, Fig. 14. In the common syncytium soon after the muscle begins to form, collagenous fibers arise, and at a still later stage elastic fibers develop. Often in a single protoplasmic mass connective tissue fibers and myofibrillæ differentiate side by side.

As the myofibrillæ form in the protoplasmic syncytium, they tend to run in longitudinal bundles, but usually they show anastomoses with neighboring bundles. Throughout development, and in most instances in the adult pig, the syncytial structure persists.

The muscle of the digestive tract of man and of chick and of the bloodvessels of chick undergoes a histogenesis very similar to that of the digestive tract of the pig, though in both these forms the syncytial arrangement in later development is not so apparent.

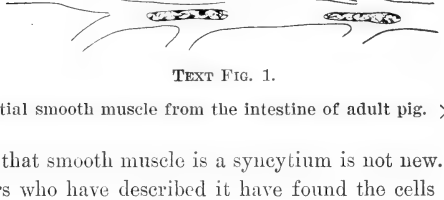
In general it may be said that complete uniformity in the structure of adult smooth muscle in different forms and even in the different organs of the same form does not exist. Adult smooth muscle may show one of two types and possibly three types of structure, McGill (2), 1907. In type 1 there is very distinct syncytial arrangement with both end and side anastomoses of the fibers, text Fig. 1, which is a persistence of embryonic condition. In Type 2 the muscle bundles show few side anastomoses, but end to end union exists either with or without terminal branching of the fibers, text Fig. 2. (3) There is possibly a third type of smooth muscle. In this type there are apparently no protoplasmic connections between the fibers. Each seems to be an independent spindle-shaped cell, text Fig. 3. Between these three types there are found all transitions. In the description each type will be taken separately.

Since syncytium as used in recent anatomical writings is a rather indefinite term, its meaning as used in this paper will be explained. By muscle syncytium is meant any tissue where there are well defined protoplasmic anatosmoses between the muscle cells. Where all of the cells are so connected the tissue is described as being a complete

syncytium. Where some of the cells are independent, others connected, the term partial syncytium is used.

a. Smooth muscle with complete syncytial structure.

When viewed in longitudinal section it is easily demonstrated that in some adult smooth muscle there is a persistence of the embryonal syncytium. The smooth muscle fibers are united by larger or smaller protoplasmic strands so that independent cells are not present. This arrangement is found in the intestine and bladder of Necturus, in the bladder of the frog, in the stomach and intestine of the chicken, and in portions of the digestive tract of dog, cat, pig and man. Muscle of type 1 is not found in all of the muscle in the regions cited. Fibers showing only end to end union often occur in portions while neighboring portions may show both end and side anastomoses.



TEXT FIG. 1.

Syncytial smooth muscle from the intestine of adult pig. × 650.

The idea that smooth muscle is a syncytium is not new. However, most authors who have described it have found the cells united only by very delicate protoplasmic strands, the so-called intercellular bridges. Among these investigators may be mentioned Leydig, 1849; Wagner, 1869; Flemming, 1878; Kultschitzky, 1888; Barfurth, 1891; Heidenhain, 1893; Werner, 1894, and Bohemann, 1895. The intercellular bridges were considered by most of these writers as of secondary origin and not as the result of the persistence of an embryonic syncytium. The improved connective tissue stains of recent years, such as Mallory's anilin-blue mixture, Van Gieson's mixture, and Mallory's phosphotungstic mixture, have shown that these fine anastomoses between the muscle cells are chiefly connective tissue strands, Figs. 20, 23, 27 and 34, and not true protoplasmic bridges.

The possibility that muscle protoplasm may surround the connective tissue strands and thus be continuous from muscle cell to muscle cell must not be forgotten. The sarcoplasm between the myofibrillæ is extremely hard to differentiate. A small amount of it may continue along the collagenous fibers and still not be seen in preparations. The collagenous fibers between the muscle cells are surrounded by protoplasm during development. In a few places this was seen in the adult. Where there is such an arrangement, the collagenous strands with surrounding protoplasm do represent true cell bridges.

A number of investigators have described a smooth muscle syncytium for adult muscle, where the fibers are united by wide protoplasmic strands. These references have been abstracted in the literature review. Drasch, 1895, in the skin glands, and Schaper, 1902, in the mesentery of Urodela, Rohde, 1905, in a number of vertebrates, and McGill (1), 1907, in the pig, have all described such syncytia.

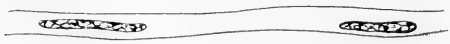
Among Amphibia rather complete syncytia were found in the urinary bladder and the digestive tract of Necturus and frog. Such an anastomosis is shown in Fig. 7, drawn from the intestine of Necturus.

In the alimentary canal of chicken only occasionally are there marked side anastomoses of the fibers, though end to end union is common.

In mammals marked side anastomosis of muscle fibers was observed in some regions in the pig, dog, cat and man. In some of these forms it is very pronounced, in others it is only occasionally met with. In the muscularis muscosæ of the small intestine of the pig is the most complete syncytium among the mammals studied, Fig. 14.

The muscle syncytium of the digestive tract of adult pig is made up of the much elongated muscle nuclei, each surrounded by a granular protoplasmic reticulum, and outside of this a layer of myofibrillæ embedded in clear protoplasm, Figs. 14, 15. The myofibrillæ are mainly arranged in spindle-shaped bundles, but from these bundles many of them pass in broad end and side anastomoses, into neighboring groups.

The granular protoplasmic reticulum surrounding the nucleus is quite variable in amount. In the two layers of the muscularis of the small intestine it is restricted to a small mass at the pole of each nucleus, Fig. 16. In the muscularis mucosæ of the small intestine there is, surrounding the nucleus, a large spindle-shaped mass of reticular protoplasm, connected by wide anastomoses with that surrounding neighboring muscle nuclei, Fig. 14 a; occasionally it is connected by similar anastomoses with the stellate-shaped connective

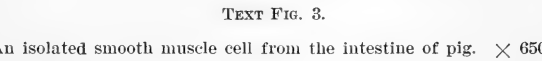


Text Fig. 2.

End to end anastomosis of smooth muscle cells. Intestine of pig. × 650.

tissue cells, Fig. 14 b. Fig. 17 shows a cross section through such muscle tissue, in which the wide area of reticular protoplasm around each nucleus is clearly indicated.

In the muscularis of the intestine of the pig the main mass of muscle consists of the myofibrillæ. They are arranged in a heavy layer around the nucleus, Fig. 16, and in heavy bundles connected with adjacent nuclei, Fig. 15 a. In the muscularis mucosæ there



Text Fig. 3.

An isolated smooth muscle cell from the intestine of pig. × 650.

is often only a thin layer of myofibrillæ around the margin of the reticular protoplasm, Fig. 13, 14, 17. Between these two types there exist all transitions.

Fig. 7 shows a portion of a smooth muscle syncytium from the intestine of Necturus. There is direct continuity between the reticular protoplasm surrounding the nuclei as well as between the myofibrillæ.

b. Smooth muscle with end to end anastomoses of fibers.

As smooth muscle develops, in many places the wide side anastomoses of the cells become less apparent, but the end to end union still persists, text Fig. 2, Figs. 10, 11, 12. The loss of side anastomoses is probably due to a rapid elongation of the central part of the fiber between the anastomoses, thus pushing the anastomoses to the ends of the fibers. It may possibly in places be due to an actual disappearance of the anastomoses themselves. Much of the muscle of

Fig. 4. A completely relaxed cell.

Fig. 5. A cell contracted at one end.

Fig. 6. A cell contracted at each end.

Fig. 7. A completely contracted cell.

Text Figs. 4-7.

Muscle cells in various stages of contraction. Carotid of ox. × 650.

the pig's intestine is in this condition. It occurs commonly in the muscle of the digestive and urino-genital tracts of dog, cat and man, and was occasionally observed in the carotid of the ox. In this type there may be continuity from cell to cell of the reticular protoplasm, Fig. 13. Most often, however, only the myofibrillæ form the anastomoses, Fig. 11. The continuity of myofibrillæ from fiber to fiber was long ago described by Rouget, 1863, and has since been mentioned by several writers.

c. Smooth muscle with apparently isolated fibers.

By far the majority of writers on the structure of smooth muscle have described it as everywhere made up of entirely free and independent spindle-shaped elements, the so-called muscle fibers, muscle cells, or muscle spindles of Kölliker, 1849. Such an element is diagrammatically represented in text Figs. 3, 4. Recent advocates of this idea are Heidenhain, 1900; Heiderich, 1902; Forster, 1904; Schlater, 1905, and Soli, 1906.

In the material studied in this investigation it was found exceedingly difficult to demonstrate isolated smooth muscle fibers. In the walls of the carotid of the ox some of the fibers appear to be free, Fig. 33. Here the fibers are long, slender structures, thicker in the vicinity of the nucleus than elsewhere. Because of their length and the curved course they take in surrounding the lumen (most of them are circular fibers) it is possible in only a few places to get them cut in exactly longitudinal section. It should be remembered that spindles appearing independent in such sections may appear so because the section is cut slightly obliquely. In these sections anastomosis of fibers end to end would not show even if present. Furthermore, a tangential section of a spindle-shaped fiber may look like an isolated fiber. Consequently it is not surprising that end to end union is hard to demonstrate in arterial muscle fibers. In many instances end anastomoses do occur, so even here there is at least a partial syncytium. It is probable, too, that the syncytium is much more nearly complete than sections would lead one to conclude. The development of arterial muscle was not studied in detail, but in both chick and pig the tissue arises as a complete syncytium. In all other muscle studied the syncytial structure in the adult is more apparent than in arterial muscle.

Most of the work of earlier investigators on the general form of the smooth muscle cell was done on macerated material. In maceration the reagent usually destroys at least the peripheral myofibrillæ, so that fine anastomoses even if present are destroyed, leaving only the central spindle-shaped portion of the cell intact. In this way are undoubtedly obtained many of the spindle-shaped cells figured in the text-books.

The relaxed muscle fiber of the carotid of the ox is a long, spindle-shaped structures, much thicker in the center than toward the poles. At the ends it may branch and as before mentioned does anastomose with neighboring fibers. It is made up of a much elongated central nucleus, Fig. 82; outside of the nucleus is a small area of reticular protoplasm, and outside of this, forming, in most cases, the bulk of the cell, is a thick layer of longitudinally running myofibrillæ, Figs. 10, 34 a. If any sarcoplasm exists between the myofibrillæ it is not demonstrable by the ordinary stains, such as hæmatoxylin, eosin, etc. Just beneath the elastica interna many of the smooth muscle cells show a large amount of reticular protoplasm around the nucleus. In such fibers the myofibrillæ are restricted to a thin peripheral layer, Fig. 9. These fibers closely resemble those already described in the muscularis mucosæ of the small intestine of the pig, Fig. 13.

2. Myofibrillæ.

In adult smooth muscle, just as in development, two types of myofibrillæ occur: (1) very fine fibrillæ, evidently corresponding to the elementary fibrils of Apathy, 1890 and 1891, and to the "Binnenfibrillen" of Heidenhain, 1898 and 1900; and (2) coarse fibrillæ which seem to correspond to the primitive fibrils of Apathy. The latter, in some particulars, also resemble the "Grenzfibrillen" of Heidenhain, in others more closely the myofibrillæ of Benda, 1902. There are often in a single muscle cell fibrillæ showing all gradations in size from the coarse to the fine myofibrillæ. In completely extended muscle the individual myofibrillæ are, throughout their entire length, of comparatively even caliber. All of the myofibrillæ stain intensely with protoplasmic stains and with iron-hæmatoxylin.

During early development in the pig and the chick all of the myofibrillæ begin as exceedingly coarse structures. These later break up, probably by longitudinal splitting, into finer fibrillæ. In the older fœtus there is undoubtedly some formation *de novo* of fine fibrillæ. In the adult muscle the coarse myofibrillæ represent either persisting embryonal structures, in which case they are usually entirely homogeneous, Fig. 13, 15, or they may be formed by the subsequent adhesion or union of the fine myofibrillæ into bundles. In this last condition the finer fibrillæ entering into their formation may, at times, be demonstrated.

The fine myofibrillæ in some muscle become very numerous in the later stages, and in the adult the muscle fiber may be well filled with them. They are often the only fibrillæ present. This is usually the case in the intestinal muscle of Necturus, Figs. 6, 23, 24 and of the chicken, Figs. 2, 11. In the muscle of the digestive and urinogenital tracts of mammals at times there are present none but fine myofibrillæ, Fig. 15. In cross section the fine myofibrillæ appear as fine dots, Figs. 16, 26.

In the adult smooth muscle coarse myofibrillæ may or may not be present. When present they are occasionally the only myofibrillæ found, as in portions of the muscularis mucosæ of the small intestine of the pig, Fig. 17, and in the carotid of the ox, Fig. 4 a. Usually, however, they are associated with fine myofibrillæ. In this relation they were found in large numbers in the carotid of the ox, Fig. 5, and in the muscularis mucosæ of the œsophagus of the pig. As occasional structures they occur throughout the muscularis of the digestive tract of dog, cat, pig, and man. No coarse myofibrillæ were found in the smooth muscle of Necturus notwithstanding the fact that in Salamander, a closely allied form, Heidenhain, 1900, found them in large numbers.

When coarse myofibrillæ alone are present, they may be arranged as a peripheral layer, Fig. 17, or they may be scattered throughout the entire diameter of the fiber, Fig. 4 a. When associated with fine myofibrillæ, they may likewise occupy a peripheral position only, Fig. 4 b. They then correspond to the "Grenzfibrillen" described by Heidenhain, 1900. More often the coarse myofibrillæ are scattered throughout the muscle cell. They then approach in arrangement more nearly the coarse myofibrillæ described by Benda, 1902.

In uncontracted muscle the coarse myofibrillæ of the adult are of approximately even caliber throughout the entire length. They stain very intensely in eosin or in iron-hæmatoxylin. Iron-hæmatoxylin stains the coarse myofibrillæ long after it has been extracted from the fine myofibrillæ so that usually in an iron-hæmatoxylin-eosin preparation the coarse myofibrillæ are stained black, the fine myofibrillæ red. In the muscle syncytium both types of fibrillæ run past the limits of one cell, through the anastomoses into neighboring cells, Figs. 13-15.

The coarse myofibrillæ, especially those lying close to the periphery of the muscle cell, have to be differentiated from the elastic fibers which, in some muscle, lie in the connective tissue immediately surrounding the muscle protoplasm, or even embedded in the peripheral protoplasm itself (Fig. 25, McGill (1), 1907). This is especially true in material stained with iron hæmatoxylin. With this stain both coarse myofibrillæ and elastic fibers are intensely black. In longitudinal section is it usually comparatively easy to tell them apart, for the elastic fibers are more wavy than the coarse myofibrillæ. Moreover, the elastic fibers are always around the periphery, while the coarse myofibrillæ may be scattered throughout the cell protoplasm. With Weigert's elastic tissue stains the elastic fibers can be differentiated from the myofibrillæ.

3. Nuclei.

The nucleus of resting smooth muscle is a much elongated, rod-shaped structure many times as long as wide. In the material studied the nuclei vary in length from twenty micra in chicken intestine to eighty micra in Necturus intestine. Schultz, in a large number of forms studied, found the shortest nuclei in smooth muscle of the dove, thirteen micra, and the longest in the intestine of Proteus, seventy-two micra. The proportion of length to width varies extremely. In muscle of a primitive type, as in the syncytia shown in Figs. 7 and 14, the nuclei are very wide in proportion to their length. In arterial muscle the nuclei are extremely long and narrow, Figs. 5, 82.

In muscle of the marked syncytial type the nuclei are usually located at the nodal points, the long axis parallel with the long axis of the bundles of myofibrillæ. In apparently isolated fibers, as in the muscle of the carotid of the ox, the nuclei usually lie close to the center of the fiber. Occasionally an eccentric position is observed. The nucleus may lie so close to one side of the fiber that one surface appears free from the cytoplasm. Figs. 19, 25. An eccentric position of the nucleus was frequently observed in intestinal muscle of Necturus, chicken, and dog. This position of nucleus was described by Kölliker in amphibian muscle and by Lenhossek in cat muscle. In the chicken the nuclei are often nearer one pole of the fiber than the

other. Typically, however, the nucleus is always surrounded by myofibrillæ.

The nuclear membrane consists of a heavy network of linin studded with fine chromatic granules. It is of smooth and even contour. Inside the nucleus is a delicate linin reticulum, with a slightly coarser but still finely meshed chromatic reticulum. Both linin and chromatin are composed of fine granules, Figs. 5, 24, 27, 82, 90. From one to several plasmasomes are present. No spiral strand of chromatin, such as is described by Münch, 1903, was found in resting muscle.

Numerous granules are present in the reticular protoplasm immediately surrounding the nucleus, but none of them could be identified as centrosomes, such as were described by Lenhossek, 1899.

4. Interstitial connective tissue.

The interstitial connective tissue of adult smooth muscle resembles as a rule ordinary areolar tissue. The connective tissue cells in some cases seem to retain their primitive relation to the protoplasmic syncytium, Fig. 14 b. The collagenous fibers may be arranged in a loose reticulum, Figs. 2, 24, 30, or as a denser reticulum, Figs. 15, 16. Where the muscle cells lie very close together the collagenous fibers may be crowded into thin membranes. Such membranes have been described by Watney, 1879, by Heidenhain, 1900, and were figured by the author in a previous paper (1), 1907.

The collagenous fibers were stained by Mallory's anilin-blue connective tissue stain and Van Gieson's stain. With either of these stains they appear extremely fine, united in may places into bundles, Figs. 28, 29, 34. Here and there in the adult they still appear to run through the protoplasm of the connective tissue cells or even in among the myofibrillæ.

The elastic fibers are rather coarse, homogeneous structures, which vary greatly in thickness, Figs. 8, 12, 15. They frequently branch and anastomose. They lie for the most part close beside the muscle fibers. Rarely some of them are embedded in the muscle protoplasm among the peripheral myofibrillæ. The intimate relations of collagenous and elastic fibers and the myofibrillæ are clearly understood

when their origin from a common protoplasmic syncytium is remembered.

V. The Gross Changes in Muscle Coats During Contraction.

1. In the digestive tract.

The gross changes of the muscle coats of the digestive tract were studied in the small and large intestine of dog and Necturus. In the large intestine of the dog during contraction there passes over the organ from before backward a series of ringlike areas of contraction. These areas include the entire circumference of the intestine. All of the muscle in a cross section of a given segment is contracted about the same amount. The gut may, however, be very firmly contracted at one point and a neighboring segment may be completely relaxed.

During contraction both layers of the muscularis thicken simultaneously. Along with this there is such a decrease in the lumen that the whole diameter of the tract decreases. The two coats contract in equal ratio so that if before contraction the ratio of the thickness of the longitudinal coat to that of the circular coat is as one to two, after contraction this same ratio is maintained. The following data are from measurements taken on sections from closely lying segments of the large intestine of dog:

Intestine 1.

a, contracted area
circular coat, 1.1 mm.
longitudinal coat, 0.6 mm.
0.6 : 1.1 = 1 : 1.8.

b, uncontracted area
circular coat, 0.3 mm.
longitudinal coat, 0.17 mm.
0.17 : 0.3 = 1 : 1.76.

Intestine 2.

a, contracted area
circular coat, 0.4 mm.
longitudinal coat, 0.12 mm.
0.12 : 0.4 = 1 : 3.3.

b, uncontracted area
circular coat, 0.2 mm.
longitudinal coat, 0.065 mm.
0.065 : 0.2 = 1 : 3.

Intestine 3.

a, contracted area
circular coat, 0.61 mm.
longitudinal coat, 0.2 mm.
0.2 : 0.61 = 1 : 3.

b, uncontracted area
circular coat, 0.34 mm.
longitudinal coat, 0.11 mm.
0.11 : 0.34 = 1 : 3.

b. Smooth muscle with end to end anastomoses of fibers.

As the large intestine of dog contracts, aside from an increase in thickness of both muscular coats and a decrease in the lumen of the tube, there is an accompanying decrease in the length of the contracting segment. The decrease in lumen is due to contraction of circular muscle, the decrease in length to the contraction of the longitudinal muscle.

In the small intestine of Necturus, dog and cat the peristalses are very irregular. The waves are short and follow each other rapidly. Seldom does a wave extend entirely around the circumference of the intestine. Thus in a single cross-section of the small intestine all gradations from completely contracted to relaxed muscle may be found. Here, as in the large intestine of dog, a very constant ratio exists between the thickness of the longitudinal and that of the circular muscle coat. In a preliminary paper (McGill (3), 1907), Fig. 2 is a cross section from a contracted area, Fig. 1 from an uncontracted area of the same section of small intestine of Necturus. Because of the incomplete contraction several measurements could be made on a single cross section. The following measurements are taken from cross sections of the small intestine of Necturus:

Section 1.

a, uncontracted area
circular coat, 0.262 mm.
longitudinal coat, 0.125 mm.

b, partially contracted area
circular coat, 0.2 mm.
longitudinal coat, 0.1 mm.

c, uncontracted area
circular coat, 0.162 mm.
longitudinal coat, 0.081 mm.

The ratio of thickness of the longitudinal to the circular coat in all three measurements is approximately as 1 to 2. A number of measurements were taken from different sections, all with like results.

As the small intestine of Necturus contracts the lumen decreases in diameter, due to the contraction of the circular muscle. The simultaneous contraction of the longitudinal muscle has, however, little effect upon the length of the intestine. A number of measurements were taken upon segments of intestine, both before and after contraction. In every instance the length remained nearly constant, though sections showed in the contracted segments distinct contraction waves in the longitudinal fibers. Though the longitudinal fibers were distinctly shortened at the contraction waves, they were unduly stretched between, which possibly accounts for no decrease in length. The work was done on animals which had been kept in captivity for some time and had been fed little, so contraction may not have been entirely normal.

2. In arteries.

In arteries during contraction there is a marked thickening of the media with accompanying decrease in the size of the lumen. When the fresh carotid of the ox is severed the proximal end contracts and the distal end relaxes. As the muscle contracts the wall of the vessel thickens and the lumen decreases in diameter, as it relaxes the reverse changes take place. Since there is little longitudinal muscle there is little change in the length of the vessel.

VI. Forms of Contraction in Smooth Muscle.

In smooth muscle two main types of contraction have been described: 1. Peristaltic contraction, where one or more contraction nodes appear in the fiber, with uncontracted areas between; 2. Total contraction where the entire fiber shortens and thickens. In this study, muscle with contraction of type 1 was easily demonstrated. Muscle of type 2 was seldom found, though in blood-vessels this type is in places nearly approached.

1. Peristaltic contraction.

Before discussing the changes which take place in smooth muscle during the peristaltic type of contraction, a few of the terms used will be defined. By contraction area is meant the entire mass of muscle, all or a part of the fibers of which have undergone active shortening

and thickening. The term contraction wave will be restricted to the deeply staining so-called homogeneous bands of firmly contracted muscle which pass irregularly across the contraction areas, Figs. 1, 2, 11, 21, 30, 37. Contraction node refers to the deeply staining thickened area in the individual muscle fiber, and internodal segment to the fibrillated, uncontracted or weakly contracted portion of the fiber between the contraction nodes.

a. The form of the contraction wave.

As the muscle of the digestive tract contracts, contraction waves appear. They are irregular in outline and branch and anastomose, Fig. 1, 30, 37. In Fig. 1 they are shown in a cross section through the contracted large intestine of dog. They are seen in the circular coats of the muscularis as irregular bands extending in places across the entire thickness of the muscle coat. The section of course shows them only in two dimensions. In serial sections their extent in the third dimension was determined. In this direction up and down the intestine they extend as irregular bands of about the same width as shown in the cross-section of the intestine. In the longitudinal coat the waves are similar in form to those found in the circular coat. In fact, usually when a contraction wave in the circular coat reaches the outer margin, there is opposite it in the longitudinal coat a similar wave, Fig. 1. When the contraction waves are cut in cross section of the muscle they appear as irregular polygonal areas studded thickly with the cut ends of contracted muscle fibers, Fig. 1, l, m. The contraction waves may extend quite obliquely across the area of contraction, Fig. 1 c. In places the contraction waves are of even contour, in other places of very diffuse and irregular outline. Not all contraction waves pass across the entire thickness of the muscularis. They may involve only a few fibers or when just beginning to form only a portion of a single fiber.

In the large intestine of dog and in the small intestine of Necturus, in tissue firmly contracted the contraction waves are quite broad, involving from one-fourth to one-half of the length of the muscle fiber, Figs. 21, 23, 30. In muscle not so firmly contracted they may be extremely narrow, Figs. 35, 39, 40. There are from one to three of these waves in each contracted fiber.

In the small intestine of chicken, Figs. 2, 11, and of mammals the contraction waves are narrower and closer together. There may be several traversing each fiber. In fact they are often so numerous as to give the fiber a distinctly cross-striated appearance. It was probably this that led many earlier writers to describe a cross-striated involuntary muscle in portions of the digestive tract.

Here and there the peristaltic type of contraction does not produce contraction waves. In such muscle there is apparently no relation whatsoever between the contraction nodes of neighboring fibers. This seems to be largely true for the smooth muscle of the urinogenital tract.

The conclusive way to study contraction phenomena is in fresh muscle. Small pieces of living muscle from the small intestine of Necturus, from the small and large intestine of dog, and from the muscular stomach of chicken were mounted as already described, in Ringer's solution or blood serum, over small electrodes on a slide and examined under the microscope. When the tissue was stimulated it contracted and the contraction waves could be observed passing over the muscle fibers, causing distinct enlargements of the fiber as they passed. The contraction is initiated almost simultaneously in neighboring fibers. Thus arise the contraction waves.

To determine whether the form of the contraction wave is due to nerve regulation or whether it is the result of some morphological connection between the muscle fibers themselves, portions of the intestine of cat were treated with atropine until the nerve endings in the smooth muscle were completely paralyzed. They were then stimulated mechanically. Marked contraction took place. Sections of such contracted material, as well as the living muscle examined directly showed identically the same form of contraction wave as did sections from the normal material used for control. The methods used are described under material and methods, so need not be described here. The following data from a single experiment show the effect of atropine:

March 14, 1908.

A large cat weighing 2700 gm. was decapitated, the abdominal cavity quickly opened and the intestines removed by clipping the

mesentery close to the tube. They were placed in a jar containing 2000 c. c. of Ringer's solution, kept at a temperature of 37° C.

2.15 P. M.—Placed the intestine in Ringer's solution.

2.45 P. M.—The intestine was contracting rhythmically, so added 4 mg. of atropine. The peristalses stopped almost immediately.

3.45 P. M.—The intestine was still quiet. Added 10 mg. more of atropine.

5.45 P. M.—The intestine was still quiet. Added 10 cg. of atropine. Contraction began again in a few minutes, probably due to the overdose.

During the progress of the experiment pieces of the intestine were removed, stimulated mechanically until completely contracted, then fixed in Zenker's fluid. Normal intestine from the same animal was similarly fixed for control. Sections from both the normal and atropinized muscle as well as the living muscle examined directly all showed contraction waves of exactly the same form.

The above experiment would lead one to conclude that the contraction waves do not depend upon nerve control. They must be transmitted to neighboring fibers through some other connection between the cells. Since the contraction waves are most distinct in muscle with most complete syncytial structure, it is possible that they are transmitted through the anastomoses between the fibers. However, in muscle with only end to end anatomoses, the contraction waves are frequently well marked. There is a possibility that the interstitial connective tissue may transmit the waves. The continuity of the contraction waves from circular to longitudinal coats, between which there is often a rather thick layer of connective tissue, would rather seem to support this view. The results of experimentation with atropine are not absolutely conclusive, for the paralyzing effect of atropine has been questioned. This was discussed under material and methods.

The staining reactions of contraction waves have already been described. In Figs. 1, 2, 11, 23, they are shown in material stained in Mallory's anilin-blue connective tissue stain, in Figs. 21, 37, in material stained in Delafield's hæmatoxylin eosin.

The behavior of the interstitial connective tissue during contraction is worthy of mention. All the changes noted are purely passive ones, caused by the decrease in length and increase in thickness of the nearby muscle fibers. During contraction the changes are similar to those described by Heiderich, 1902. In uncontracted areas the collagenous fibers form a loose reticulum, Figs. 2, 13, 15, 24, etc.; elastic fibers run straight, Fig. 12. In the contraction waves the collagenous fibers become much condensed, Figs. 20-23, 28-30. This explains why, by earlier workers on intercellular bridges, all of whom considered the collagenous fibers protoplasmic connectives, the bridges were described as being more numerous and larger in contracted than in uncontracted muscle. If the pressure of the contracting fibers in the contraction waves be great enough the collagenous fibers become packed into distinct membranes, Fig. 31. The elastic fibers where contraction waves pass over the muscle become distinctly wavy, due to passive shortening, Fig. 12.

Well defined contraction waves do not occur in arterial muscle. Frequently a contracted fiber will be entirely surrounded by uncontracted fibers. More often an irregular area, including anywhere from a few fibers to the entire thickness of the muscle coat, is contracted, while neighboring muscle is relaxed. In firm contraction practically every fiber in the arterial wall may be contracted.

b. The form of the contraction node.

In peristaltic contraction the muscle fiber is traversed by one or more thickened areas, the contraction nodes. They represent the portion of the fiber included within the contraction wave, consequently they are just as numerous in the fiber as are the contraction waves passing over it. In the large intestine of dog and the small intestine of Necturus, when firmly contracted the single contraction nodes may include as much as one-half of the entire length of the fiber, Figs. 21, 23, 30. When less firmly contracted the contraction nodes are not so wide, Figs. 35, 40. In the smooth muscle of the small intestine of mammals and in the muscular stomach of chicken the contraction nodes are shorter and more numerous. Fig. 11. In the muscle of the œsophagus of the pig they are often so close together as to make the fiber appear distinctly cross-striated.

While the internodal segments of the fibers are lightly staining and distinctly fibrillated, as in Figs. 2, 5, 11, 20-22, 35, 36, the contraction nodes in ordinary material appear homogeneous and are deeply staining. This condition has been described by numerous investigators and by them has been variously interpreted. Kölliker, 1849; Roulé, 1890, 1891; Schaffer, 1899; Heiderich, 1902, and Soli, 1906, 1907, considered the homogeneous nodes the contracted portions of the fiber. Henneberg, 1901, because he found that these nodes in his fixed and stained preparations were usually of smaller caliber than the internodal segments, described them as uncontracted, the internodal segments as the contracted portions of the fiber. His work was done upon the carotid of the ox. Heiderich demonstrated quite conclusively on the same material that the homogeneous nodes are nodes of contraction. That Henneberg found them smaller in cross section of fixed material than the internodal segments Heiderich explained as due to their being more subject to shrinkage in some reagents than are the fibrillated internodal segments. He tested a large number of fixatives. With some the internodal segments were larger than the homogeneous areas, with others smaller. In fresh material the homogeneous areas were always of greater caliber. The fact that the nuclei shorten and thicken at the homogeneous areas while the elastic fibers run more wavy and the collagenous fibers are more condensed at these points he took as additional evidence that the deeply staining homogeneous nodes are the contraction nodes. Soli, 1906, 1907, in the stomach muscle of birds found in fixed material that the deeply staining homogeneous areas are invariably of greater caliber than are the fibrillated portions of the fiber, so he considers the former the contraction nodes.

In most of the material investigated by the author the contraction nodes resemble those described by Soli, 1906. That is, they occur as deeply staining thickened nodes of the fiber, Figs. 2, 11, 12, 20-22, 30, 37. With ordinary stains, such as hæmatoxylin eosin or in iron-hæmatoxylin, after the usual differentiations they appear homogeneous. Muscle of this type was found throughout the digestive tract of chicken and mammals and in the urino-genital tracts of mammals. The most pronounced thickening of the fiber at the contraction node

was found in the muscle of the digestive tract of chicken, Fig. 2, of dog, Fig. 21, and in that of the urino-genital tract of man. Figs. 3 and 31 show the marked thickening which may take place in the fiber during contraction. In Fig. 3 the contraction nodes are stained black, in Fig. 31 orange. The mortising of the contraction nodes of neighboring fibers together, as is shown in the contraction wave in Fig. 2, is further proof of the large amount of thickening of the fiber which takes place at this point. The occurrence of thickening of the contraction nodes in fixed material is probably due to the fact that the fixative used (Zenker's fluid) did not produce the shrinkage of the contraction nodes which was obtained by Henneberg and Heiderich in their work.

When the contraction nodes are seen in cross section many of them are oval or elongated, indicating that the fiber at this point is distinctly flattened. The flattening is probably due to unequal pressure of neighboring nodes. It is seen most frequently where there is little connective tissue separating the nodes. In the circular muscle of both intestine and blood-vessels especially nearest the lumen the contracted fibers show this flattening. In cross section of these fibers the long diameter extends from nearest the lumen outward. Since, when the muscle of these tubes is contracted the lumen is narrowed, this direction of flattening is just what one would expect if it be due to pressure. The uncontracted fibers, internodal segments and even the contraction nodes, when they are separated by much loose connective tissue, are typically round in cross section.

In the muscle from the contracted areas of the small intestine of Necturus the deeply staining contraction nodes are of just about the same thickness as the internodal segments. In this the muscle approaches that described by Henneberg. But even here when the nodes and internodal segments of the contracted areas, Fig. 23, are compared with the fibers in completely relaxed muscle, Fig. 24, it will be seen clearly that both are of much greater caliber than are the relaxed fibers. Furthermore, from the shortening of the nuclei and the condensation of the connective tissue, it will be seen that the fibrillated internodal segments have also undergone some contraction. So even in Necturus muscle it seems that at all times the contracted fibers are of greater caliber than are the relaxed fibers.

The contraction nodes can be demonstrated in fresh material. Small pieces of muscle from the stomach of chicken, mounted in physiological solutions and examined under the microscope show them even when the muscle is not stimulated electrically. With some electrical stimulation they may be demonstrated in almost any living smooth muscle. They appear under the microscope as marked homogeneous thickenings of the fiber.

Though all previous investigators of contractility in smooth muscle have described the contraction nodes as homogeneous, it is possible in material stained in iron-hæmatoxylin and properly differentiated, to trace the myofibrillæ through them and to show the continuity with the myofibrillæ in the internodal segments, Figs. 35, 40. This will be discussed more fully later when the behavior of the myofibrillæ during contraction is described.

As proof that the homogeneous, deeply staining nodes are the contracted portions of the smooth muscle fiber may be mentioned the following: In living material they show as distinctly thickened areas. In fixed material when the fixative used is one which does not produce unequal shrinkage of the nodes and the internodal segments, the homogeneous nodes show (as in fresh material) as thickenings of the fiber. In both fresh and fixed material the nuclei are drawn closer together and are shorter and thicker in the contraction nodes than in the internodal segments. Figs. 20-22, 37. Around the contraction nodes the collagenous connective tissue is condensed. Figs. 20-23, 30. In the neighborhood of the nodes the elastic fibers run a very wavy course, while through the internodal segments they are comparatively straight, Fig. 12. And, as will be described more fully later, the myofibrillæ, when they can be traced through the contraction nodes, run straight and are thicker there than they are in uncontracted internodal segments. A spiral winding of smooth muscle during contraction, such as is described by Forster, was not observed.

2. Total contraction.

Total contraction, as it has been described, is characterized by the decrease in length and increase in thickness of the entire muscle fiber, text Figs. 4, 7. This form of contraction was observed by Kölliker,

Heidenhain, Henneberg and Heiderich in the muscle of blood-vessels. It is not the only type of contraction described for blood-vessels. Heiderich, 1902, in the umbilical vessels found peristaltic contraction.

In the material studied in this investigation typical total contraction was not observed. In the smooth muscle of the blood-vessels of dog, cat, pig, ox and man and in the sphincter pylori of Necturus and dog, the contraction approaches the total type, Figs. 33, 34. The contraction often involves all but the tips of the fibers, Fig. 33. Here, as in determining the form of arterial muscle, the interpretaiton of sections is difficult. A fiber contracted in the middle, as in Fig. 33 and Fig. 34 a, if cut through slightly obliquely or tangentially, would appear in section as a short, completely contracted fiber, Fig. 34 b. In rare instances the contraction may involve the entire fiber. More frequently the contraction passes over the fiber in broad nodes involving from one-half to two-thirds of the fiber, Fig. 34, text Fig. 5. Such fibers are well differentiated in material stained in Mallory's anilin-blue connective tissue stain. The contraction node stains orange, the uncontracted portion of the fiber red. It seems highly probable that arterial contraction is only a modified contraction where the contraction nodes are much longer and involve more of the fiber than is usual in the peristaltic contraction in the muscle of the alimentary canal or the urino-genital tract. In some places in the carotid of the ox two or even more contraction nodes are present in a single muscle fiber, thus producing a typical partial contraction, Fig. 5, text Fig. 6. The contraction nodes in the muscle fibers of blood-vessels are not as deeply staining as are those in the muscle of the digestive and urino-genital tracts. Consequently the fibrillæ in material stained in either iron-hæmatoxylin or Mallory's anilin-blue connective tissue stain can in almost every instance be traced through the contraction nodes, *cn* in Figs. 33, 34. Fig. 34 shows them in cross section of a contraction node. The cut ends of the fibrillæ show as fine dots. It is only when overstained that the contraction nodes appear homogeneous as described by Henneberg and Heiderich.

VII. The Behavior of the Myofibrillae During Contraction.

Although the myofibrillæ have long been considered the contractile elements of smooth muscle, previous investigators have not demonstrated that they are such. The myofibrillæ can be easily seen, even in fresh material, in uncontracted muscle and in the internodal segments of contracted muscle, Fig. 2, 5-36. The contraction nodes, even by recent workers on smooth muscle, have been described as entirely homogeneous, Henneberg, 1901; Heiderich, 1902; Soli, 1906. The author in a preliminary paper, McGill (3), 1907, showed that in material properly differentiated the myofibrillæ may be traced through the so-called homogeneous nodes, and that they apparently thicken as they pass through.

In material fixed and stained in the ordinary manner these nodes do appear perfectly homogeneous, Figs. 2, 3, 5, 11, 20, 22, 31. However, in muscle fixed in Zenker's fluid, then over-stained in iron-hæmatoxylin and the hæmatoxylin carefully extracted, it is possible in many instances in the contraction nodes to show a distinct fibrillation, *cn* in Figs. 6, 18, 33, 34, 35, 36, 39, 40. When examined under high magnification, the individual fibrillæ may sometimes be traced continuously through one contraction node and internodal segment into the next contraction node, Fig. 40.

The myofibrillæ when they can be traced through the contraction nodes run just as straight a course as in the uncontracted muscle or in the internodal segments of contracted muscle, Figs. 6, 33, 34, 39, 40. If they were not contractile elements one would expect them to be folded and wavy as are the elastic fibers when the muscle fiber shortens.

When uncontracted muscle fibers are caught between contracted fibers the myofibrillæ, as well as the whole uncontracted fiber, may be thrown into zigzag waves due to passive shortening. Likewise when small pieces of muscle are removed and allowed to contract in the absence of tension, the fiber and consequently the myofibrillæ even in contracted muscle may take a wavy course. But in muscle contracted with normal amount of tension, then fixed and sectioned, the myofibrillæ in both the contracted and uncontracted fibers run comparatively straight. The zigzag form of contraction so frequently

observed and described by earlier workers was as a rule undoubtedly brought about by removal of the tension under which the fibers normally contract. They studied only small isolated bits of tissue, so their results are easily explained.

As the myofibrillæ enter the contraction nodes they often appear to thicken distinctly, Figs. 5, 6, 35, 40. The amount of thickening depends upon the amount of contraction. The nodes labeled *a* in Figs. 39, 40, are just beginning to contract, so there is only a slight increase in diameter of the myofibrillæ. Nodes labeled *b* in Figs. 35, 40, are more firmly contracted and show more marked enlargement.

In muscle where the contraction nodes come very close together, as in pig's œsophagus when the tissue is just beginning to contract, the myofibrillæ may appear segmented, as shown in Fig. 26. Even in wide contraction nodes, as in those of arteries, the contracting myofibrillæ may thicken unequally, giving a segmented appearance, Fig. 18, from the carotid of ox. Where this condition is marked it may give an appearance closely simulating the myofibrillæ of striated muscle. The segmentation of the myofibrillæ of the smooth muscle in the mesentery of Urodela, described by Schaper, 1902, was probably due to mild contraction.

However, it should be remembered that iron-hæmatoxylin and orange G, the only stains by which the myofibrillæ of contracted muscle could be demonstrated, are largely physical stains. Instead of there being a true enlargement of the myofibrillæ themselves at the point of contraction, the physical condition of the inter-fibrillar substance immediately surrounding the myofibrillæ may be so altered as to make it stain as intensely as the myofibrillæ, and thus produce an apparent though not a real thickening of the fibrillæ. Were the physical condition of all the inter-fibrillar substance so altered, the stained material would show the whole contraction node homogeneous. On the other hand, the homogeneous appearance of contraction nodes may be due to the crowding together of enlarged myofibrillæ. If closely packed, in material deeply stained, the myofibrillæ would not be demonstrated.

VIII. The Behavior of the Nuclei During Contraction.

1. The form of the contracted nucleus.

Two ideas have been advanced as to the form of the contracted nucleus. The first is that during contraction there is an active shortening and thickening of the nucleus, so that it changes from a rod-shaped to an oval-shaped structure. This is maintained by Henneberg, Heiderich and Soli, among recent workers. The other is that during contraction the nucleus is passively or perhaps actively folded or twisted into a spiral. This type was described by Forster and Schlater.

In all the smooth muscle studied, during contraction the nuclei are drawn closer together in the contraction waves than in the uncontracted areas. They undergo distinct decrease in length and increase in thickness, Figs. 22, 37. This can be observed as well in fresh as in fixed material. In fact when living smooth muscle is stimulated to contraction under the microscope the contraction of the nucleus can be observed. The nuclei (with the possible exception of the extremely long nuclei in arteries) do not undergo folding or spiral twisting during contraction. However, in normal contraction, when an uncontracted fiber is caught between fibers that have contracted, both it and its nucleus may be passively twisted or folded.

Frequently in the walls of contracted arteries spiral nuclei were observed, Figs. 81-84, from the carotid of ox. But Figs. 60, 61, 78-80, show conclusively that shortening and thickening of the nuclei do occur. It is probable that when both nucleus and fiber contract at equal rates there occurs only the type of contraction shown in Figs. 78-80. If, however, as undoubtedly may happen in muscle with extremely long nuclei, the fiber contracts more rapidly than does the nucleus, various forms of folding or twisting of the nucleus will result. Figs. 81, 82, show nuclei, which are shorter and thicker as well as spirally wound. Of course, this might be due to passive shortening of a partly contracted nucleus. Many of the spiral nuclei belong to fibers passively contracted. However, they do occur in the contraction nodes of actively contracted fibers.

Figs. 27-29 and 85-89 show two series of nuclei from the smooth muscle of the intestine of Necturus, which illustrate well the decrease in length and increase in thickness which occurs during contraction. The following measurements taken from nuclei of both contracted and uncontracted smooth muscle of Necturus, serve as further illustration of the point in question:

	Length of Nucleus.	*Greatest Width of Nucleus.*
No. 1	82 micra	5 micra
No. 2	72 micra	8 micra
No. 3	62 micra	9 micra
No. 4	55 micra	9 micra
No. 5	43 micra	10 micra
No. 6	35 micra	15 micra
No. 7	30 micra	17 micra
No. 8	29 micra	16 micra
No. 9	28 micra	18 micra

Figs. 42-53 is a similar series from the smooth muscle of the large intestine of dog, Figs. 55-58 from a small artery from the mesentery of pig, Figs. 65-67 from the bladder of cat, Figs. 68-72 from the muscular stomach of chicken, and Figs. 77-80 from the carotid artery of ox.

During contraction the nucleus changes from rod-shaped to oval-shaped or elliptical. The nuclear membrane which in the resting nucleus is of very even contour, in the contracted nucleus is often distinctly serrated at the ends, Figs. 28, 29, 88, 89. It often has the appearance of being indented by the contracting fibrillæ. Whether this is actually the case is uncertain.

The contraction node passes along the smooth muscle fiber, causing a distinct enlargement as it goes. As it approaches a nucleus, the nucleus begins to thicken at the end nearest the node, Figs. 20, 21, 28, 50, 61, 86. When the whole nucleus is included in the contraction node, it assumes its completely contracted, oval form, Figs. 19, 21, 23, 29, 89. Occasionally each end of a nucleus may be caught in a contraction node while the middle lies in an internodal

segment. Then the ends of the nucleus contract, while the center remains unchanged. This frequently occurs in the intestinal muscle of Necturus, where the nuclei are extremely long, Fig. 28.

2. The behavior of the chromatin during nuclear contraction.

In the resting smooth muscle nucleus the chromatin is arranged as a very delicate central reticulum and as a thin layer just beneath the nuclear membrane, Figs. 22, 24, 27, 42, 77, 85. During contraction the chromatin is massed at the two ends of the nucleus. There is left a space relatively free from chromatin at the middle of the nucleus, Figs. 28, 29, 51, 89. As the chromatin masses at the two ends of the nucleus there is a streaming and rearrangement of the meshes. At the same time it becomes much more deeply staining.

The massing of the chromatin at the ends of the contracted nuclei is most marked in the intestinal muscle of Necturus and dog, Figs. 28, 29, 51, 52. Necturus material is very favorable for study because the nuclei are so extremely large.

As the nucleus begins to enlarge at the point of contraction the chromatin reticulum breaks up into very fine threads, Fig. 86. These pass, as if by distinct streaming, toward the poles of the nucleus where they arrange themselves in loops or festoons, Figs. 28, 87, 88. As the process continues the fine strands of chromatin fuse to form exceedingly coarse threads. These may remain in a loose festoon at the end of the nucleus or else break up there into a coarse reticulum, Figs. 29, 59, 89. At the same time some of the chromatin collects in a heavy layer just beneath the nuclear membrane. The strands of chromatin in the fully contracted nucleus are much coarser than are those in the resting nucleus, Figs. 27, 28, 29. Figs. 93-95, 96-99, show similar chromatic changes in the nuclei of Necturus where the smooth muscle had been stimulated to contraction by pilocarpine.

The chromatic changes in the nuclei of smooth muscle of dog intestine are quite similar to those observed in Necturus, Figs. 42-63. However, in resting nuclei the chromatin strands tend to run as fine longitudinal fibrils, Fig. 42. These are more pronounced in partially contracted nuclei, Figs. 43-46. As the nucleus contracts

these fibrils become coarser, Figs. 47-49. Instead of running straight, as they do in resting nuclei, they may run a wavy course or even twist up into distinct spirals, Figs. 48-49. Finally these chromatin strands collect at the ends of the nuclei and fuse there to form a coarse reticulum, Fig. 51. The chromatin spirals are never as distinctly marked as were those described by Münch, 1903.

In the stomach of the chicken the nuclei contract in about the same manner as described for Necturus, Figs. 68-72. In the nuclei of the smooth muscle fibers of arteries there are fewer chromatic changes than were observed in other muscle, Figs. 54-64, 77-80. The dark bands shown in Figs. 81-84 are due to folding of the nuclei and not to condensation of the chromatin.

The whole behavior of the chromatin of the nuclei during contraction indicates that the contraction of the smooth muscle nucleus is a very active process. It is highly improbable that the changes described could be brought about passively by the contraction of the extra-nuclear portion of the muscle fiber.

3. The effect of contraction on the volume of the nucleus.

An attempt was made to determine by actual measurement whether there is change in the volume of smooth muscle nuclei during contraction. Not enough data have been obtained to determine the point definitely. From the results at hand it seems that there is no change in the volume during contraction.

4. The effect of fatigue on the nucleus.

Gilman, 1903, showed that when striated muscle is completely fatigued the nuclei are shrunken, crenated, more lightly staining and less granular than in muscle not so fatigued. Similar experiments were made by the author on smooth muscle. Strips from the muscularis of the intestine of Necturus were suspended in a moist chamber, arranged for electrical stimulation, and were stimulated until no further contractions could be obtained. Forty contractions was the maximum number obtained from one piece. When the strip was completely fatigued, it was fixed and sectioned. The sections were studied, along with like sections from control muscle, for comparison. In none of the material was there any indication of shrinkage or crenation of the nuclei. In every instance the

nuclei in the fatigued and in the control muscle showed precisely similar structure. At the same time there was no apparent change in the myofibrillæ, the exhausted muscle appearing in no wise different from resting muscle in structure.

5. The effect of drugs on nuclear contraction.

The effect of a number of drugs on the contraction of smooth muscle nuclei was observed. Among those employed were cocaine, pilocarpine, apomorphine, adrenalin and atropine. Atropine and cocaine are in certain doses muscle narcotics. In muscle placed in these solutions until the tissue is completely relaxed, the nuclei show the structure of typical resting nuclei. Figs. 90-92 are nuclei from intestinal muscle of Necturus, relaxed by placing in a 1 per cent cocaine solution. Pilocarpine was the usual stimulant employed. Figs. 93-95, 96-99, are from intestinal muscle of Necturus contracted by placing in pilocarpine solution. The changes brought about by contraction with this drug are precisely similar to those caused by the other stimuli used.

IX. Chemical Changes in Smooth Muscle During Contraction.

The deep staining of the entire contraction node as compared with the light staining of the internodal segments would seem to indicate that during contraction there is more change in the muscle fiber than can be attributed to increase in the thickness of myofibrillæ alone. The difference in staining reaction between the contracted and uncontracted muscle is so striking as to indicate a marked chemical change. With iron-hæmatoxylin the contraction nodes stain readily and intensely black. Figs. 1, 2, 3, 5, 11, 23. Of course since iron-hæmatoxylin is largely a physical stain, this may be due to the condensation of the fiber and swelling of the myofibrillæ at the contraction node. But the contrast seems entirely too sharp to be due to this alone. With Delafield's hæmatoxylin and a counterstain of eosin the contraction nodes stain much more intensely with eosin than do the internodal segments. Figs. 20-22. The picric acid in Van Gieson's mixture gives a like effect, Fig. 28. Mallory's anilin blue connective-tissue stain is a valuable dif-

ferential. With it the myofibrillæ of the uncontracted portions of the fiber stain red in the fuchsin, the contraction nodes bright yellow with orange G. Figs. 30-34. This stain cannot be entirely relied upon as a chemical test, since orange G. is also known to be a physical stain. However, the exceedingly sharp differentiation of the contraction nodes with all the above stains indicates marked chemical changes in the smooth muscle fiber during contraction.

An attempt was made by using neutral red and phenolphthalein in physiological solution as indicators, to determine whether there is a change in the alkalinity of smooth muscle during contraction. If there be it should show at the contraction nodes. Small pieces of the fresh tissue were mounted on a slide in solutions of the indicator. When stimulated the tissue contracted, but there was no apparent change in the color of the indicator. It should be remembered that none of the indicators, neutral red, rosolic acid, etc., in use at present are delicate enough for microscopic tests unless the reactions be very marked. Schultz, 1907, in studying this same question in smooth muscle *en masse* determined that there was no decrease in the alkalinity during contraction, such as has been pointed out for striated muscle.

X. Development of Contractility.

The development of contractility in smooth muscle was studied in the digestive tract in a series of chick embryos ranging in age from three days to twenty-one days of incubation. First a series of sections of embryos at different ages was made to determine when smooth muscle arises. Smooth muscle begins to form between the third and fourth day by an elongation of the cells in the mesenchymal syncytium surrounding the epithelial tube. By the fifth day the myofibrillæ are rapidly forming as thick varicose structures. They run for long distances through the cytoplasm without regard for cell boundaries. By the seventh day the muscle nuclei are well elongated. The tissue is still a distinct syncytium and remains as such throughout development. The structure of the smooth muscle in the chick after hatching was not studied, so later development remains to be treated.

Next was determined experimentally when contraction begins. The interesting question to decide here is whether or not contraction is dependent upon the myofibrillæ. If it be, one would expect it not to appear until after the myofibrillæ are formed. The intestine and muscular stomach of the chick embryo were removed from the living embryo, kept at a temperature of 37° C., and stimulated mechanically, electrically or by heating. The tissue was stimulated while in focus under the low power of the microscope. The first contraction of the intestine observed occurred on the seventh day. It was merely a very slow contraction arising after marked stimulation. By the twelfth day, when the temperature is raised to 43° C., the intestine contracts rhythmically. By mounting small living pieces on a warm slide and examining under the high power of the microscope it was hoped that the finer details of contraction could be made out. Until the late embryo there are so many yolk granules present that this was not accomplished.

Sections from the stimulated intestine were fixed and examined microscopically. No contraction changes were observed in the fiber until the twelfth day. Sections from contracted muscle at this time show many of the nuclei slightly shorter and broader than in uncontracted muscle. Figs. 73-76 show the degree of shortening observed in the contracted intestine of a fourteen-day chick embryo. On the twenty-first day, aside from shortening of the nuclei, the muscle of the contracted intestine shows in many fibers irregular areas staining slightly deeper with eosin than does the rest of the fiber. In these areas the nuclei are shorter than elsewhere. They probably represent developing contraction nodes. At this time there are no nodes which stain differently with iron-hæmatoxylin or Mallory's anilin-blue connective-tissue stain. Everywhere the myofibrillæ can be traced uninterruptedly through the muscle syncytium. The author hopes soon to be able to trace the later development of contraction in the chick after hatching.

Soli, 1907, traced the development of smooth muscle in the stomach of the chick. He obtained the first elongation of mesenchyme on the seventh day of incubation. He did not observe fibrillæ until the seventeenth day. Contraction nodes he found appearing on the ninth day after hatching.

In a section through the large intestine of an eight-month human fœtus the author observed rather distinct contraction nodes. At that age the contraction waves are quite irregular in outline.

Summary.

A. Structure of resting smooth muscle.

1. Smooth muscle in chick, pig and man arises as a syncytium and the syncytial structure persists in most instances in the adult.

2. Adult smooth muscle may show one of two and possibly of three types of structure. In type 1 there is a very distinct syncytial arrangement. The fibers are joined by both side and end anastomoses. In type 2 the muscle fibers show few side anastomoses, but end to end union still persists. There is possibly a third type of muscle with no anastomoses between the fibers, each forming an individual cell.

3. Muscle of type 1 was found in portions of the digestive and urino-genital tracts of adult Necturus, chick, pig, cat, dog and man and of the arterial muscle of pig, ox and man. The tissue consists of the much elongated muscle nuclei, each surrounded by a granular protoplasmic reticulum and outside of this a layer of myofibrillæ embedded in clear protoplasm. Both granular protoplasm and myofibrillæ may be continuous from cell to cell. Where there is much granular protoplasm there are few myofibrillæ and vice versa.

4. In muscle of type 2 the loss of side anastomoses is probably due to rapid elongation of the central part of the fiber during histogenesis, crowding the anastomoses toward the ends. This type was found here and there in all muscle studied. The myofibrillæ pass continuously from cell to cell through the broad end anastomoses. Where there is much granular protoplasm it may also help to form the anastomoses.

5. Muscle of type 3, though it is the type usually described, was found hard to demonstrate. Frequently, especially in arterial muscle, what appear to be isolated muscle cells are seen. They are long, spindle-shaped elements, each with a central nucleus and surrounding this the sarcoplasm filled with myofibrillæ. The cell body is

thickest in the neighborhood of the nucleus and tapers toward the poles. When such isolated fibers appear in section, absence of end anastomoses may be due to the fact that the section is cut slightly obliquely. In macerated material the anastomoses are usually destroyed, and there results the spindle-shaped smooth muscle cell described in the text-books.

6. In adult smooth muscle, just as in development, two types of myofibrillæ occur; very fine fibrillæ corresponding to the elementary fibrillæ of Apathy and to the "Binnenfibrillen" of Heidenhain; coarse fibrillæ similar to the primitive fibrillæ of Apathy which in some respects resemble the "Grenzfibrillen" of Heidenhain, in others more nearly the coarse myofibrillæ of Benda. Some muscle fibers have only fine myofibrillæ, others only coarse myofibrillæ, while still others have both types. When coarse myofibrillæ are present they may be arranged as a peripheral layer similar to the "Grenzfibrillen" of Heidenhain or they may be scattered throughout the sarcoplasm, as are the coarse myofibrillæ of Benda. Each myofibrillæ throughout its length is fairly uniform in caliber. The myofibrillæ run continuously from cell to cell through the anastomoses.

7. The nucleus of resting smooth muscle is a much elongated spindle-shaped structure ranging in length in the material studied from twenty micra in the digestive tract of the chicken to eighty micra in Necturus. An eccentric position of the nucleus was frequently observed. The nuclear membrane is of even contour. The chromatin is arranged in fine granular reticulum supported by a fine linin network. From one to five plasmasomes are present.

8. The connective tissue of resting smooth muscle appears like ordinary areolar tissue, in the meshes of which are embedded the muscle fibers. The branched connective-tissue cells anastomose with each other and occasionally with the muscle cells. The collagenous fibers are arranged as a loose reticulum, as a heavy reticulum or as distinct membranes. The elastic fibers form a loose network. The fibers which run parallel with the muscle fibers are comparatively straight.

B. The structure of contracted muscle.

1. During the contraction of the digestive tract both layers of

the muscularis contract simultaneously. As they contract they thicken and in constant ratio. Aside from the thickening of the muscularis the lumen as well as the whole diameter of the tube decreases, due to the shortening of the circular fibers, and the length of the segment decreases, due to the shortening of the longitudinal fibers. In the carotid of ox during contraction there is thickening of the coats accompanied by a decrease in diameter, both of the lumen and the whole tube. There is little change in length, for there are few longitudinal muscle fibers.

2. Two types of contraction have been described: peristaltic, where a series of wave-like thickenings cross the fiber; total, where the entire fiber shortens and thickens. In the material studied all the muscle seems to belong to the first type, although in arterial muscle there is in places a near approach to total contraction.

3. In peristaltic contraction in the digestive tract contraction waves pass over the muscularis. These are irregular anastomosing areas of contracted tissue between which there are areas of resting muscle. The contraction waves vary much, both in length and width as well as in number. In length they may cross only a few muscle fibers or they may include the entire muscularis. In width they may be only narrow bands or they may be so wide as to include most of the length of a muscle fiber. Where narrow and close together they give the tissue a distinctly striated appearance. In the contraction waves the muscle fibers are shorter, thicker and more deeply staining than in the uncontracted areas; the nuclei are crowded closely together, the connective tissue is much condensed. From the material studied there is no evidence that the propagation of contraction waves is due to nerve control. They are probably conducted from fiber to fiber by some protoplasmic connection between the cells themselves. In syncytial muscle the protoplasmic anastomoses probably serve this purpose. In arterial and urino-genital muscle there are no distinct contraction waves. Here there are merely scattered contracted fibers or groups of fibers among uncontracted tissue.

4. The portion of the muscle fiber in the contraction wave is the contraction node, that in the uncontracted area is the internodal

segment. The contraction nodes, both in living and in fixed material, show as conspicuous thickenings of the fiber. In arterial muscle they are long, so that a single one may include most of the length of the muscle fiber. In the muscle of the digestive tract they are usually narrower and a number may be present in each fiber. The nodes stain more distinctly with eosin and iron-hæmatoxylin than do the internodal segments. With Mallory's anilin-blue connective-tissue stain the nodes are colored orange, the internodal segments red. With ordinary differentiation the nodes appear homogeneous, the internodal segments are distinctly fibrillated. Although the contraction nodes of smooth muscle have always heretofore been described as homogeneous, when the material is carefully stained in iron-hæmatoxylin the myofibrillæ may be traced through the nodes and be shown to be continuous with the myofibrillæ of the internodal segments. As the myofibrillæ enter the nodes they show a distinct increase in caliber. Where the contraction nodes are narrow and close together the myofibrillæ appear segmented. That the contraction nodes represent contracted portions of the muscle fiber is shown by the following: In both fresh and fixed material the smooth muscle fibers are thicker at the contraction node than elsewhere. The myofibrillæ run straight through the nodes and as mentioned above apparently thicker as they pass. In the nodes the nuclei are shorter and thicker than in the internodal segments. In the region of the contraction node the collagenous fibers are condensed and the elastic fibers take a wavy course.

5. During contraction the nuclei of smooth muscle undergo an active shortening and thickening. This change in shape can be seen in living muscle. Typically, with the possible exception of some arterial nuclei, and the nuclei of passively shortened muscle fibers, there is no folding or spiral winding of muscle nuclei during contraction.

6. In general, as the nucleus contracts there is a rearrangement of the chromatin. The fine strands collect into coarser fibers, which arrange themselves in loops or festoons at the two ends of the nucleus. During the process there is an apparent streaming of the chromatin toward the poles.

7. There appears to be no change in the volume of smooth muscle nuclei during contraction.

8. Fatigue was shown to cause no apparent change in the structure of the nuclei.

9. Nuclei in muscle contracted by pilocarpine, adrenalin, atropine, etc., have the same structure as nuclei contracted by electrical or mechanical stimuli.

10. During contraction more changes take place in smooth muscle than can be attributed to morphological causes, such as thickening of the myofibrillæ, etc. At the contraction nodes the staining reactions would indicate that there is a marked chemical reaction taking place also.

11. The development of contraction was studied in chick and pig embryos. The muscle cells become contractile soon after the myofibrillæ appear. Thus in the chick, where the myofibrillæ arise on the fifth day, distinct contraction was observed on the seventh day. This, together with the fact already mentioned that the myofibrillæ become shorter and thicker during contraction, would seem to indicate that the myofibrillæ are the contractile elements. Contraction waves do not appear until comparatively late in development.

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ABBREVIATIONS.

l. m., longitudinal muscle coat.
c. m., circular muscle coat.
c. w., contraction wave.
c. n., contraction node.
i. s., internodal segment.
i. c., interstitial connective tissue.
c. f., collagenous fibers.
e. f., elastic fibers.
g. p., granular protoplasm.
p. s., protoplasmic syncytium.
c. mf., coarse myofibrillæ.
f. mf., fine myofibrillæ.
t. mf., thickened myofibrillæ.
nu., nucleus.
r. nu., nucleus of resting muscle.
ch. f., chromatin festoon in contracted nucleus.

All figures were drawn by means of a camera lucida. The following microscopic lenses were used. Zeiss achromatic D and apochromatic 2 mm. 1:30 objectives with compensating oculars 4, 8 and 12; Leitz $^1/_{12}$ in. oil immersion and Bausch and Lomb $^1/_{16}$ in. oil immersion objectives with 1 in. ocular.

EXPLANATION OF FIGURES.

FIG. 1. Cross-section of the entire muscularis of the contracted large intestine of dog—l. m., longitudinal muscle; c. m., circular muscle; c. w., contraction wave; i. s., internodal segments of muscle fibers; c., an oblique contraction wave. Zenker's fluid. Iron-hæmatoxylin. × 390.

FIG. 2. Longitudinal section of a group of muscle fibers from the muscular stomach of chicken showing a portion of a contraction wave (c. w.) with uncontracted areas on either side. The enlargement of the muscle fibers in the contraction wave is marked. In the contraction wave (c. w.) the contraction nodes (c. n.) appear homogeneous and are stained very deeply. In the uncontracted areas the internodal segments (i. s.) are fibrillated and lightly staining—i. c., interstitial connective tissue; f. mf., fine myofibrillæ; r. nu., an elongated resting nucleus. Zenker's fluid. Iron-hæmatoxylin. × 1275.

FIG. 3. Cross-section of a portion of the circular muscle coat of the contracted large intestine of dog. This section shows plainly the enlargement and deep staining of the muscle fibers at the contraction nodes (c. n.) as compared with the uncontracted internodal segments (i. s.). The myofibrillæ in the internodal segments are only indistinctly shown—i. c., interstitial connective tissue. Zenker's fluid. Iron-hæmatoxylin. × 2250.

FIG. 4. Cross-section of a portion of the media of the contracted carotid of ox. c. n., contraction node. The contraction nodes in this section are distinctly fibrillated. In them the cut ends of the coarse myofibrillæ are shown, c. mf. In some of the fibers not firmly contracted (d.) both coarse and fine myofibrillæ (f. mf.) appear—i. s., either an entirely relaxed fiber or an internodal segment of a contracted fiber; i. c., interstitial connective tissue; r. nu., resting nucleus; c. nu., contracted nucleus. Zenker's fluid. Iron-hæmatoxylin. × 1650.

FIG. 5. Longitudinal section of a portion of a contracted muscle fiber from the media of the carotid of ox, showing two contraction nodes (c. n.)—i. s., internodal segment; r. nu., resting nucleus; c. mf., coarse myofibrillæ. Zenker's fluid. Iron-hæmatoxylin. × 1650.

FIG. 6. Section of a portion of a partially contracted muscle fiber from the intestine of Necturus, showing the continuity of the myofibrillæ through the contraction nodes (c. na. and c. nb.). The enlargement of the myofibrillæ is shown in contraction node c. nb. This cell has only fine myofibrillæ—g. p., granular protoplasm; f. mf., fine myofibrillæ; c. f., collagenous fibers; c. n. b., a contraction node just beginning to form; c. n. a., a more firmly contracted node. Zenker's fluid. Iron-hæmatoxylin. × 2250.

FIG. 7. Cross-section of a small portion of the circlar muscle coat of Necturus taken near the submucosa, showing the continuity of both granular protoplasm and myofibrillæ from cell to cell. The tissue is a complete syncytium—g. p., granular protoplasm; f. mf., fine myofibrillæ. Zenker's fluid. Iron-hæmatoxylin. × 2250.

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Fig. 8. Longitudinal section of a portion of a muscle fiber from the carotid of ox—just beginning to contract, showing both fine and coarse myofibrillæ—g. p., granular protoplasm; c. mf., coarse myofibrillæ; c. f., collagenous fibers; e. f., an elastic fiber showing the wavy course due to contraction of adjacent muscle fibers. Zenker's fluid. Iron-hæmatoxylin. × 1275.

Fig. 9. Cross-section of three uncontracted muscle fibers from just outside the elastica interna of the carotid of ox, showing two types of fibers, one with a large amount of granular protoplasm (g. p.) and only a peripheral layer of myofibrillæ, the other with no granular protoplasm and closely packed myofibrillæ—i. s., internodal segment; c. f., collagenous fibers. Zenker's fluid. Iron-hæmatoxylin. × 1375.

Fig. 10. Section of a portion of a muscle fiber from the internal iliac artery of man, showing two nuclei in one mass of granular protoplasm (g. p.). This fiber has only fine myofibrillæ (f. mf.). Formalin. Heusen's hæmatoxylin. × 1275.

Fig. 11. Longitudinal section of muscle fibers from the circular layer of the cæcum of chicken, showing regular contraction waves. The number of contraction nodes in each fiber gives the tissue a cross-striated appearance. The sharp contrast between deeply staining homogeneous contraction node and lightly staining internodal segment is well shown; (a) shows end to end union of the muscle fibers. The shortening of the nuclei in contraction nodes is indicated—c. n., contraction node; i. s., internodal segment; c. f., collagenous fibers. Opposite the contraction nodes the collagenous tissue is condensed. Zenker's fluid. Iron hæmatoxylin. × 1275.

Fig. 12. Cross-section of a portion of the circular coat of the large intestine of a dog to show the straight course of the elastic fibers (e. f.) in the uncontracted muscle and the wavy course through the contraction waves. The enlargement of the muscle fibers in the contraction waves is marked. The union of muscle fibers end to end may also be noted—i. s., internodal segment; c. n., contraction node. Zenker's fluid, Weigert's resorcin, elastic tissue stain. × 925.

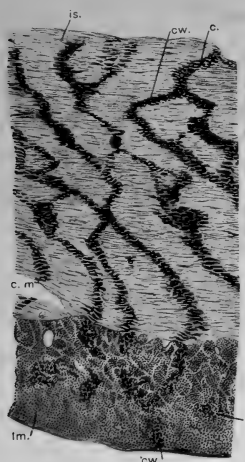

Fig. 1

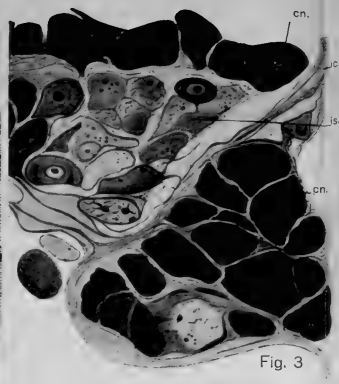

Fig. 3

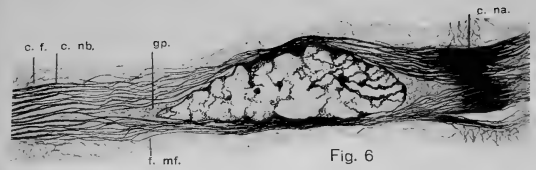

Fig. 6

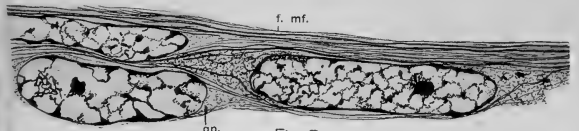

Fig. 7

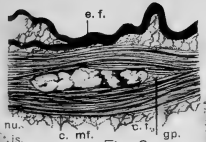

Fig. 8

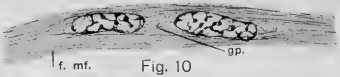

Fig. 10

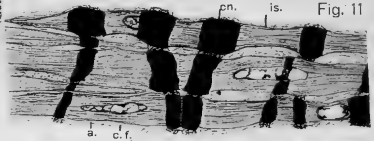

Fig. 11

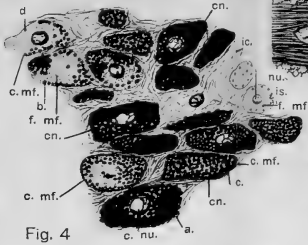

Fig. 4

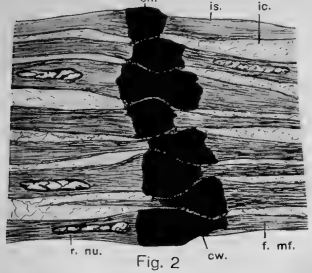

Fig. 2

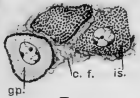

Fig. 9

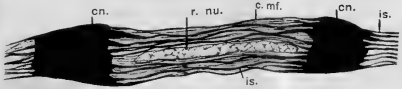

Fig. 5

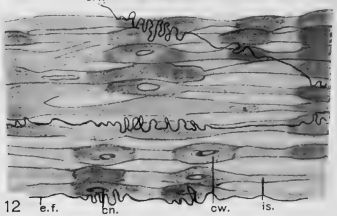

Fig. 12

Fig. 13. A portion of the uncontracted muscularis mucosæ of the small intestine of pig to show the continuity from cell to cell of both granular protoplasm and myofibrillæ, through end anastomoses. The large amount of granular protoplasm and the few peripheral myofibrillæ give the tissue the appearance of primitive muscle—a., protoplasmic anastomosis; i. c., interstitial connective tissue; g. p., granular protoplasm; c. mf., coarse myofibrillæ. Gilson's fluid. Iron-hæmatoxylin-eosin. × 1760.

Fig. 14. From the same region as Fig. 13, but showing in addition anastomoses between granular protoplasm of muscle cells and that of connective tissue cells (b.). The whole tissue is distinctly syncytial—g. p., granular protoplasm; p. s., protoplasmic syncytium; c. mf., coarse myofibrillæ; c. f., collagenous fibers. Gilson's fluid. Iron-hæmatoxylin-eosin. × 1760.

Fig. 15. Section through the circular muscle coat of the small intestine of adult pig, showing the syncytial structure of the tissue. This material was slightly contracted, probably by the fixative, so the nuclei are oval instead of rod shaped, although no distinct contraction nodes are present—a., myofibrillæ, continuous from cell to cell; g. p., granular protoplasm; e. f., elastic fibers; f. mf., fine myofibrillæ. Gilson's fluid. Iron-hæmatoxylin. × 1760.

Fig. 16. Cross-section of muscle from the same region as is shown in Fig. 15. Each muscle cell contains a large number of fine myofibrillæ, which in the cross-section appear as fine dots—mf., myofibrillæ; i. c., interstitial connective tissue; e. f., elastic fibers. Gilson's fluid. Iron-hæmatoxylin-eosin. × 1380.

Fig. 17. Cross-section through muscle similar to that shown in Fig. 13. Figs. 13-17 are all drawn from the same section. The peripheral arrangement of fibrillæ as "Grenzfibrillen" is shown in Fig. 17—i. c., interstitial connective tissue; g. p., granular protoplasm; c. mf., coarse myofibrillæ. Gilson's fluid. Iron-hæmatoxylin-eosin. × 1760.

Fig. 18. Portion of a partially contracted muscle fiber from the carotid of ox, showing the varicosity of the myofibrillæ in beginning contraction. The coarser myofibrillæ are in the main black, the finer, red—t. mf., thickening of a coarse myofibrilla. Zenker's fluid. Iron-hæmatoxylin-eosin. × 1380.

Fig. 19. A portion of a fiber from the large intestine of dog to show the peripheral position sometimes taken by the nucleus—c. nu., contracted nucleus; c. n., homogeneous appearing contraction node; i. s., fibrillated internodal segment. Zenker's fluid. Hansen's hæmatoxylin-eosin. × 520.

Figs. 20-22. Portions of muscle from the circular layer of the contracted large intestine of dog. The intestine was contracted from exposure to the air. The contraction nodes (c. n.) in all three sections are apparently homogeneous and are stained very intensely in eosin. The internodal segments are lightly stained and show the myofibrillæ distinctly. The connective tissue (c. f.) is condensed at the contraction waves. The nuclei in the internodal segments are

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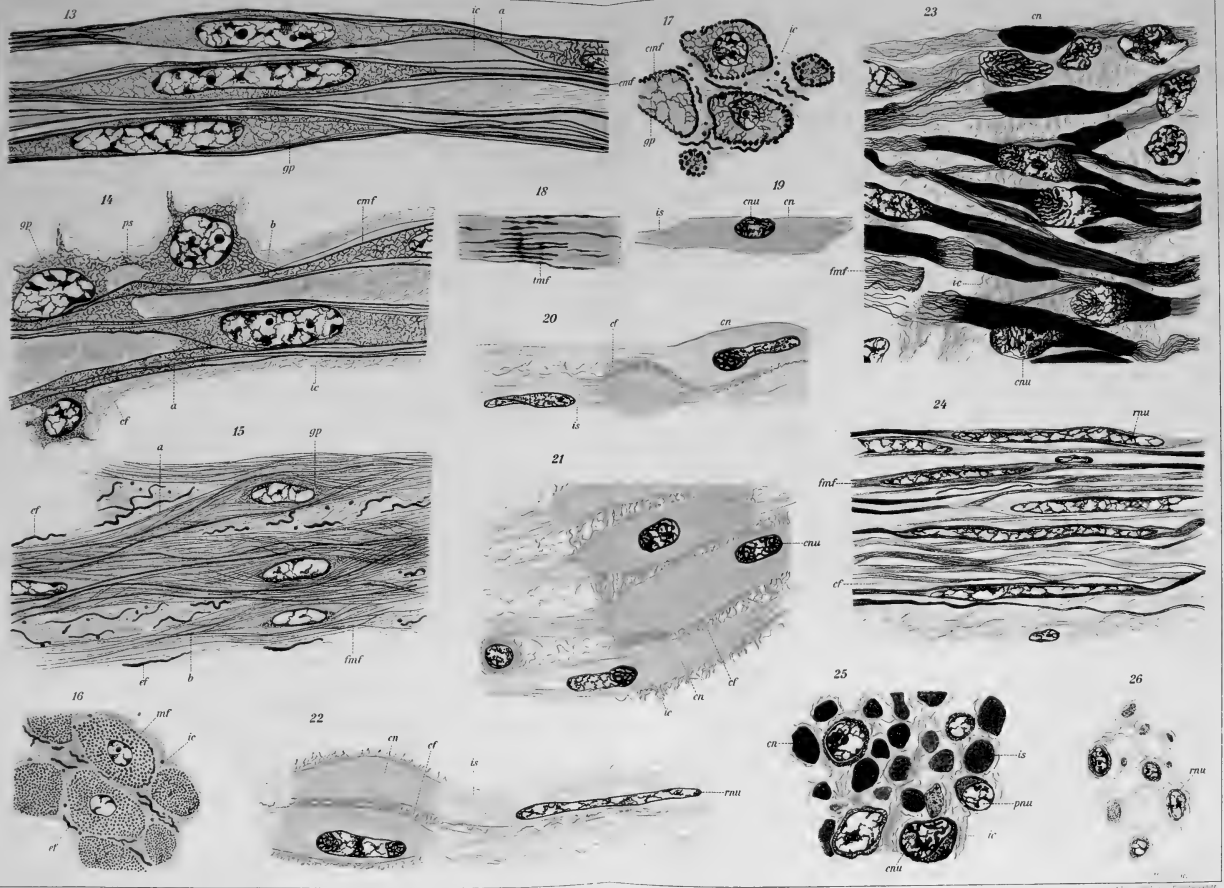
long, rodlike structures with delicate chromatin reticulum (r. nu.). In the contraction nodes they are shorter and thicker with the deeply staining chromatin massed at the ends (c. nu.). Zenker's fluid. Hansen's hæmatoxylin-eosin. × 1760.

Fig. 23. A contracted portion of the circular coat of the small intestine of Necturus, contracted by electrical stimulation. In the contraction wave the fibers stain deeply with the hæmatoxylin, the nuclei are short and thick with the chromatin massed at the ends, the connective tissue is condensed—c. n., contraction node; i. c., interstitial connective tissue; f. mf., fine myofibrillæ; c. nu., contracted nucleus with coarse chromatin reticulum. Zenker's fluid. Iron-hæmatoxylin-eosin. × 880.

Fig. 24. An uncontracted portion of the circular muscle coat taken from the same section and magnified the same amount as Fig. 23, showing the structure of the relaxed muscle. r. nu., resting nucleus with fine chromatin reticulum; fmf., fine myofibrillæ; cf., fine collagenous fibers. Zenker's fluid. Iron-hæmatoxylin-eosin. × 880.

Fig. 25. Cross-section of a portion of the longitudinal muscle coat of intestine of Necturus, muscle contracted—c. n., contraction node; i. s., internodal segment showing myofibrillæ; p. nu., a muscle fiber with the nucleus at the periphery; c. nu., contracted nucleus; i. c., interstitial connective tissue. Zenker's fluid. Iron-hæmatoxylin-eosin. × 880.

Fig. 26. Cross-section of uncontracted muscle taken from the same section as Fig. 25 and drawn to the same scale. When Figs. 25 and 26 are compared the marked thickening of both fibers and nuclei during contraction is very apparent—r. nu., resting nucleus. Zenker's fluid. Iron-hæmatoxylin-eosin. × 880.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Figs. 27-29. These drawings were all taken from the same region of the intestine of Necturus. One side of the intestine was firmly contracted, the other was relaxed. Fig. 27 is from resting muscle—r. nu., resting nucleus; f. mf., fine myofibrillæ; i. c., loose interstitial connective tissue. Fig. 28 is from muscle beginning to contract. Two contraction waves cross the section. The portions of the nuclei in the contraction nodes are contracted, showing distinct shortening, thickening and massing of the chromatin. The portions in the internodal segments are still relaxed. The rearrangement of the chromatin meshes during contraction is shown well in this preparation. Each end of nucleus (a) is in a contraction node, so shows contraction phenomena, the center lies in an internodal segment, so has the structure of a resting nucleus. The condensation of the connective tissue at the contraction node is shown—c. n., contraction node; i. s., internodal segment; i. c., interstitial connective tissue. Fig. 29 is from a firm contraction wave. The nucleus (c. nu.) is short and thick. The chromatin is arranged in loops or festoons (ch. f.) at the two ends—i. c., interstitial connective tissue. Ammonia alcohol, Van Gieson's stain. × 1980.

Fig. 30. A section of a portion of the longitudinal muscle coat of the œsophagus of pig stained in Mallory's anilin-blue connective tissue stain. The contraction nodes (c. n.) are stained orange and appear for the most part homogeneous. The myofibrillæ in the internodal segments are stained bright red (f. mf.). The massing of the blue stained collagenous fibers in the contraction waves is well demonstrated (c. f.). In the region of the internodal segments the collagenous fibers are in a loose meshwork. The enlargement of the muscle fibers in the contraction wave is distinctly shown. In this preparation the nuclei are not well differentiated. Zenker's fluid. Mallory's anilin-blue connective tissue stain. × 1100.

Fig. 31. Cross-section of a portion of a contraction wave and uncontracted area from the œsophagus of pig. In the contraction waves the contraction nodes (c. n.) appear homogeneous and are stained orange. The internodal segments (i. s.) are of less diameter and show the cut ends of the myofibrillæ stained red. The nuclei in contraction nodes are larger in cross-section than they are in the internodal segments. In the contraction wave the connective tissue (c. f.) is much condensed. Zenker's fluid. Mallory's anilin-blue connective tissue stain. × 1100.

Fig. 32. Cross-section of muscle fibers from the contracted carotid of ox—c. n., a contraction node. It is stained orange, but at the same time shows the cut ends of myofibrillæ—i. s., either internodal segments of a contracted fiber or an uncontracted fiber. The red myofibrillæ are seen throughout the entire sarcoplasm—e. f., elastic fiber; c. f., collagenous fibers. Zenker's fluid. Mallory's anilin-blue connective tissue stain. × 1700.

Fig. 33. Portion of a contracted muscle fiber from the carotid of ox. Around the nucleus is a wide contraction node (c. n.). The end of the fiber is uncontracted (i. s.). The contraction node is stained orange. The

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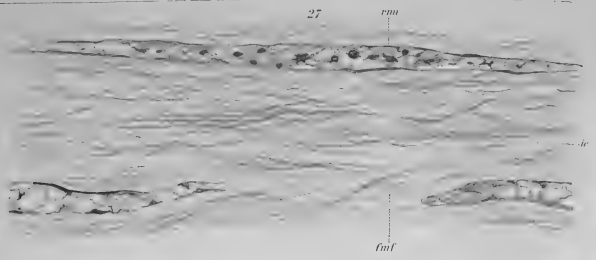
myofibrillæ may be seen indistinctly here. The internodal segment is distinctly fibrillated and the myofibrillæ are stained red. The connective tissue is condensed in the region of the contraction node (c. f.). Zenker's fluid. Mallory's anilin-blue connective tissue stain. × 1700.

FIG. 34. Portion of the media of contracted carotid of ox, showing a contracted fiber and a portion of an uncontracted fiber (a)—c. n., contraction node; i. s., internodal segment; f. mf., fine myofibrillæ; c. f., collagenous fibers; e. f., elastic fibers. Zenker's fluid. Mallory's anilin-blue connective tissue stain. × 650.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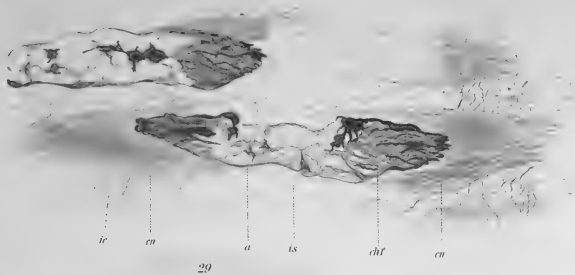
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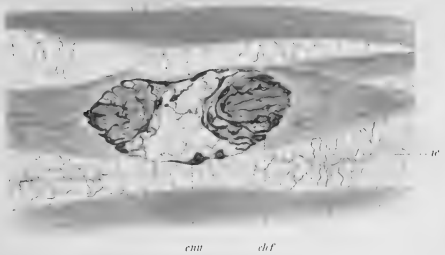
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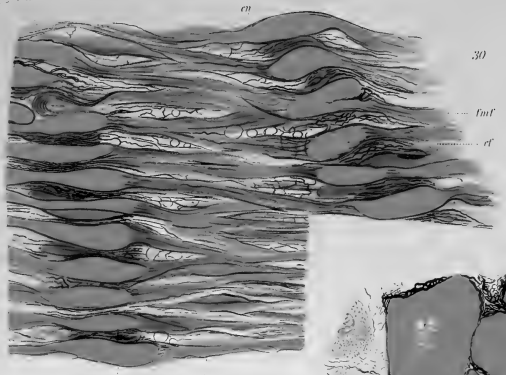
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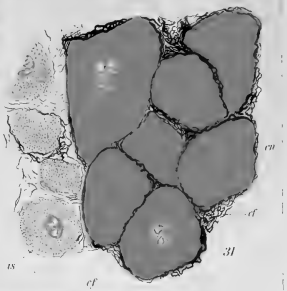
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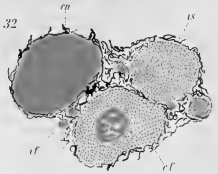
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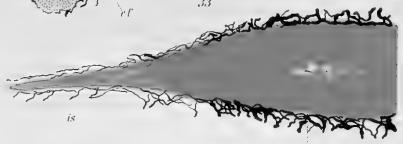
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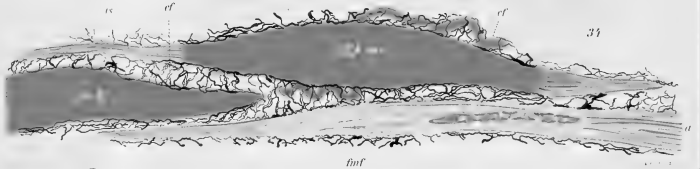
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Fig. 35. Portion of a muscle fiber from the contracted large intestine of dog, showing two small contraction nodes (c. n.). The myofibrillæ run continuously from internodal segment to internodal segment through the contraction nodes, apparently thickening in the region of the nodes. (t. mf.) shows such a thickening—r. nu., a resting nucleus; c. f., collagenous fibers. Zenker's fluid. Iron-hæmatoxylin-eosin. × 3000.

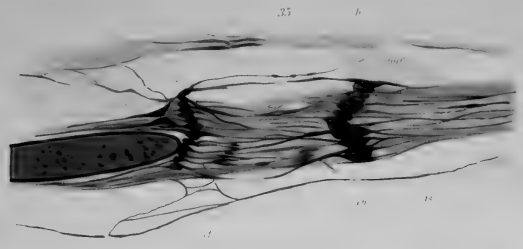
Fig. 36. Is taken from the same section as Fig. 35 and shows similar structure. The contraction nodes are wider than those shown in Fig. 35. Zenker's fluid. Iron hæmatoxylin-eosin. × 3000.

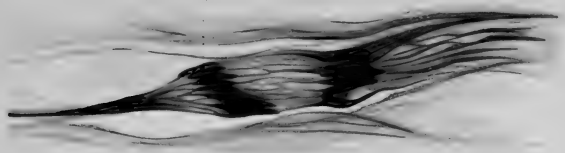
Fig. 37. Cross-section of a portion of the circular muscle coat of the intestine of dog. In the contraction wave (c. w.) the thickening of the fibers, the change in staining reaction, the clumping together of the nuclei, along with the shortening and thickening of each nucleus, are clearly shown—r. nu., resting nucleus; c. nu., contracted nucleus; c. n., contraction node. Zenker's fluid. Delafield's hæmatoxylin-eosin. × 880.

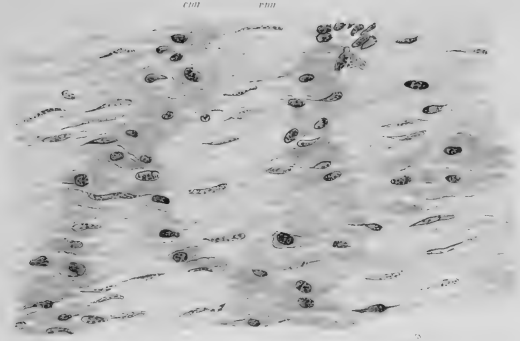
Fig. 38. Is taken from the same section as Fig. 35—c. n., contraction node deeply stained so that no myofibrillæ show; i. s., internodal segment; c. mf., coarse myofibrilla thickening as it enters the contraction node. Zenker's fluid. Iron-hæmatoxylin-eosin. × 3000.

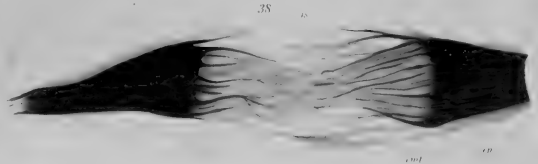
Figs. 39-40. Drawn from the same section as Fig. 35. Portions of muscle fibers showing beginning contraction nodes (c. n.) with the myofibrillæ enlarged in the contraction nodes (a, b)—t. mf., thickened myofibrillæ. Zenker's fluid. Iron-hæmatoxylin-eosin. × 3000.

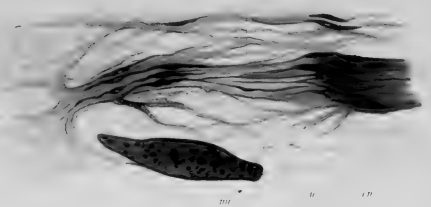
Fig. 41. A group of myofibrillæ from a partially contracted muscle fiber in the large intestine of dog, showing numerous enlargements of the myofibrillæ—t. mf., thickened myofibrillæ. Zenker's fluid. Iron-hæmatoxylin. × 3000.

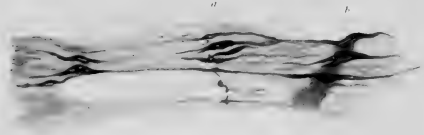








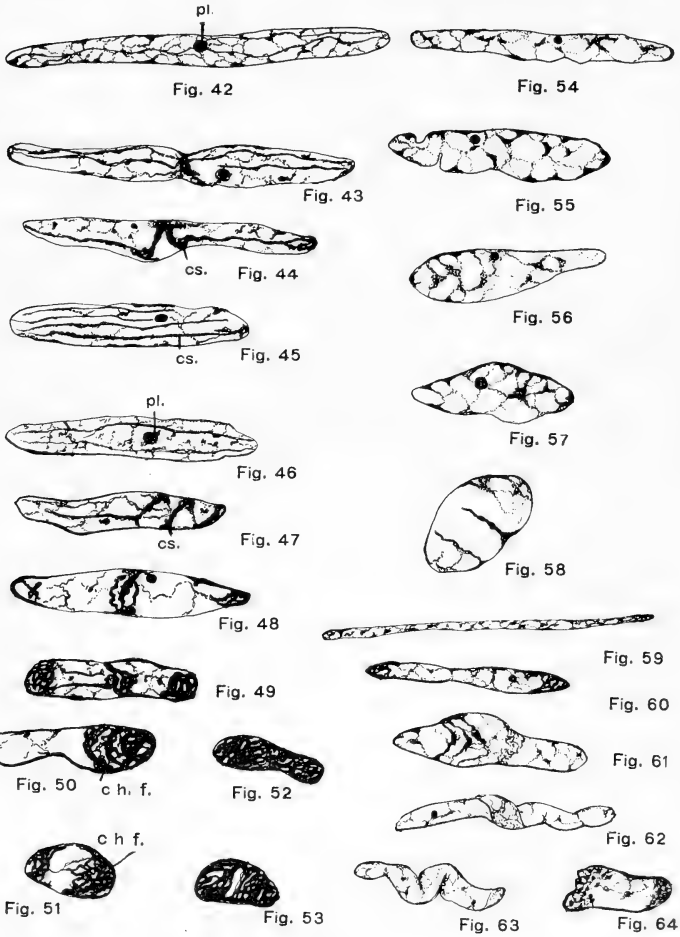




FIGS. 42-53. A series of nuclei from muscle cells in different stages of contraction from the circular muscle of the contracted large intestine of dog. Fig. 42 is a resting nucleus from an internodal segment. The chromatin is in a fine reticulum. A few fine strands run longitudinally, giving the appearance of longitudinal chromatic fibers—pl., plasmasome. Figs. 43, 44 show nuclei just beginning to contract. The chromatin strands have thickened. Figs. 45-48 show further stages in the contraction of the nucleus. In Fig. 49 the chromatin has drawn into three masses with very thick strands. In Fig. 50 one end of the nucleus was in a contraction node, so is enlarged and deeply staining, the other end was in an internodal segment, so has the structure of a resting nucleus. Figs. 51, 53 show the thickened chromatin strands massed at each end of the completely contracted nucleus. Zenker's fluid. Hansen's hæmatoxylin. × 3000.

FIGS. 54-58. Nuclei in various stages of contraction from a contracted mesenteric artery of pig. During contraction the nuclei shorten and thicken, Figs. 54, 57. Fig. 58 shows a folded nucleus occasionally met with. There are few chromatic changes in these nuclei during contraction. Gilson's fluid. Iron-hæmatoxylin. × 3000.

FIGS. 59-64. Contraction stages of the nucleus from the internal iliac artery of man. During contraction the nucleus shortens and thickens. Accompanying this is some massing of chromatin at the poles. A certain amount of folding or spiral winding may be normal for the contraction of arterial muscle, Figs. 62, 63. Formalin. Hansen's hæmatoxylin. × 1700.


pl.
Fig. 42
Fig. 54
Fig. 43
Fig. 55
cs.
Fig. 44
Fig. 56
cs.
Fig. 45
Fig. 57
pl.
Fig. 46
Fig. 58
cs.
Fig. 47
Fig. 48
Fig. 59
Fig. 49
Fig. 60
Fig. 50
c h. f.
Fig. 52
Fig. 61
Fig. 62
c h f.
Fig. 51
Fig. 53
Fig. 63
Fig. 64

Figs. 65-67. Three nuclei from the bladder of cat, showing degrees of shortening of the contracted nucleus. Zenker's fluid. Iron-hæmatoxylin. × 3000.

Figs. 68-72. Nuclear contraction in the muscular stomach of chickens. Fig. 68 shows a resting nucleus. The remaining figures show the changes in shape and the arrangement of the chromatin at various phases of contraction. Zenker's fluid. Iron-hæmatoxylin. × 3000.

Figs. 73-76. Stages in the contraction of the nucleus in the muscular stomach of a 14-day chick embryo. There is during contraction a decrease in length. There are few chromatin changes. Zenker's fluid. Iron-hæmatoxylin. × 2200.

Figs. 77-84. Nuclear contraction phases in the carotid of ox. Fig. 77 shows a resting nucleus. Figs. 78-80 show shortening and thickening of the nuclei. Figs. 81-84 show folding or spiral winding of the nucleus which may possibly be typical at times for arterial nuclei. Figs. 81, 82, along with coiling, have also undergone shortening and thickening. Zenker's fluid. Iron-hæmatoxylin. × 3000.

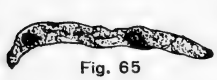


Fig. 65

Fig. 77

Fig. 66

Fig. 78

Fig. 79

Fig. 67

Fig. 80

Fig. 68

Fig. 81

c h f.

Fig. 69

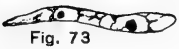


Fig. 73

Fig. 82

Fig. 74

Fig. 70

Fig. 75

Fig. 71

Fig. 83

Fig. 76

Fig. 72

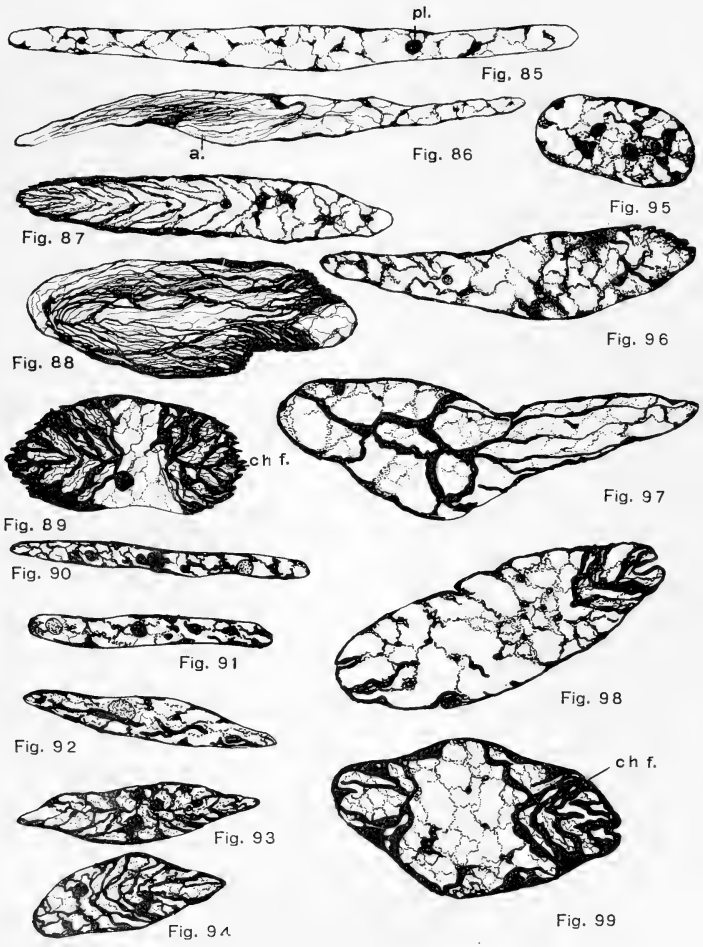
Fig. 84

Figs. 85-89. Nuclei from the small intestine of Necturus, contracted by electrical stimulation. Fig. 85 is a completely relaxed nucleus. There is a fine chromatin reticulum and a single plasmasome (pl.). In Fig. 86 a nucleus is shown, one end of which is just beginning to contract. The chromatin has changed from a granular reticulum to fine longitudinal strands (a). Fig. 87 shows the chromatin arranged in loops at one end of the nucleus. At the same end the nuclear wall is serrated. Fig. 88 shows a festoon of thick chromatin strands. In Fig. 89 is shown a completely contracted nucleus. The heavy chromatin strands are arranged in festoons at either end (ch. f.). The ends of the nucleus are serrated. Zenker's fluid. Iron-hæmatoxylin. × 1700.

Figs. 90-92. Resting nuclei from muscle of intestine of Necturus kept for 20 minutes in 1 per cent cocaine solution. Zenker's fluid. Iron-hæmatoxylin. × 1100.

Figs. 93, 95. Contracted nuclei from muscle of the small intestine of Necturus. The tissue was contracted by placing it in pilocarpine solution. Zenker's fluid. Iron-hæmatoxylin. × 1100.

Figs. 96-99. Nuclei from the same source as those shown in Figs. 93-95. Instead of fixing the tissue frozen sections were made and stained in methylene-blue. Figs. 96-98 are partially contracted nuclei. The chromatic changes are marked. Fig. 99 is a completely contracted nucleus. Its structure is almost identical with that shown in Fig. 89—ch. f., chromatin festoon. Frozen sections. Methylene-blue. × 2200.


pl.
Fig. 85
a.
Fig. 86
Fig. 95
Fig. 87
Fig. 96
Fig. 88
ch f.
Fig. 97
Fig. 89
Fig. 90
Fig. 91
Fig. 98
Fig. 92
ch f.
Fig. 93
Fig. 99
Fig. 94