ON THE ORIGIN OF THE LYMPHATIC SYSTEM FROM THE VEINS AND THE DEVELOPMENT OF THE LYMPH HEARTS AND THORACIC DUCT IN THE PIG.

BY

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WITH 12 TEXT FIGURES.

Although considerable attention has been given of late to the study of the development of lymph glands, only two writers have led up to the discovery of the origin of the lymphatic system as a whole, Budge[1] and Ranvier.[2]

In 1880 Budge published an account of a canal system which he had discovered in the mesoderm of early chick embryos; and in 1887, after Budge's death, His published a further but necessarily incomplete account of this work from Budge's notes and pictures.

Budge injected the false amnion of chicks three days old, and found that the fluid ran out into the area vasculosa as if in ducts. He then injected along the arteries in chicks from nine to eighteen days old and obtained beautiful injections of undoubted lymphatics. These two experiments are related to one another in the text by the following theory: Budge thought that there were two lymphatic systems, and that the first or primitive system was present in the three-day chick. He thought that the false amnion and cœlom being continuous, there were ducts within the body wall connected with the cœlom, analogous to those of the area vasculosa which he had injected from the false amnion. The ducts within the body lying along the dorsal line became pinched off from the cœlom and united to form a thoracic duct. With the thoracic duct began the second or permanent lymphatic system, which he had injected along the arteries in nine-day chicks. This idea of relating the lymphatic system to the serous cavities has remained but a theory and the gap between the two systems of Budge has never been filled.

[1] Budge: Arch. f. Anat. u. Phys., Anat. Abthg., 1880 and 1887.
[2] Ranvier: Comptes Rendus, 1895 and 1896; Archiv d'Anatomie, Tome 1, 1897.

Having repeated Budge's experiments I am convinced that while his injections of the ducts along the arteries are fundamental in the study of a certain stage of the development of lymphatics, the spaces connected with the false amnion have no connection whatever with the lymphatic system. In injecting the false amnion some minor changes in the methods of Budge are an advantage. Instead of a hypodermic syringe it is better to use a glass tube drawn out to a fine point and to introduce the fluid slowly under the even pressure of a low column of mercury. Budge used Berlin blue and found it necessary after filling the false amnion, to stroke the embryo gently in order to force the fluid into the area vasculosa. India ink is, however, a better fluid, for it is so finely divided that it runs of its own accord. I inserted the needle into the false amnion according to Budge's directions, given in his first paper, and injected the ink until the cavity was just full, then floated the embryo on to a glass slide with the dorsal surface upward. The fluid will now enter the area vasculosa and in places will run to its edge. This can be watched under the microscope. The fluid runs by putting out blunt processes simulating canals and so interpreted by Budge; at first these processes anastomose, making the network shown in Budge's pictures, but, as a rule, the meshes soon fill in and the fluid advances as a solid column with processes projecting in every direction. In other words, the fluid runs just as it would if forced between two glass plates held closely together. In serial sections through these injected specimens it is found that the upper and lower layers of the area vasculosa are connected here and there by delicate fibrils which are really processes of scattered mesenchyme cells and that the injected fluid passes into the spaces thus made and not into preformed channels lined by endothelium.

Notwithstanding the fact that Budge's theory of the origin of the thoracic duct is not correct, this theory led to the discovery of the true origin. For it was by injecting into the side of the neck in early embryos in the hope of reaching Budge's spaces behind the aorta, that the cervical lymph heart was injected, and the lymph hearts give the key to all the superficial lymphatics.

Between the years 1895 and 1897, Ranvier published a long series of articles on the development of the lymphatic system. He worked chiefly on the frog, and on pig embryos from 9 to 18 cm. long. From his injections of the lymphatic capillaries in the skin and in the villi of the pig embryos he made an important discovery; namely, that the lymphatics within the capillary plexus grow by budding. He says that

from the side of a duct appears a bud which is at first solid, but soon has a lumen. The lumen becomes larger, while at the same time the bud advances until it reaches a second duct. It opens into this duct by a process of absorption of the endothelium, and at the point of junction a valve is made. These points are beautifully seen in injected specimens for the larger duct forms, as Ranvier says, a collarette for the smaller. Thus he says the ducts grow from centre to periphery, while the valves necessarily open in the opposite direction. He says that as soon as lymphatics can be recognized in mammals they are furnished with valves. He also worked on frogs and described injections of the subcutaneous lymph sacs. From these sacs fluid can be made to run centrally to one of the lymph hearts and thence to the vein, and peripherally to minute ducts in the web of the feet. Ranvier states that these small ducts develop from the great lymph sacs.

His general conception of the lymphatic system is that it is a great gland of which the lymphatic capillaries correspond to the secreting portion, while the lymphatic ducts are the excretory canals. He says that the lymphatic system may be considered as a great vascular gland which takes its origin embryologically in the venous system and pours into the veins the product of its secretion which is lymph.[3]

Ranvier's great contribution to the study of the lymphatic system is the discovery of the fact of the growth of the ducts by budding in contradistinction to the generally accepted theory that the lymphatic system develops out of tissue spaces. Gulland[4] states this theory clearly as follows: The fluid of the blood exudes from the veins into the tissue spaces which gradually dilate, flow together and form the first lymphatic ducts. The walls of these ducts are made from the connective tissue which becomes compressed around them, and the ducts subsequently open into the veins. The most recent statement of this theory is that of Sala.[5] He describes the development of the lymph hearts and the thoracic duct in the chick as follows: That the first trace of the lymphatic system is the appearance of lymph hearts or spaces in the mesenchyme just lateral to the caudal myotomes. These spaces flow together and join the thoracic duct, which forms as two cords of mesenchyme cells which extend from the level of the thyroid glands to the level of the celiac axis. In the centre of these

[3] Ranvier: Comptes Rendus, Tome 121, 1895, p. 1109.

[4] Gulland: Journal of Pathology and Bacteriology, Vol. II, 1894, p. 466.

[5] Sala: Ricerche n. lab. di anat. norm. d. r. Univ. di Roma, Vol. VII, 1900, pp. 263-269. Reviewed in Archives Italiennes de Biologie, Tome 34, 1900, p. 453.

cords develops the lumen of the thoracic ducts which forms connections with the ductus Botali, the aorta and the superior vena cava.

In the present communication, Ranvier's hypothesis that the lymphatic system takes its origin from the veins will be proved. He missed the proof because he thought there were no lymphatics in pig embryos under 9 cm.[6] As a matter of fact the ducts have spread over nearly the whole body in a pig 5.5 cm. long.

In the study herein reported, I have been greatly aided by a manuscript of Dr. W. G. MacCallum's which I was privileged to read. From this paper, which is on "The Relations between the Lymphatics and the Connective Tissue," and is soon to be published in the Archiv für Anatomie, certain observations and conclusions which aid my work are quoted with the author's permission.

He injected the subcutaneous lymphatics of embryo pigs for the most part between 5 and 15 cm. long and has given most graphic and accurate descriptions of these injections, and of the lymphatics both in fresh and stained preparations. He noted as Ranvier had the growth of the lymphatic capillaries within the plexus by budding, and describes the long sprouts or strands of endothelial cells growing out from the ducts, and how the lumen of a duct gradually opens into the sprouts. He discovered the fact that the early lymphatics have no valves, and made an important addition to the method of injection, by stripping off the skin, placing it on a slide and injecting it under the microscope. He noted that the fluid injected ran into perfectly definite walled channels and that there was no extravasation until the pressure was too great, when the walls of the ducts would suddenly and explosively burst and the fluid would then pass into the meshes of the connective tissue. His conclusion was that the lymphatic ducts in the skin of the embryo pigs are closed ducts.

Inasmuch as in this communication the lymphatic system of the mammal will be traced in its development up to the stage represented in the frog, it will be necessary to keep in mind the amphibian lymphatic system. In the frog the large subcutaneous lymph sacs communicate by ducts with four lymph hearts or sacs, two in the neck and two in the inguinal region. From these sacs, ducts empty into the veins in four places, two in the neck at the junction of the subclavian and cardinal veins, and two in the inguinal region, where the femoral and sciatic veins join to enter the Wolffian body as the renal portal system. There are no valves except where the ducts enter the veins and there are no lymph glands.

[6] Ranvier: Comptes Rendus, Tome 121, 1895, p. 1106.

Up to the time when the pig embryo reaches the fish stage, that is to say, when the four visceral arches are plainly seen (see Keibel's Normaltafeln. 1. Das Schwein., Fig. 19), there are no lymphatics. This is true of embryos up to 14 mm. long, which corresponds to a human embryo of about five weeks. There are, however, in these early stages certain areas in which loose connective tissue, bounded by zones of denser tissue, forms channels of least resistance for fluid injected under pressure. For example, there is such an area around the central nervous system. If Prussian blue is injected just dorsal to the spinal cord near the tail it will not only outline the cord and the brain but will also surround the peripheral nerves as far as they have developed. Sections of such specimens, especially if thick, give deceptive pictures, for the blue granules lying in the meshes of the connective tissue look as if they were in definite ducts. This is, however, not the case, and though these wide intercellular spaces, being full of lymph, may be called lymph spaces, and may have an important relation to the nourishment of the nervous system, they are not a part of the lymphatic system. In sections this loose tissue often breaks away, especially around the nerves, and gives the false appearance of empty spaces. It is in a similar way possible to outline the Wolffian body at least in part by injection.

Another of these areas of loose tissue bounded by zones of denser tissue is found beneath the skin. If one injects Prussian blue into the tissue beneath the skin of the embryo pig, there will be at the point of injection a mass of the blue fluid from which straight, blunt processes reminding one of Budge's canals, run out often in parallel lines. These processes have no resemblance to the true lypmhatic ducts which lie at a more superficial level and can be injected over them, but are due simply to the separation of the connective-tissue cells and show that the intercellular spaces are lines of least resistance for fluid injected under pressure. These spaces are artificially widened by injection. The distance one can inject these spaces depends on the looseness of the connective tissue, and as the meshes of the connective tissue are widest around the central nervous system, it is here that one can inject the farthest.

Serial sections of several embryos of stages before the lymphatic system has begun, that is of pigs up to 14 mm. long, have been made. In these specimens the blood-vessels are injected and from a study of the sections it is clear that all the spaces in the body walls can be proven to be blood-vessels except the spaces between the individual cells. There are no spaces along the dorsal line in connection with the cœlom, which could form a thoracic duct as Budge supposed. In this stage, before there are any lymphatics, many of the blood-vessels widen out into sinusoids instead of capillaries.[1] Some of these sinusoids are beneath the skin, and since they are many times the width of the capillaries, and since the endothelium which lines them is thinner than that of the capillaries, they look much like lymphatics. However, they are readily distinguished by their evident connection with the veins and by the fact that they contain blood.

The development of the lymphatic system was found in this way. We have an abundant supply of pig embryos at the Anatomical Laboratory. Every day large numbers of embryos of all sizes from under 10 mm. upwards are brought to the laboratory. Moreover, we are so near the abattoir that the embryos are often brought with the heart still beating. It is essential in injecting lymphatics to have fresh embryos, for after an embryo is once thoroughly cold it is impossible to get good injections. The best results are always obtained while the heart is still beating. The embryos must be injected immediately after removing them from the uterus and the skin must be kept moist while injecting.

I began with the study of the lymph glands and made the first injections of them by introducing the needle into the foot pads. If in pigs about 10 cm. long, the needle is inserted into the foot pads of the hind feet, ducts are readily injected which run to a gland in the inguinal region, while from the fore feet the ducts run up to a gland in the front of the neck. As younger pigs were taken, for example, below 6 cm., it became impossible to inject any ducts from the foot pads; and still younger, at 4 cm., it became impossible to inject the ducts subcutaneously in the side of the leg. In order to get younger stages of the glands it was thus necessary to inject nearer to them and it was found that in stages when no lymphatics could be injected in the legs they could still be injected with ease in the body wall. These ducts in the body wall run to two other glands, one over the crest of the ileum and one in the posterior part of the neck.

These injections gave the first idea of the gradual growth of the lymphatic system from the centre; because at a certain stage when lymph ducts could always be injected in the body wall, none were ever injected in the feet or legs; that is to say, the legs had not yet received

[1] Minot: On a hitherto unrecognized form of blood circulation without capillaries in the organs of vertebrata. Proceedings of the Boston Society of Natural History, Vol. XXIX, No. 10, April, 1900, pp. 185-215.

lymphatics. From this time on my attention was turned to the development of the ducts rather than of the glands, because I was passing to stages before the lymph glands were formed.

At this time, following the suggestion of Budge's theory of the thoracic duct developing from spaces behind the aorta, a needle was introduced into the side of the neck of a very young embryo, and passed behind the heart. The injection obtained proved to be venous, but by taking larger embryos lymph ducts began to radiate out from the point of injection; for example, in a pig 18 mm. long a few ducts could be injected just at the point of puncture. For this injection the needle was introduced straight inward at a point midway between the ear and the upper border of the arm. At this stage, while it was always possible to inject the small tuft of ducts at the neck, it was never possible to inject ducts in any other part of the skin. Taking a stage a little larger, a wider area or zone of lymphatics could be injected from the same point in the neck, but none were injected in any other place in the skin until the pig was 3 cm. long, when the ducts could be injected just at a point over the crest of the ileum. Taking a stage still larger a wider injection could be made from both of these two points in the skin, one at the neck and one over the crest of the ileum. The zone that could be injected at each stage was both definite and constant and an increase of the pressure simply ruptured the ducts at their tips instead of injecting them farther. Moreover, at any point within a zone, ducts could always be injected subcutaneously in fresh specimens, while at all points beyond these zones they could never be injected.

Thus two points had been discovered as the result of many injections, from which the superficial lymphatics spread or radiated out to cover the skin of the body and head. At a little later stage two other points were found from which the ducts grew to the legs, one of these being in the front of the neck, the other being in the inguinal region. A large number of injections were made at the two primary points until a very complete series of the zones possible to inject at each stage was obtained. This series included the stages from the time when the ducts can just be injected at the side of the neck in a pig 18 mm. long, up to the time when the ducts from the two points of radiation have met and anastomosed over the side of the body of a pig 5.5 cm. long.

The early stages of this development have been embodied in a diagram or composite picture, Fig. 1. The picture includes pigs of four different lengths, 1.8 cm., 2 cm., 3 cm., and 4 cm. Later stages are omitted to avoid confusion. The injections for the diagram were all made from two points, marked a for the neck and c over the crest of the ileum. The letters are used to mark the area which can be injected in a pig of a given size. For example, c corresponds to a pig 3 cm. long and shows that the ducts are just beginning over the crest of the ileum, while from the neck they have already grown over the head and thorax, and over the face either side of the eye.

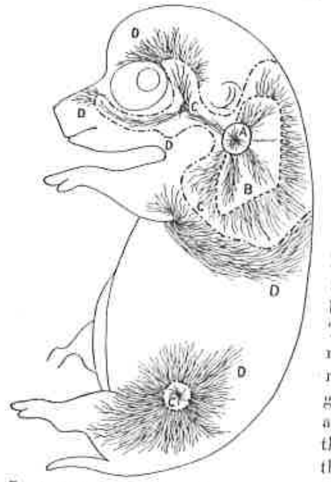


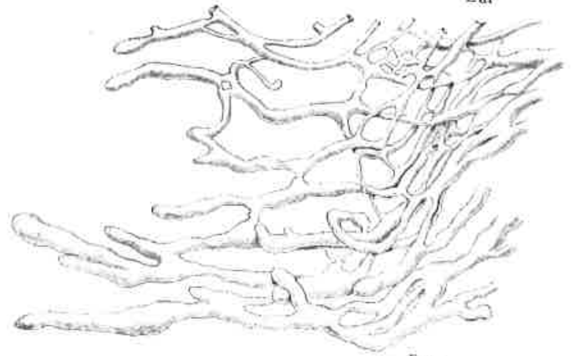
Fig. 1. Composite picture of the spreading of the superficial lymphatics in the embryo pig. A, area of lymphatics in a pig 18 mm. long; b, area in a pig 2 cm. long; c, area in a pig 3 cm. long; d, area in a pig 4 cm. long.

The areas without any ducts in the diagram represent the areas which have not received lymphatics in a pig 4 cm. long. In a pig 5.5 cm. long the two systems of ducts shown in the diagram have met and anastomosed over the body wall, and ducts have grown down the legs nearly to the feet, but there are still areas of the skin which have not yet received lymphatics; for example, the top of the head, the foot pads and the tail. The details of how these areas receive lymphatics as well as the relation of these ducts to the glands that form subsequently are given later in order to relate them to the lymph hearts. At this time we will limit the attention to the study of the successive zones of lymphatics and their relation to the areas without lymphatics.

The method of obtaining these injections is important. In the neck it was found that the best way to obtain maximum injections was to introduce the needle perpendicular to the skin, and it appeared later that this is due to the fact that one often enters the cervical lymph heart, a large sac from which all the ducts radiate. The method used was as follows: A glass tube drawn out to a fine point, the size of which should vary with the size of the pig, is held in a firm clamp which can be moved in three directions by screws. The glass tube is connected by rubber tubing with a flask of Berlin blue or India ink, and this flask is again connected with a pressure flask. The pig is placed on the

stage of a dissecting microscope, just enough pressure is used so that the fluid will drop slowly and the needle is screwed down into the side of the neck half way between the ear and the upper border of the arm. If the pig is not longer than 3 cm., tie the umbilical cord so as to fill the veins with blood and then introduce the needle into the side of the neck to a point just outside the anterior cardinal vein. For the point of radiation of the ducts for the lower part of the body, namely, over the crest of the ileum subcutaneous rather than deep injection are better for, as will appear later, the posterior lymph hearts are situated very deep.

Ear



Eye

Fig. 2. Terminal lymphatics of the skin between the eye and the ear in a pig 5 cm. long. × 16.

It now became necessary to study more carefully these successive zones which had been injected, to prove that the ducts in the border of the zones were terminal and to note their relation to the skin areas not yet invaded by lymphatics. Figure 2 gives a picture of the border zone taken from the ducts injected over the side of the head between the eye and the ear in a pig 5 cm. long. This is a favorable place for study, for the ducts are larger and readily seen with the unaided eye. In general the ducts are the largest in pigs from 4 to 5 cm. long and they decrease in size as the pig grows larger. Other good places for studying the border zone are over the shoulders and over the side of the body. The ducts from the two sides of the neck meet and anastomose between the scapulæ when the pig is 3 cm. long, so that the border zone is early obliterated here. Figure 2 shows that in the border zone the ducts grow out in advance of the plexus. In injecting, when the pressure is increased, these advance ducts always burst at the rounded tips, showing that they are the ends of the ducts. If now the ducts are just filled, care being taken not to burst them and each duct is touched just at the point where it leaves the plexus, with a small glass rod, it will be noted that the duct expands and contracts as the pressure is varied, or in other words, the wall is continuous and elastic. Occasionally it happens that one of these blunt ends, which may even be bulbous, is not really the end of a duct, for by a little pressure the injecting fluid can be forced out into a long thread-like process. That this is really a duct is shown by the fact that it can be filled and refilled by varying the pressure. These fine ducts or sprouts represent the process of growth at the border zone. In areas where the ducts are growing more rapidly than they do over the side of the head the terminal ducts are smaller than those shown in Fig. 2, and almost every one will have one or more long sprouts running out in advance.

It has already been said that no lymphatics have ever been injected in the areas beyond these zones of lymphatics in the different stages. Beside these injection experiments to prove that the ducts in the border zone are terminal and that no lymphatics can be injected beyond, complete serial sections have been made showing, first, the zone itself, with its rich capillary plexus, second, the zone of the growing tips, and third, the areas not yet invaded by lymphatics. These early ducts are so large that there is no mistaking them in sections; they are many times the size of blood capillaries. Uninjected specimens stained in acid fuchsin show them especially well in contrast with the blood capillaries.

I now considered the point proved that the lymphatics gradually invade the skin, for by making injections from either of the two radiating points in successive stages one can inject a wider area as larger pigs are taken and around these areas there is always a border zone of terminal ducts, which burst at the tips if pressure is used. The tips of these ducts are growing points and often have sprouts running out from them and finally, beyond this zone, there are no lymphatics, as has been proven both by their absence in sections and by a large number of negative injection experiments.

The next step in the growth of the lymphatics was to find out how they reached the surface. This was studied first in the neck. The

lymph ducts in pigs 4 to 6 cm. long were injected and the specimens were dissected so as to follow the ducts to the vein. When, however, the injection had gone over into the veins extensively the lymph ducts could not be distinguished from them. To overcome this difficulty the veins were filled with cinnabar gelatine and then the lymphatics were injected with a small amount of fluid, just enough to enter the vein. It is easy to see when the Berlin blue enters the subclavian vein, for the vein lies so near the surface. These specimens showed that the duct accompanies the anterior cardinal vein. In embryos between 18 mm. and 2.5 cm. long, if one injects subcutaneously in the side of the neck, small ducts pass inward and open into a large sac just external to the cardinal vein. The sac when injected is easily seen from the surface. Figure 3 is a section through it in an embryo 2.5 cm. long. This sac, which corresponds to the anterior lymph heart of the frog, is the key to the study of the subcutaneous lymphatics for the anterior half of the body, for they all radiate from it. In serial sections of this stage, the duct from the sac to the vein was traced to the junction of the anterior cardinal and subclavian veins.

FIGURE 3. Transverse section through the neck of a pig 2.5 cm. long, showing the anterior lymph hearts, to be compared with Fig. 8. × 4.5. Acv, anterior cardinal vein; alh, anterior lymph heart; P, pharynx; t, trachea.

This method was sufficient as long as the sac could be injected from the surface, but in an embryo below 18 mm. in length it was again difficult to distinguish the lymphatics from the veins. To overcome this difficulty the veins were now injected. This can be done in three ways: First, if the umbilical cord is tied, there will be a natural injection of blood; second, if one injects into the liver the entire venous system will be injected, and third, in the younger embryos in which the liver is too small the Wolffian body answers the same purpose. By this method the sac which, being empty, contrasted with the injected vein was traced in serial sections in an embryo 15 mm. long.

Serial sections were now cut of embryos 12, 13 and 14 mm. long and showed no lymphatics whatever. However, in an embryo 14.5 cm. long a minute lymphatic sac connected with the vein was found. There are well marked differences in development between embryos 14, 14.5 and 15 mm. long. An embryo 15 mm. long shows the ear (See Keibel's Normaltafeln, No. 1. Das Schwein. Fig. 23), while an embryo of 14 mm. corresponds more to Keibel's Fig. 21, and shows indistinctly all four visceral arches. In the sections of the embryo 14 mm. long the subclavian vein is represented only by a constriction on the side of the cardinal vein. At 14.5 mm. two visceral arches show on the surface and in sections the subclavian vein reaches the root of the arm bud. In this specimen the lymphatics are two small buds extending 228 μ from the vein. Figure 4 is the opening of this sac into the vein and shows that the entrance is guarded by a valve.

The valves which guard the openings of the lymphatic ducts into the veins have required considerable study. Serial sections of five different stages of embryos between 14.5 mm. and 3 cm. have been cut. The sections are stained on the slide with hæmatoxylin and a combination of eosin, 6 parts, aurantia, 1 part and orange G, 4 parts. In this stain the endothelium of the lymph ducts contrasts much better with the connective tissue than in sections stained with carmine. In all the sections of the openings of the lymphatics into the veins, the duct lies for some distance against the vein, the two being separated only by a double layer of endothelium, one for the vein and one for the lymph duct. Finally, in each series one can see that, just at the edge of the lymph duct, these two layers are continuous, see Fig. 4. But it was necessary to prove that this was actually an opening and for a long time I could not inject from the lymphatics to the veins in very young embryos. The reason for this is plain in Fig. 7. In injecting from the periphery one is injecting through small ducts into a large sac, and it is impossible to get pressure enough to force the fluid from the sac into its narrow efferent duct. One could easily fill the sac by puncturing it but the vein lies too near to be sure of puncturing the sac alone. However, by filling the sac carefully from the peripheral ducts one can then press gently with the finger against the neck and see that the fluid actually goes into the vein at the point of the junction of the subclavian and cardinal veins. It takes some pressure to open the valve, and the heart should be beating in the embryo used for the experiment. Having opened the valve, if serial sections are cut through it, the double fold of endothelium will be found a bit raised and smeared with India ink. India ink is a better fluid for these injections than Prussian blue. It runs farther and easier for its granules are smaller and they do not clump in the lymph. This has been done in embryos from 3.5 cm. down to 2.2 cm. long.

FIG. 4. Relation of the lymph duct to the cardinal vein in a pig 14.5 mm. long. Alh, anterior lymph heart. × 170.

In general, when a vein buds off from another vein, it grows out at nearly a right angle, while when a lymphatic buds from a vein, it grows at the smallest possible angle, or, in other words, it grows parallel to the vein. This difference in the direction of the growth of the lymphatic bud from the vein makes the valve at its orifice. (Fig. 4.) In the paper of Dr. W. G. MacCallum referred to above, he emphasizes the continuity of the endothelium in the lymphatic system and the fact that each endothelial cell comes from a preceding endothelial cell. This idea is here fully confirmed and carried a step farther, namely, that the endothelium of the lymphatic system buds off from the endothelium of the veins. In the stages considered in this paper none of the ducts nor sacs of the lymphatic system have any wall except a single layer of endothelial cells.

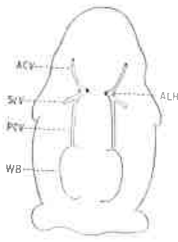


FIG. 5.

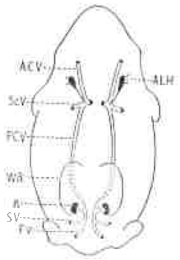


FIG. 6.

FIG. 5. Diagram of the lymphatic system in an embryo pig 14.5 cm. long. × 3. Acv, anterior cardinal vein; alh, anterior lymph heart; pcv, posterior cardinal vein; Wb, Wolffian body.

FIG. 6. Diagram of the lymphatic system in an embryo pig 15 mm. long. × 3. Alh, anterior lymph heart, fv, femoral vein; k, kidney; sv, sciatic vein.

The proof that the lymphatic ducts bud off from the veins is as follows: It has been established that the ducts invade the skin from four points, two anterior and two posterior, and that the growth is from centre to periphery. Starting from the time when the ducts have completely covered the skin, every stage has been followed backward until the ducts are extremely small and extend only a short distance from the vein. In this stage the opening into the vein is just as perfect as in the later stages. Moreover, previous to the stage in which this bud connected with the vein is found, there is no trace of a lymphatic duct or sac, as there would be if the sac formed first and subsequently joined the vein.

The general course of the early development of the lymphatic system in the embryo pig has been embodied in a series of diagrams, Figs. 5 to 11. They are made from actual injections of the lymphatics except in Figs. 5 and 6, for which the veins were injected. All of the specimens except the last two have been cut in complete serial sections. The lymphatics are drawn to scale except in the first one, in which the sac actually measures 228 μ and so had to be exaggerated a little to show in the diagram.

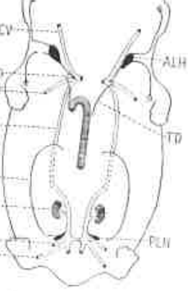


FIG. 7. Diagram of the lymphatic system of an embryo pig 2 cm. long. × 3. Alh, anterior lymph heart; plh, posterior lymph heart; rld, right lymphatic duct; td, thoracic duct.

The first diagram, Fig. 5, from an embryo 14.5 mm. long, shows the two lymphatic buds previously described, and their relation to the subclavian and cardinal veins. At this stage the posterior cardinal vein enters the Wolffian body. Figure 4 shows the sac and the valve which guards the entrance into the vein in this specimen.

The second diagram, Fig. 6, from an embryo 15 mm. long, shows the rapid growth of the ducts anteriorly. It is now evident that they open into a definite sac, corresponding to the cervical or anterior lymph heart of the frog. Small sprouts from this sac have started toward the skin. In an embryo 18 mm. long they have reached the skin and can be injected subcutaneously.

The third diagram, Fig. 7, is from an embryo 2 cm. long. The lymph heart is now large. Figure 3 shows its size in an embryo 2.5 cm. long. The ducts in the neck have now begun to spread in the skin, one tuft grows up behind the ear and another grows downward over the scapular area. This stage marks the beginning of the thoracic duct. From the two cervical lymphatic ducts which we have been following, two ducts start out, very near the opening into the vein, and grow posteriorly. The vagus nerve lies just behind the junction of the subclavian and cardinal veins and these two ducts follow the nerve on either side, and lie just internal to it. These two ducts are taken from sections and have not been injected at this stage.

The same specimen shows also the two posterior lymph hearts. In embryos from 15 to 19 mm. long there are no vessels in the lower part

of the body which are not definitely blood-vessels proved by injections. In the embryo 2 cm. long there are two lymphatic sacs connected with the posterior cardinal vein below the Wolffian body. At this point the sciatic and femoral veins unite in the posterior cardinal vein which enters the Wolffian body as the renal portal system. The two sacs, corresponding to the posterior lymph hearts of the frog, lie close to the vein, and have already sent sprouts out toward the skin. Their opening into the vein is also guarded by a valve so that they remain empty in a complete venous injection. Figure 12 is a section passing through the posterior lymph hearts in a pig 2.5 cm. long, and shows also the ducts reaching part way to the skin.

The next stage, Fig. 8, is from a pig 2.7 cm. long. It shows a further development of the superficial lymphatics. At the same time it is the fundamental stage in the development of the thoracic duct and explains the asymmetry of the lymphatic system. The duct which grows downward on the left side meets the aorta and relying on its support grows more rapidly than the duct on the right side and so becomes the thoracic duct. It passes downward not only on the left side of the aorta but also sends a branch behind the aorta to pass downward on the right side. This may be called the right thoracic duct, to distinguish it from the right lymphatic duct. The duct which started out from the cervical duct on the right side remains as the right lymphatic duct, and in serial sections of a pig 3 cm. long has been traced to the root of the right lung. At this stage there are two buds starting from the left thoracic duct toward the heart and the lung. The thoracic ducts shown in this diagram as well as in all the succeeding ones, are from injections.

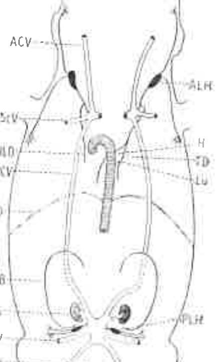


FIG. 8. Diagram of the lymphatic system in an embryo pig 2.7 cm. long. × 3. Alh, anterior lymph heart; d, diaphragm; h, ducts to the heart; lu, ducts to the lung.

The method of injecting the thoracic duct is as follows. Occasionally, it is possible to inject the thoracic duct completely from the periphery in a pig about 4 cm. long, Fig. 10. In general, however, in studying any area in the lymphatic system, one must inject close to the area. This is due to two facts: first, the ducts are very irregular and many exceedingly narrow ducts here and there check the flow of the fluid. These minute channels represent simply the process of growth and are not valves, for at this stage fluid always runs in both directions from the point of injection. Secondly, the lymph clumps the granules of the injecting mass and so closes the ducts. India ink will always run farther than Prussian blue. The study of the development of the thoracic duct depends on being able to inject it centrally.

The thoracic duct in these early stages lies actually in the edge of the wall of the aorta. The side of the thorax is cut away from the embryo, cutting the ribs close to their origin. The lower tip of the lung is freed and pushed aside, thus exposing the aorta and the posterior cardinal or azagos vein lying close beside it. The needle must now be introduced between the aorta and the vein and it must actually pick up the edge of the wall of the aorta. It is inserted just behind the intercostal branches of the aorta. In pigs 4 cm. long and upwards a fine hypodermic needle can be used, but in pigs smaller than 4 cm. a needle is too large and glass tubes drawn out to a fine point are necessary. At these stages, if one simply enters the loose connective tissue between the aorta and the vein without picking up the wall of the aorta, the injected fluid will extravasate. In older pigs, however, for example, over 20 cm. in length, the thoracic duct is separate from the aorta and can be injected by inserting the needle between the aorta and the vein. At this stage there is a plexus of lymphatic ducts in the aorta wall from which the thoracic duct can also be injected. The right lymphatic duct can be injected by dissecting the neck and introducing the needle behind the junction of the subclavian and cardinal veins.

Figure 9, from a pig 3 cm. long, shows the completion of the thoracic duct. In the thorax the two branches of the thoracic duct, lie either side of the aorta, between it and the azagos vein, but between the two kidneys they widen out to make a double receptaculum chyli and lie close together just behind, or dorsal to the aorta. The thoracic ducts on either side have now joined with the posterior lymph hearts and so the lower connection of the lymphatic system with the veins has been given up.

It will be noted that there has been a change in the venous system at the same time, for the femoral and sciatic veins now pass to the vena cava instead of following the old course into the Wolffian body as

the renal portal system. The area between the two kidneys is so rich in blood-vessels that a brief description of the veins will aid in locating the lymphatics in sections. This description is taken from a specimen prepared in this way. A fresh embryo of this stage, namely, 3 cm. long, was injected with carmine gelatine into the liver, and by this means the venous system was filled with red. The lymph ducts were then injected subcutaneously over the scapular area with India ink. The ink entered the vein and passed to the heart, which, as it was still beating, pumped the aorta full. A little of the black ink entered the vena cava, but not enough to obscure the picture. The embryo was hardened, cut in two parts in the median sagittal plain, cleared in creosote and mounted in balsam. In this specimen, the femoral and sciatic veins join to make a large median vein, the vena cava, which lies directly dorsal to the aorta opposite the posterior half of the Wolffian body. This median vein is connected by many branches with the posterior cardinal vein on the left side. Opposite the middle of the Wolffian body the vein is deflected to the right and comes to lie beside the aorta instead of behind it. The branch of the vena cava from the left kidney passes ventral to the aorta as in the adult. At this level the double receptaculum chyli is directly dorsal to the aorta. Opposite the anterior end of the Wolffian body the vena cava turns ventralward in the root of the mesentery and runs directly to the liver. Thus, in sections of this stage, opposite the posterior part of the Wolffian body it is the vena cava behind the aorta, while opposite the anterior half of the Wolffian body it is the double receptaculum chyli. This area shows especially well that in studying the lymphatic system either the veins or the lymphatics must be injected. The lymphatic

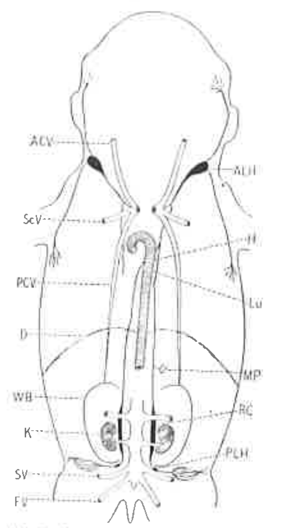


FIG. 9. Diagram of the lymphatic system in an embryo pig 3 cm. long. × 3. Alh, anterior lymph heart; h, ducts to the heart; lu, ducts to the lung; mp, ducts to the mesenteric plexus; plh, posterior lymph heart; rc, receptaculum chyli.

ducts often have a rim of coagulated lymph which stains in eosin, and this together with the absence of blood cells aids in distinguishing them.

To return to the diagrams of the lymphatic system, Fig. 9 shows that the ducts from the posterior lymph hearts have just reached the skin. If a needle is inserted rather deeply over the crest of the ileum, the receptaculum chyli can be injected through them. The diagrams of the lymph hearts show why it is so much easier to inject the anterior one directly than the posterior one, namely, that the anterior one is superficial. To inject the posterior lymph heart directly, the abdominal wall must be opened and the needle inserted just below the kidney. There are so many veins in this region, however, that it is rare to get a pure lymphatic injection in this way. The receptaculum chyli is much more easily injected directly than the lymph heart.

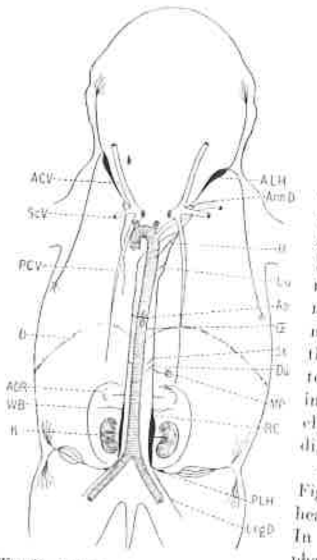


Fig. 10. Diagram of the lymphatic system in an embryo pig 4 cm. long. × 3. Adr, adrenal; alh, anterior lymph heart; ao, lymphatic plexus to the aorta wall; arm d, deep lymphatics to the arm; du, ducts to the duodenum; h, ducts to the heart; oe, ducts to the œsophagus; leg d, deep lymphatics to the leg; lu, ducts to the lung; mp, ducts to the mesenteric plexus; plh, posterior lymph heart; rc, receptaculum chyli; st, ducts to the stomach.

At the stage of this diagram, Fig. 9, the lymph ducts to the heart have encircled the auricles. In an embryo 3.7 cm. long, lymphatics have been injected covering the inner surface of the left lung, while in serial sections of an embryo 3 cm. long there is a plexus of lymph ducts in the root of the mesentery, and the right lymphatic duct can be traced to the right lung. At this stage there has also been a new development in the superficial lymphatics at the neck. The diagram shows simply the two systems growing behind the ear and over the back. The ducts now grow both between the eye and the ear and in front of the eye. This shows in Fig. 1.

The last two diagrams show the further development both of the

thoracic duct and of the superficial lymphatics. In Fig. 10, from an embryo 4 cm. long, there is a plexus of ducts in the front wall of the aorta just above the diaphragm. Branches have been injected in this stage to the heart, lung, aortic wall, stomach, duodenum, adrenal, Wolffian body and kidney. At the same time ducts are growing along the subclavian and femoral arteries to make the deep lymphatics of the arm and leg. In Fig. 11, from an embryo 5.5 cm. long, there are additional branches to the reproductive organs. At the same time the superficial lymphatics have met and anastomosed over the sides of the body. The lymph hearts are now much smaller and are no longer definite sacs, but rather wide ducts.

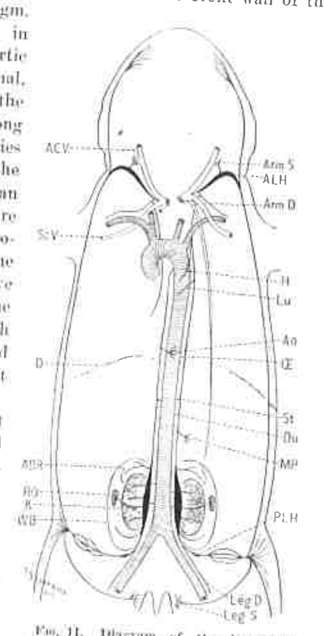


Fig. 11. Diagram of the lymphatic system in an embryo pig 5.5 cm. long. × 2. Alh, anterior lymph heart; arm d, deep lymphatics to the arm; arm s, superficial lymphatics to the arm; ao, plexus to the aorta wall; du, ducts to the duodenum; h, ducts to the heart; oe, ducts to the œsophagus; leg d, deep lymphatics to the leg; leg s, superficial lymphatics to the leg; lu, ducts to the lung; mp, ducts to the mesenteric plexus; plh, ducts to the posterior lymph heart; ro, ducts to the reproductive organs; st, ducts to the stomach wall.

From a study of the diagrams it will be plain that the superficial lymphatics starting from the veins primarily and from the four lymph hearts secondarily follow in general the course of the veins; in the neck the anterior cardinal, in the groin the sciatic. On the other hand, from the time the thoracic duct meets the aorta, the deep ducts grow along the arteries. This distinction was noted in the adult as far back as 1836 by Breschet.[7]

The spread of the superficial lymphatics in embryos up to 4 cm. long is shown in Fig. 1. As has been said, all the ducts shown in the picture have been injected from two points, one in the neck marked a, which corresponds to the posi-

[7] Breschet: Le Systeme Lymphatique, Paris, 1836.

tion of the anterior lymph heart, and one over the crest of the ileum marked c. When the ducts first reach the skin in the side of the neck, one tuft grows behind the ear and the other grows over the scapular area. This is shown in the area marked b, corresponding to an embryo 2 cm. long. At the point of radiation of these two groups of ducts a plexus is formed which eventually becomes a lymph gland and corresponds to the group of glands in the posterior part of the neck of the adult.

From the anterior lymph heart another group of ducts grows to the angle of the jaw and there divides, one group growing over the head in front of the eye and the other growing between the eye and the ear. This second point of radiation, seen in a pig 3 cm. long in the area marked c in the diagram, is likewise a plexus, which becomes a lymph gland. A third point of radiation is made in the axilla for the ducts of the wall of the thorax. The ducts from this plexus are present in a pig 4 cm. long, as is shown in the area marked d. Finally, at the same stage, namely, in a pig 4 cm. long, a fourth point of radiation develops nearer the front of the neck for the superficial ducts of the arm and for the ducts over the front of the neck. This plexus has been injected in a pig 4 cm. long and by 4.5 cm. the ducts from it have reached the middle line of the neck in front and below the elbow on the surface of the arm. In Fig. 11 is seen the connection of this point of radiation with the anterior lymph heart; it is marked arm s.

Fig. 12. Transverse section of an embryo pig 2.5 cm. long at the posterior end of the Wolffian body showing the posterior lymph hearts, to be compared with Fig. 8. × 5.5. A, aorta; c, cœlom; ld, lymphatic ducts growing to the surface; plh, posterior lymph heart; v, vein formed by the junction of the femoral and sciatic veins as shown in Fig. 8.

These four points of radiation, one in the posterior part of the neck or scapular, a second behind the angle of the jaw or maxillary, a third in the axilla or axillary, and a fourth in the anterior part of the neck or clavicular, are all connected in the depth with the anterior lymph heart, and can be injected from it. The branches that radiate out from the four plexuses anastomose so freely in the skin that they can also be injected subcutaneously. Certainly all the ducts in the anterior half of the body of a pig 5.5 cm. long can be injected by one puncture over the scapular area except possibly the ducts down the arm, which are too far away from the point of injection. In general, each one of these four points of radiation is a plexus which develops into a lymph gland. Each gland then drains an area represented by the ducts that grew out from the plexus.

In the groin there are only two points of radiation for the superficial lymphatics. The first or primary one is over the crest of the ileum, and sends ducts over the back and sides of the body as far up as the axilla, and down over the hip. The ducts from this plexus do not grow to the middle line of the body in front, but anastomose freely with those of the other side across the back. The second centre, which comes a little later, is in the inguinal region and sends its ducts to the leg and to the ventral abdominal wall. In a pig 4.5 cm. long these ducts have grown to about the level of the umbilicus and extend part way down the leg. All the superficial lymphatics, after they have once covered the skin, anastomose freely; for example, one can inject in a pig 5.5 cm. long from the surface of the hip up to the axilla.

The deep lymphatics follow the arteries, which gives the key for the study of their development. By injecting along the aorta the ducts to the following organs have been injected: the heart, lung, œsophagus, stomach, duodenum, mesentery, adrenal, kidney, Wolffian body, reproductive organs, pancreas, spleen and liver. The ducts to the pancreas and spleen have only been injected in a pig 7.5 cm. long. I have, as yet, no early injection of the ducts of the liver. Fig. 11 shows the deep lymphatics of the arm and leg following the arteries. In a pig 7.5 cm. long the ducts following the umbilical arteries into the cord have been injected, that is, the lymphatics do not pass over to the umbilical cord from the abdominal wall but enter rather with the arteries. The right lymphatic duct has been injected in a pig about 10 cm. long and its branches pass to the heart and anastomose with those from the thoracic duct to that organ.

It has now been shown that the lymphatic system in the embryo pig begins as two blind ducts which bud off from the veins in the neck. At the very start the openings of these ducts into the veins are guarded by valves formed by the direction which the endothelial bud takes as it grows from the vein. In the ducts themselves there are no valves at first. From these two buds and later from two similar buds in the inguinal region ducts grow toward the skin and widen out to form four sacs or lymph hearts and from these sacs the lymphatics grow to the skin and cover its surface. At the same time there is a growth of ducts along the dorsal line following the aorta to make a thoracic duct from which the lymphatics grow to the various organs. Thus the ducts of the lymphatic system gradually invade the body, but there are certain tissues which they never reach even in the adult, for example, the cornea and cartilage.

The development of the lymphatics has here been traced for the most part in pig embryos. We have, however, a rat embryo which corresponds with Fig. 6. The veins are filled with blood and so the lymph hearts are easy to find. Saxer[9] evidently has a cow's embryo 2.5 cm. long cut in serial sections, which shows the anterior lymph hearts. He describes the specimen as follows, that in the side of the neck are small lymphatic ducts which collect into two symmetrical cystic spaces, from which a duct narrows down rapidly and opens into the vein. Several of the human embryos of Professor Mall's collection have the veins well filled with blood, so that it has been possible to find the lymphatics in them and to confirm some of the steps of the development of the lymphatic system in the human embryo.

The lymphatic system, at the stage to which it has been traced in this work, represents about the stage of the adult frog. That is, it is a system of ducts without valves except at the openings into the veins and generally speaking, without glands. The plexuses which are to form the glands are present in many places.

The function of such a system is clearly suggested by a pathological specimen reported by Smith and Birmingham.[10] The specimen was of twins prematurely born, one of which was normal, while the other was so œdematous that it was simply a round ball. By good fortune the writers studied the lymphatic system in both fœtuses. In the normal one the thoracic duct and lymph glands were easily found, while in the œdematous one there was no trace of a thoracic duct and no lymph glands.

The lymphatic system is a modification of the circulatory system, dependent both in its origin and, in large measure, in its development on the blood-vessels. It returns to the vascular system the fluid exuded into the tissue spaces from the blood-vessels. Speaking more generally, it is a system of absorbents. The lymph glands which develop by the increase of connective tissue around plexuses of ducts come later; they occur only in birds and mammals and do not begin to develop in mammalian embryos until the ducts or capillaries they drain are well formed.

This study was begun at the suggestion of Professor Franklin P.

[9] Saxer: Anatomische Hefte 1, VI, 1896, S. 370.

[10] Smith and Birmingham: Absent thoracic duct causing œdema of a fœtus. Journal of Anatomy and Physiology, Vol. 23, 1889, p. 532.

Mall. It was his idea that by repeating and extending the experiments of Budge it might be possible to fill in the gap between the two lymphatic systems of Budge and clear up the subject of the origin of the lymphatic system, and this has been indeed the case. It is a pleasure to thank him for many suggestions and for his continued interest in the work. The opportunity for the work was made by a fellowship offered to the Johns Hopkins University by the Baltimore Association for the Promotion of University Education of Women. It is also a pleasure to express my gratitude to them.