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

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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.

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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 verschlieszen, 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 schlieszlich zu einem Lymphgefässe umbildet, ferner die rege Zellvermehrung im Gebiete des sich entwickelnden Lymphherzens und schlieszlich 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

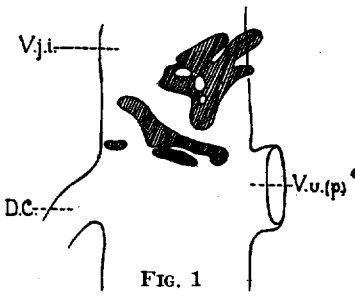


FIG. 1

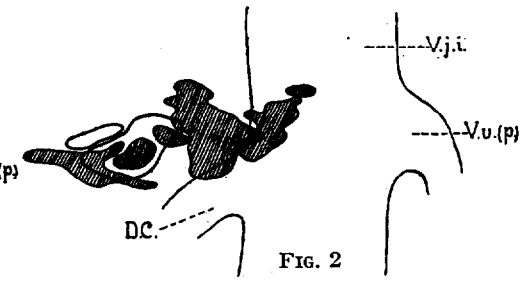


FIG. 2

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

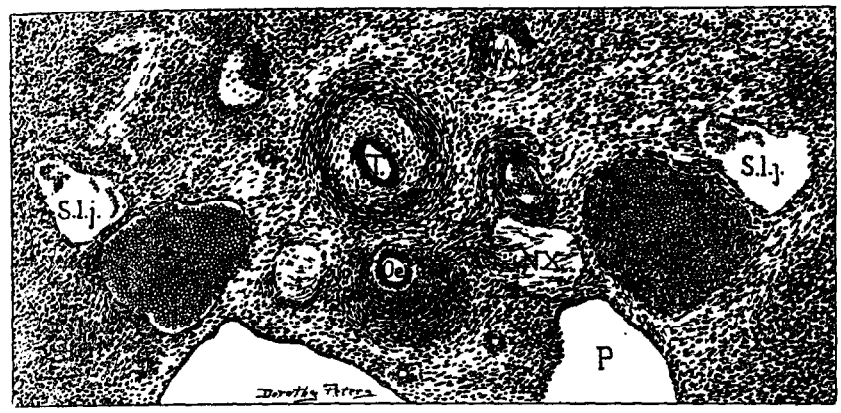
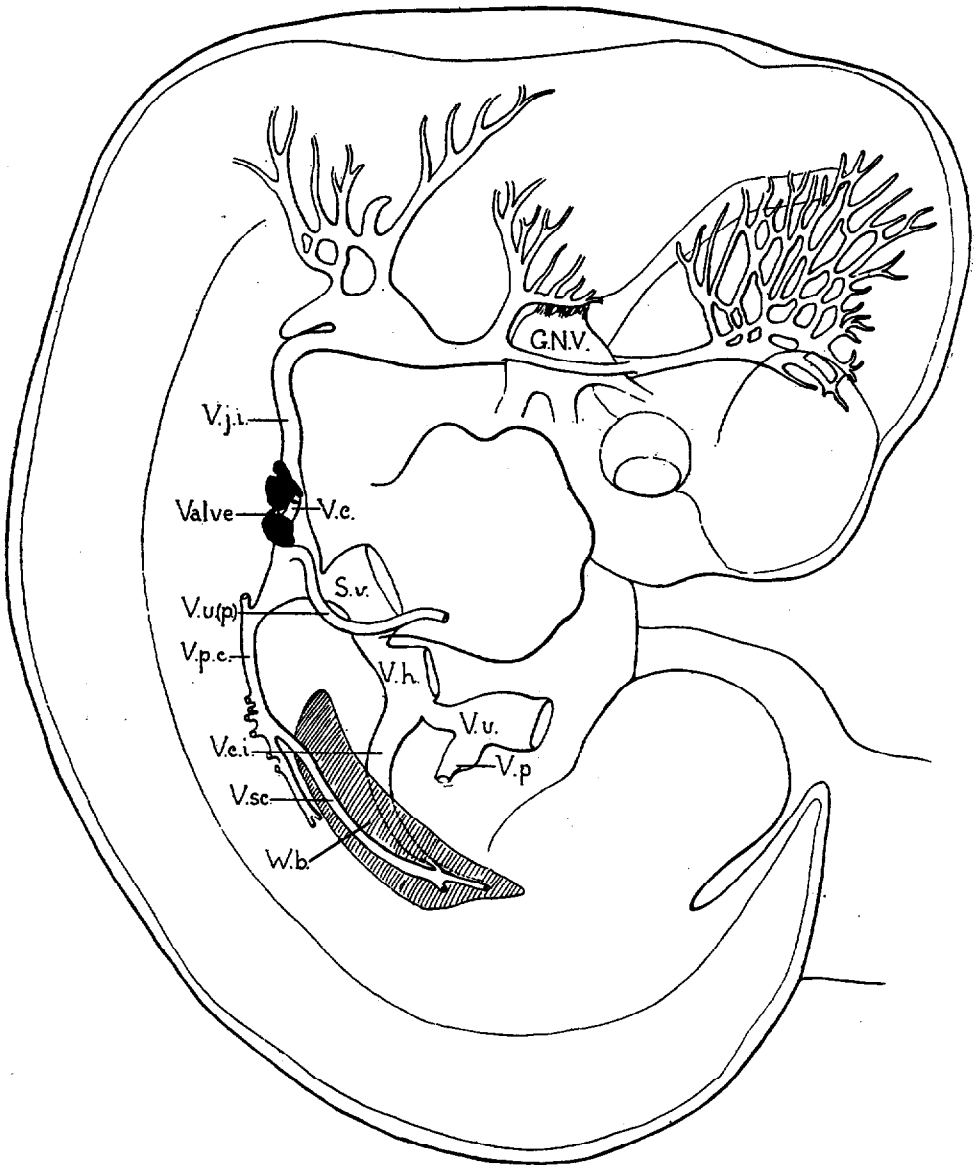


FIG. 3. Transverse section through the neck of a human embryo, 10.5 mm. long, showing the symmetrical jugular lymph sacs. Mall collection, No. 109. A., artery; N. X., n. vagus; m. s., n. sympatheticus; Oe., œsophagus; P., pericardium; S. l. j., saccus lymphaticus jugularis; T. trachea. The V. jugularis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


G.N.V.
V.j.i.
Valve
V.c.
S.v.
V.u(p)
V.p.c.
V.h.
V.u.
V.p
V.c.i.
V.sc
W.b.

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.

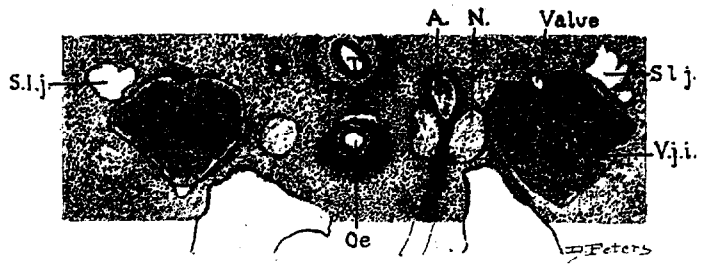


FIG. 5. Transverse section through the jugular sacs of a human embryo, 10.5 mm. long. Mall collection, No. 109, to show the left valve. × 28. A., artery; N., nerve; Oe., œsophagus; P., pericardium; S. l, j., vena jugularis; V. j. i., vena jugularis interna.

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

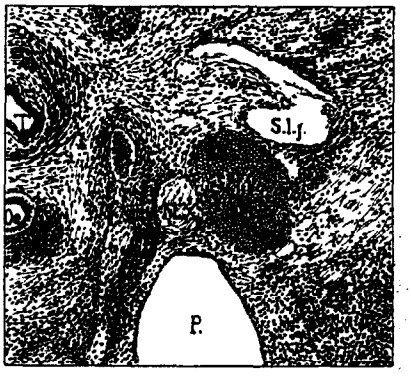


FIG. 6. Transverse section through the left jugular sac to show the possible beginning of a vessel growing down to form the upper part of the thoracic duct. × 40. A., aorta; N. X., N. vagus; Oe., œsophagus; S. l. j., saccus lymphaticus jugularis; V. j. i., vena jugularis interna.

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.

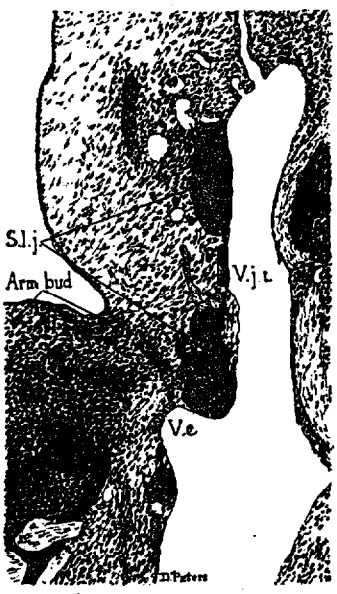
The next point of interest is the location of the valve. In Figs. 7 and 8, it will be seen that the beginning cephalic vein is easily recognized by its position opposite the upper curve of the arm bud. The lymphatic sac runs deep into the angle between the cephalic and the internal jugular veins, Fig. 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



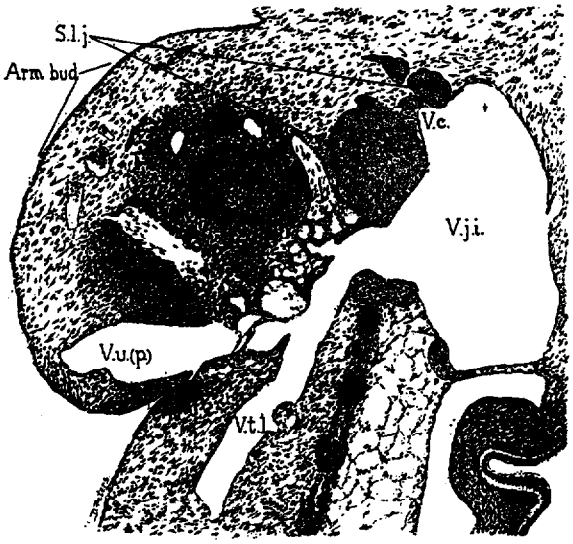


FIG. 7 FIG. 8

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.

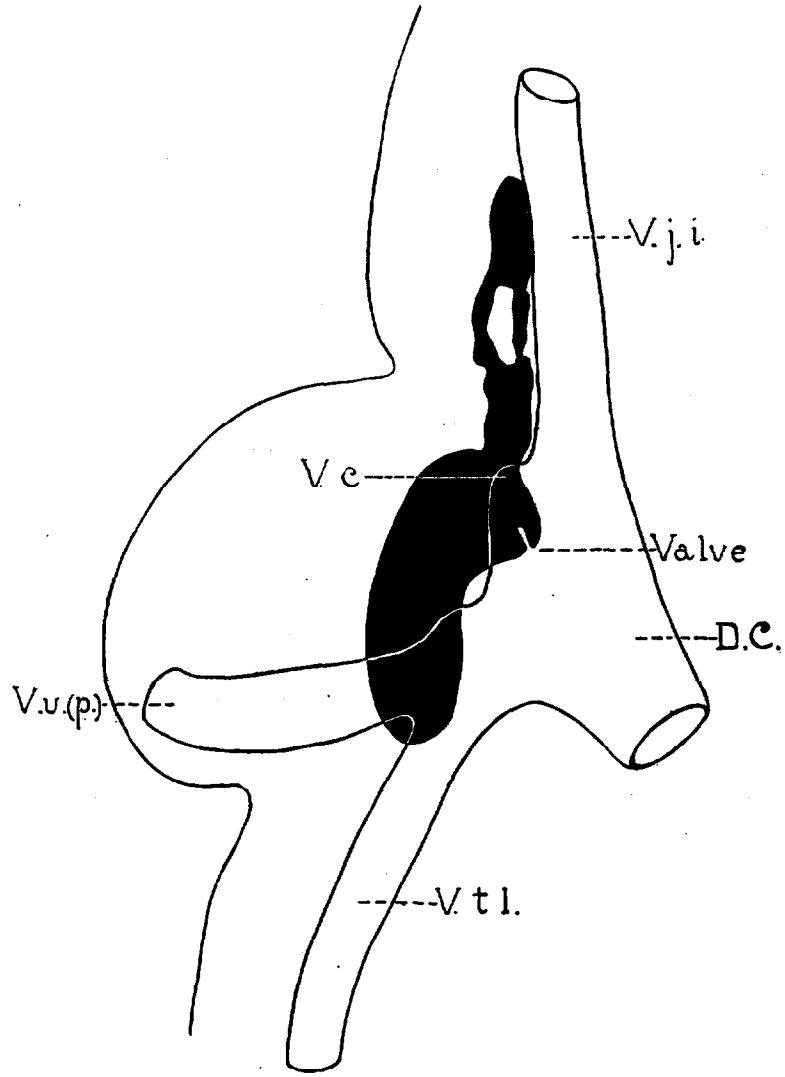


FIG. 9. A composite diagram made by superimposing the sections showing the jugular sacs, as shown in Figs. 16 and 17, of human embryo, 11 mm. long, Mall collection. × about 36. D. C., ductus Cuvier; V., position of valve; V. j. i., vena jugularis interna; V. t. l., vena thoracicus lateralis; V. u. (p.)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

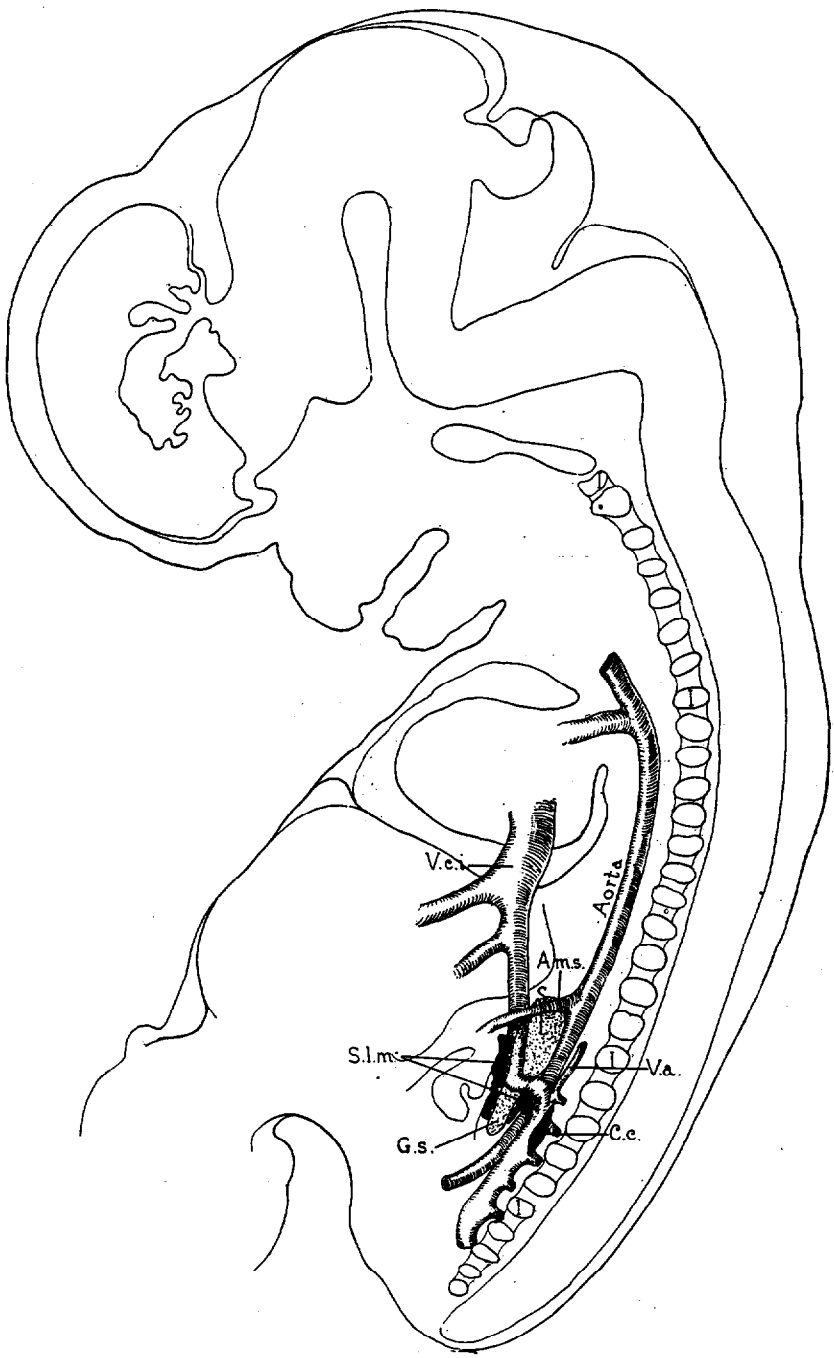


FIG. 11. A composite diagram made by superimposing the sections showing the relations of the mesenteric sac and cisterna chyli to the veins, in a human embryo measuring 23 mm., Mall collection, No. 382. X about 8. A. m. s., A. mesenterica superior; C. c., cisterna chyli; G. s., ganglii sympathetica; 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

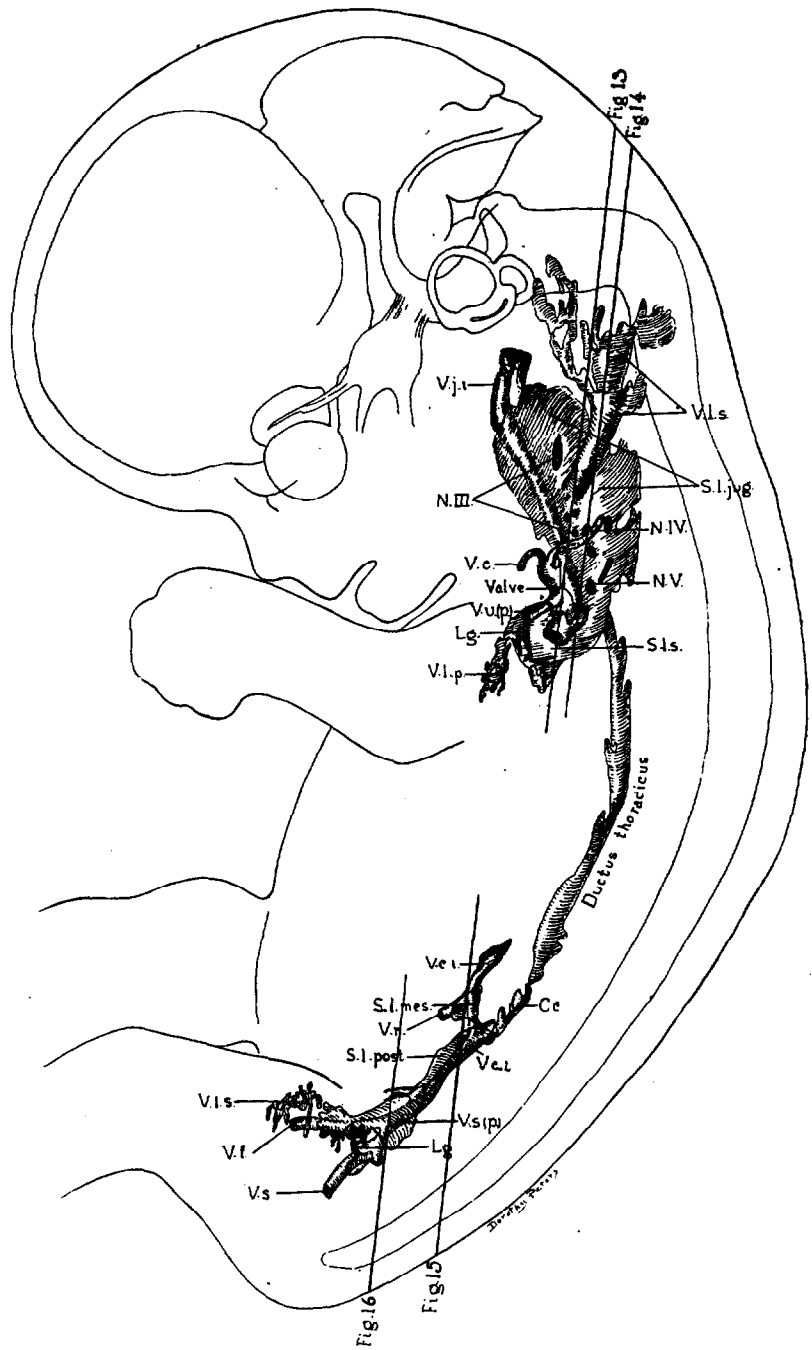


FIG. 12. Flat reconstruction of the primitive lymphatic system in a human embryo, 30 mm. long, Mall collection, No. 86. X about 5.4. C. c., cisterna chyli; L. g., lymphoglandula; N. III., N. IV., and N. V., Nn. cervicales; S. l. jug., saccus lymphaticus jugularis; S. l. mes., saccus lymphaticus mesentericus; 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-

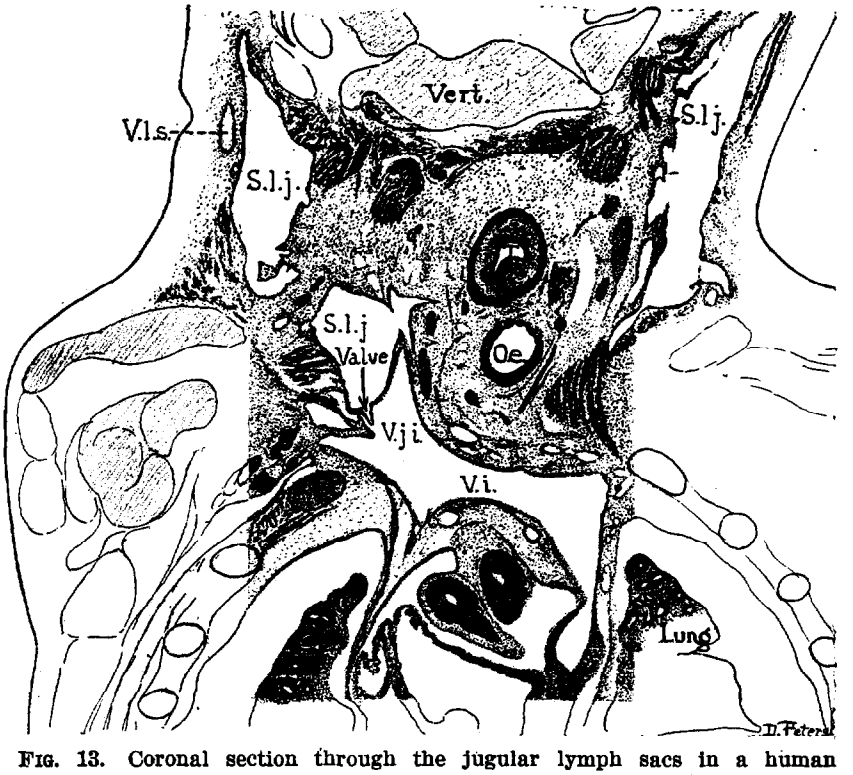


FIG. 13. Coronal section through the jugular lymph sacs in a human embryo of 30 mm., Mall collection, No. 86. × about 11. The level of the section is shown on the reconstruction of Fig. 21. The section shows the complete lymph sac on the right side and is cut to show the valve on the left. S. l. j., 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.

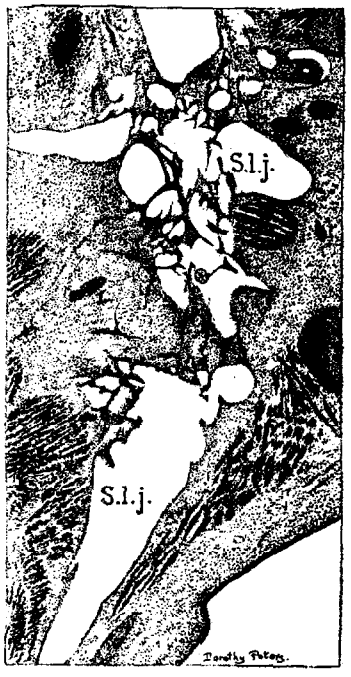


FIG. 14. Coronal section through the jugular lymph sac of the same embryo, at the level shown in Fig. 21, to show the simple bridging of the sac which is the anlage of the first lymph node. × about 19. S. l. j., saccus lymphaticus jugularis.

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

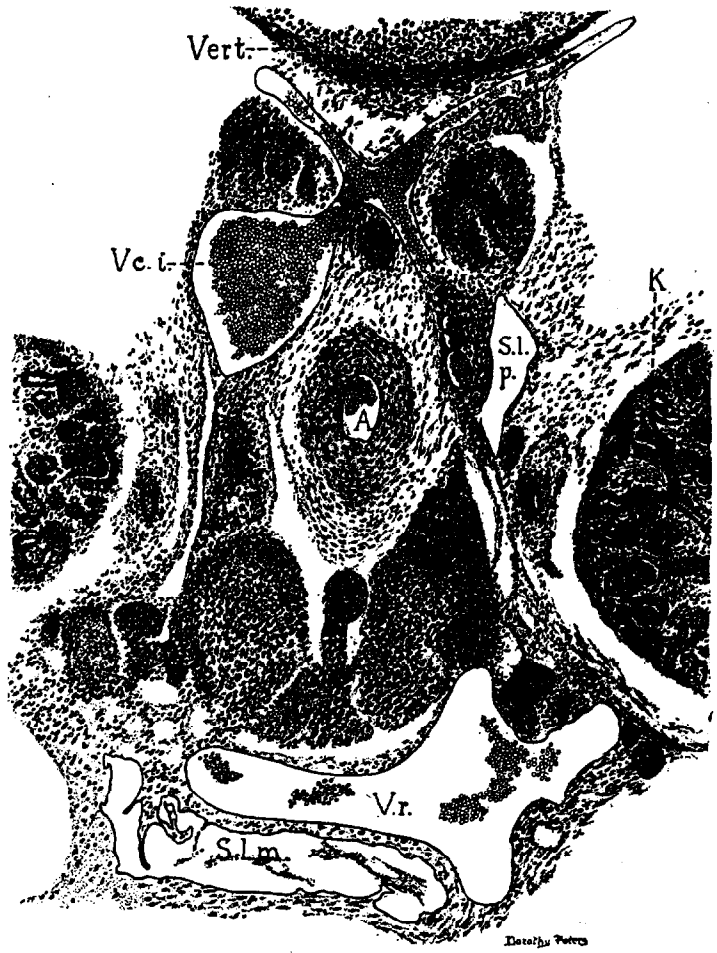


FIG. 15. Coronal section through the retroperitoneal sac of the human embryo, at a level indicated on Fig. 21. × about 39. A., aorta; K., kidney; S. l. m., saccus lymphaticus mesenterica; S. l. p., saccus lymphatica posterior; V. c. i., vena cava inferior; V. r., vena renalis.

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

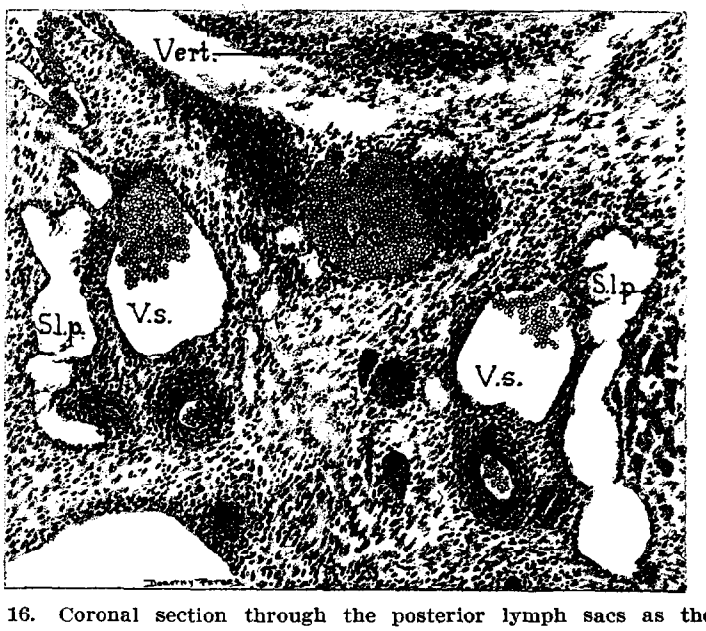


FIG. 16. Coronal section through the posterior lymph sacs as they lie along the primitive sciatic veins, of the same embryo, at a level shown in Fig. 21. × about 49. S. l. p., saccus lymphaticus posterior; V. c., vena candalis; V. s., vena sciatica primitiva.

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

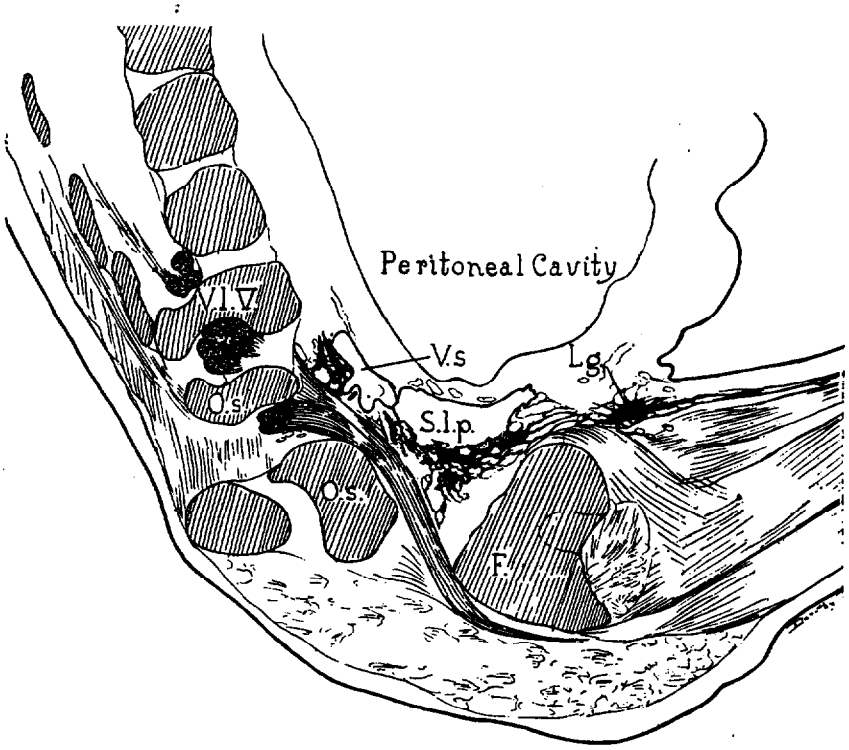


FIG. 17. Sagittal section of a human embryo measuring 50 mm., Mall collection, No. 96, showing the posterior lymph sac within the pelvis and its extension along the femoral vein. × about 8. F., femur; Lg., lymphoglandula (femoralis); O. s., os sacrum; S. l. p., saccus lymphaticus posterior with lymph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

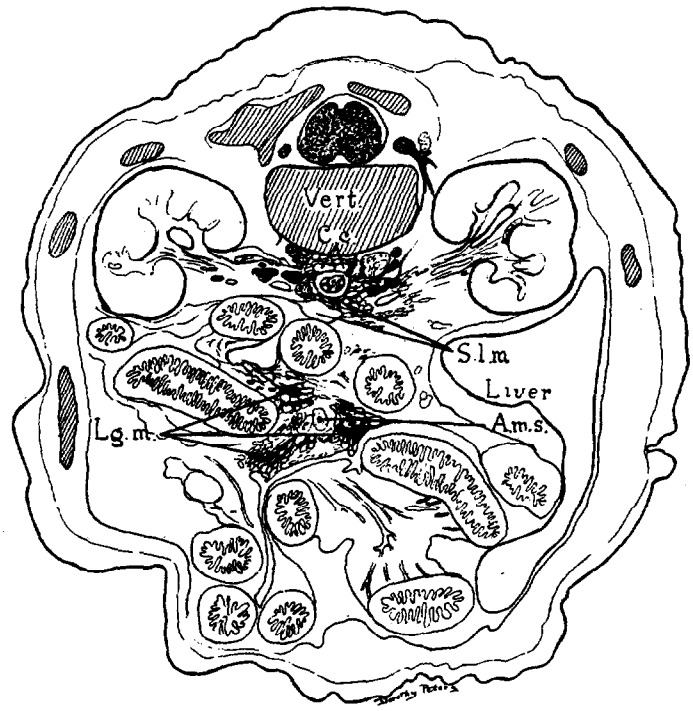


FIG. 18. Transverse section through the abdominal cavity of a human embryo, 80 mm. long, Mall collection, No. 172. It shows the kidneys, a little of the liver, and many loops of the intestine. × about 8. A. m. s., arteria mesenterica superior; C. c., cisterna chyli at its lower border; Lg. m., lymphoglandulae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

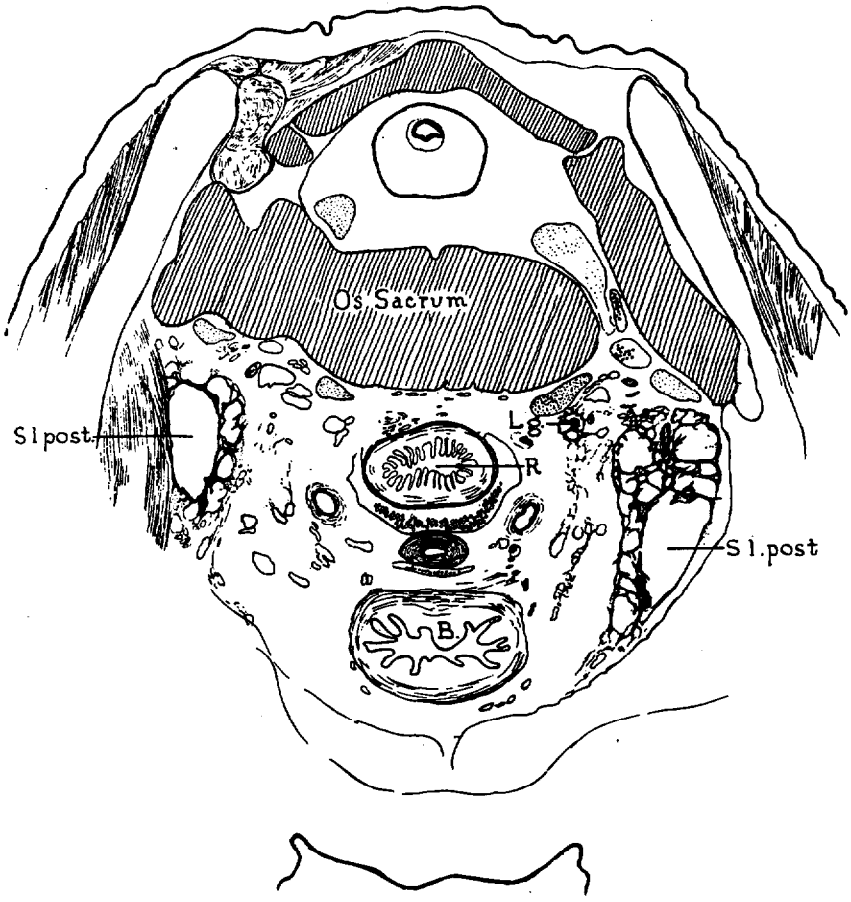


FIG. 19. Transverse section through the pelvis of a human embryo, 80 mm. long. Mall collection, No. 172, to show the posterior lymph sacs. × about 9. B., bladder; Lg., lymphoglandula; R., rectum; S. l. post., saccus lymphaticus posterior.

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.