THE DEVELOPMENT OF THE LYMPHATIC SYSTEM IN RABBITS.

BY

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WITH 8 TEXT FIGURES.[1]

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

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

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

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

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

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

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

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

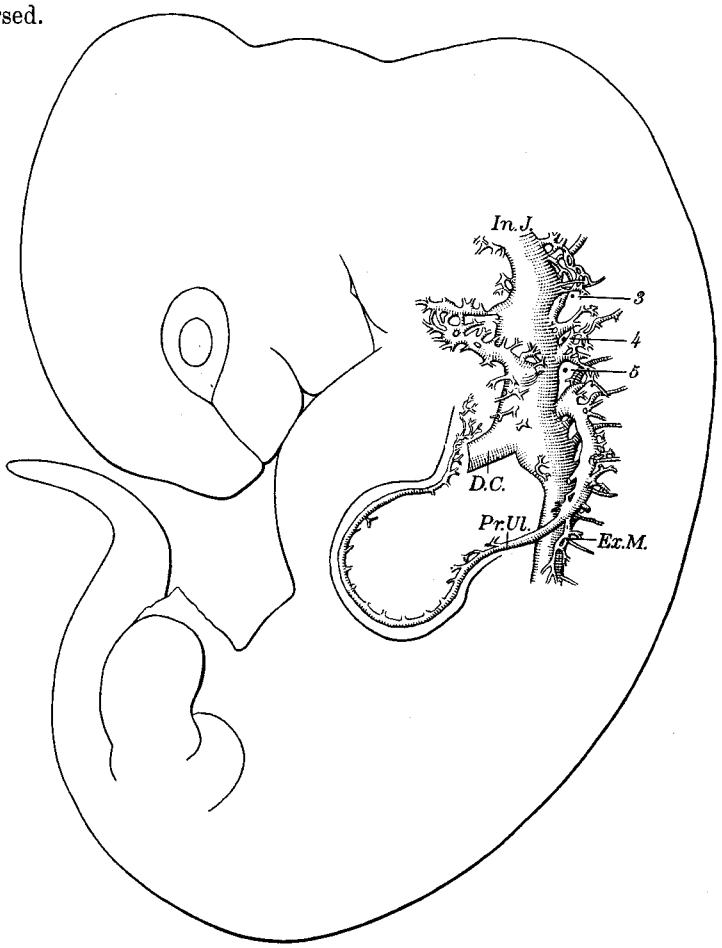

FIG. 1. Rabbit, 13 days, 9.5 mm., Harvard Embryological Collection, Series 498, × 13 diams. 3, 4, and 5 indicate the position of the corresponding cervical nerves in this, as in the following figures. The veins shown are those of the left side: *D. C.*, duct of Cuvier; *Ex. M.*, external mammary; *In.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

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

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

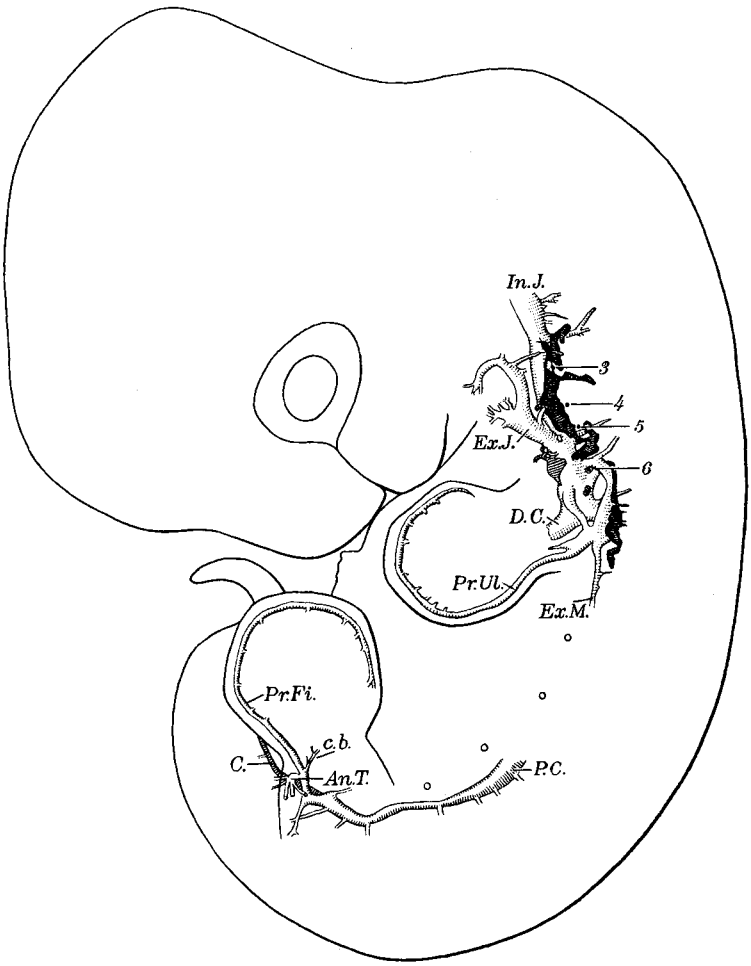

FIG. 2. Rabbit, 14 days, 10 mm., H. E. C., Series 155, × 13 diams. The lymphatic vessels are heavily shaded, as in all the following figures. The veins are those of the left side: *An. T.*, anterior tibial; *C.*, caudal; *c. b.*, "connecting branch"; *D. C.*, duct of Cuvier; *Ex. J.*, external jugular;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

In a rabbit of 13 days, 9.5 mm., no lymphatics could be found. The reconstruction, Fig. 1, shows the veins along which the first lymphatics are soon to appear. The internal jugular vein receives a great many small

branches. One of these, nearly parallel with the dorsal border of the vein and wider than the others, opens into the vein at either end. It is in relation with the third cervical nerve. From its position and appearance it is believed that this branch of the vein becomes a lymphatic vessel.

The second reconstruction is a 10 mm. embryo of 14 days. In this specimen a chain of lymphatic spaces has appeared along the internal jugular and the dorsal root of the primitive ulnar veins. The most anterior segment of the chain extends back to the third cervical nerve. It sends out short blind sprouts like a vein and contains many blood corpuscles. The partition between it and the jugular vein is very thin, and at one point there is a suggestion of communication between the two, as shown in the figure. No opening into the vein can be demonstrated, however. The second segment of the chain, proceeding posteriorly, extends to the fifth nerve. It equals the internal jugular vein in diameter, and is closely applied to its wall. Behind the third nerve it sends a blind diverticulum around the ventral end of the dorsal body muscles, into the deep subcutaneous tissue of the back. This diverticulum, not matched on the opposite side of the embryo, contains blood which apparently entered it from rough treatment in preserving the specimen. The third segment of the chain, between the fifth and sixth nerves, seems to connect with the root of the ulnar vein. This connection, however, lies in the plane of section, and a thin intervening wall may have been carried away in the process of cutting. A detached lymph space follows the dorsal root of the ulnar vein. A small and somewhat questionable one, not matched on the opposite side, rests against the superior vena cava, between the roots of the ulnar vein. The most significant structure found in this embryo is a space filled with blood, which opens into the external jugular vein near its junction with the internal jugular. This space lies quite near the third segment of the lymphatic chain. On the opposite side of this embryo, and in the following one, this blood-filled sac connecting with the vein appears to be replaced by a lymphatic space, detached from the vein, but connecting with the chain.

Fig. 3, from an embryo of 14 days, 11 mm., shows the fusion of all the lymphatics of the previous stage into one large sac which encircles the external jugular vein. On neither side could this sac be seen to communicate with the veins. No lymphatic vessels were found which did not connect with the jugular sacs. The dorsal subcutaneous extension, described in the preceding stage, occurred on both sides. In the posterior part of the embryo, no lymphatics were found. The reconstruction of the cardinal veins is that already figured in this journal, Vol. I, Plate 2, Fig. 5 (following p. 244).

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

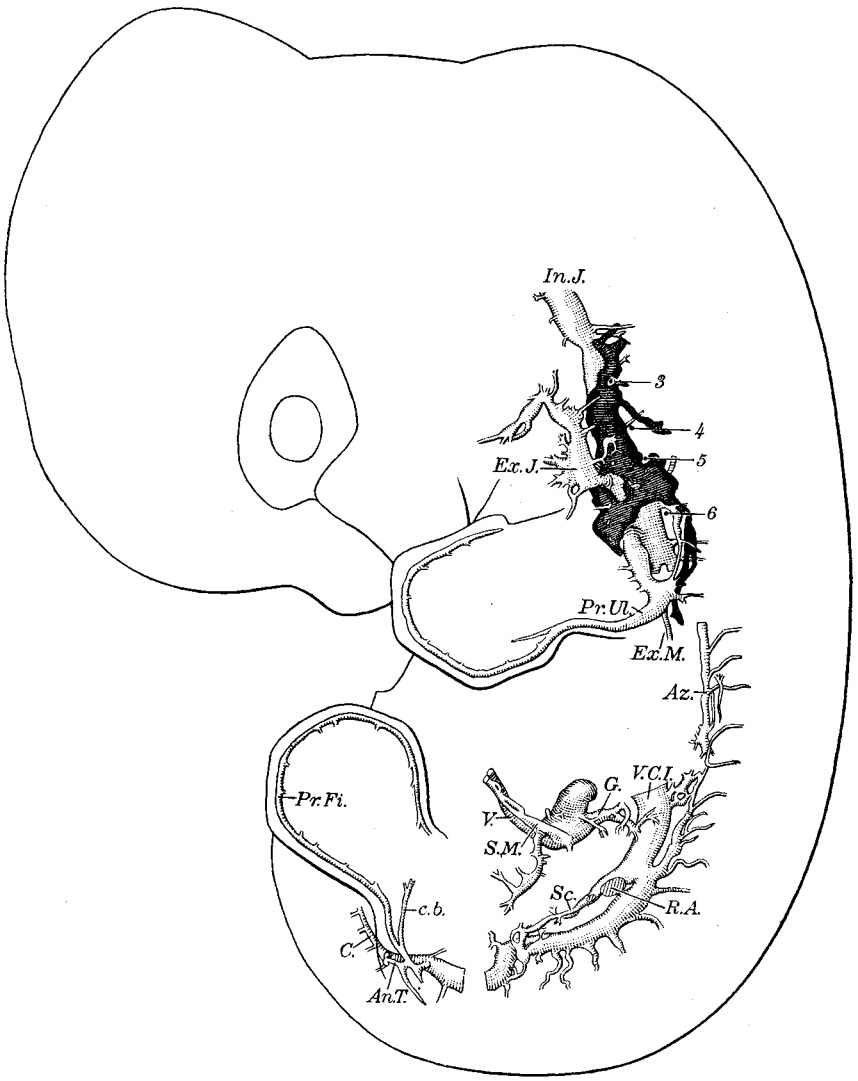

FIG. 3. Rabbit, 14 days, 11 mm., × 13 diams. The structures drawn are the same as in Fig. 2, except that in the trunk of the embryo the following veins, belonging to the median plane and to the right side, have been added: *Az.*, azygos; *G.*, gastric; *R. A.*, renal anastomosis of the subcardinal veins;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

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

The jugular lymph sac in Fig. 4 has completely surrounded the third and fourth cervical nerves. It envelops two-thirds of the circumference of the internal jugular vein. On the right side of the embryo, in one section (No. 476), a minute orifice connected the sac and the vein. It was not in the position of the adult opening between these structures, and was not matched on the opposite side. The deep subcutaneous outgrowth from the jugular sac has become greatly dilated in its distal portion. Near the beginning of the external mammary vein, a large lymph space is found wedged between two converging venous branches. This space is not connected with the veins. It may be a remnant of the lymphatic vessels which in the preceding stage accompanied the dorsal root of the ulnar vein. A few slender detached lymphatics follow the external mammary vein. Finally there are two lymphatics which appear to have arisen from branches of the azygos vein, one near the vagus nerve (Fig. 4, x) and the other along the aorta (Fig. 4, y). The former connects with a small vein, the latter ends blindly not far from one. Obviously when a connection with a vein is well preserved the structure in question would be considered a venous branch; and after becoming detached, were it not for its endothelial wall, it might be called a mesenchymal excavation. The study of this and the following specimens seems to show that the lymphatics along the aorta (thoracic ducts) are derived in part from the azygos veins; below, from the subcardinals; and above, from the jugular sacs.

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

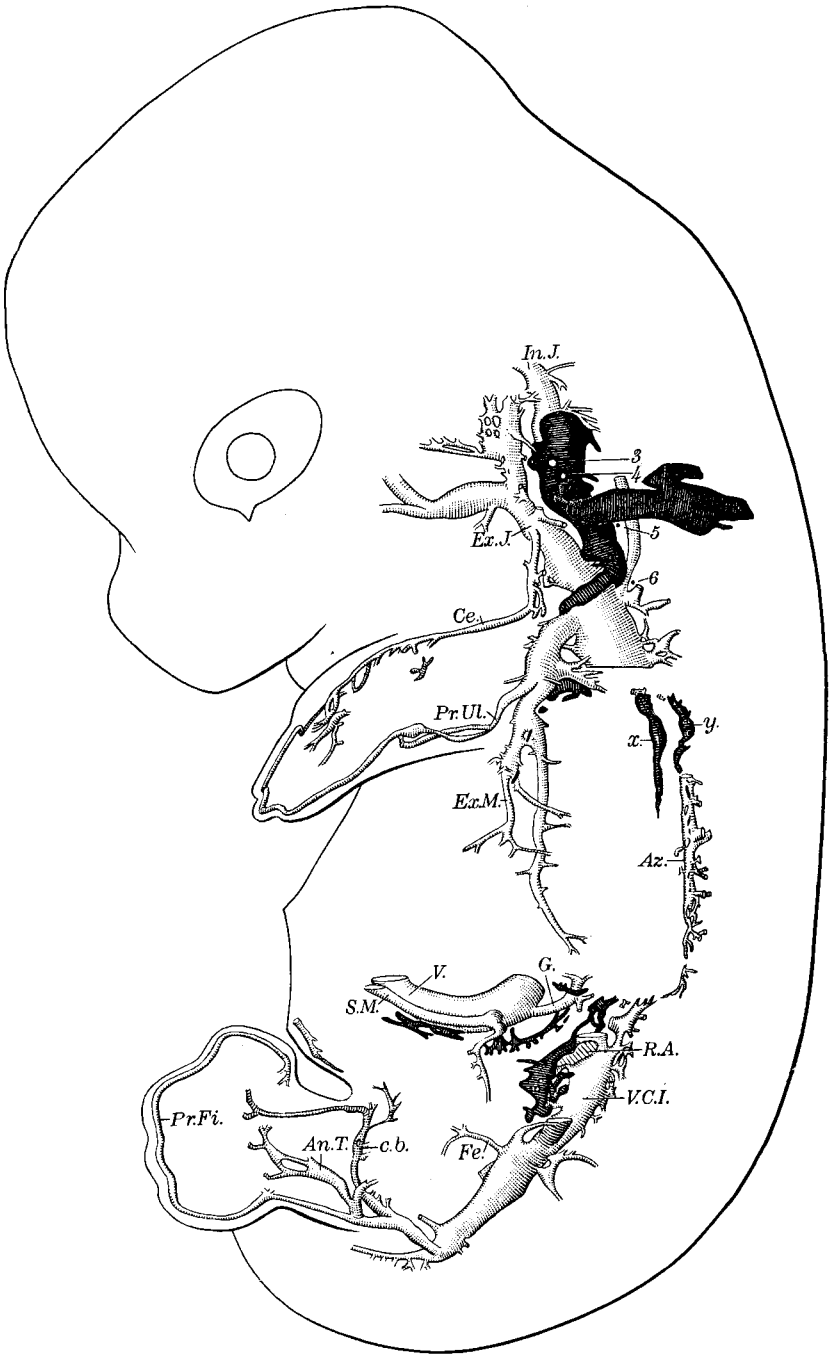

FIG. 4. Rabbit, 14 days 18 hours, 14.5 mm., H. E. C., Series 143, × 13 diams. *x* designates a lymphatic vessel accompanying the left vagus nerve; *y*, a lymphatic along the aorta. The veins of the arm are: *Ce.*, cephalic; *Pr. Ul.*, primitive ulnar. Those of the leg are: *An. T.*, anterior tibial;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

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

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

Considering its lymphatic development the pig of 21 mm. is less advanced than the rabbit of 14.5 mm., since there are no lymphatic vessels along the external mammary vein nor in the mesentery. The cat of 15 mm. is more advanced than either. In this embryo the D formed by the jugular sac is almost bisected diagonally. The second, third, and fourth nerves pass through its aperture, but the fifth penetrates the posterior section of the sac by a separate opening. There are two deep subcutaneous diverticula corresponding with the single one in the rabbit and several in the pig. In one section (266) a branch of the jugular sac may enter the innominate vein a little anterior to the subclavian, but it is not clear that an actual opening exists and none can be found on the opposite side.

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

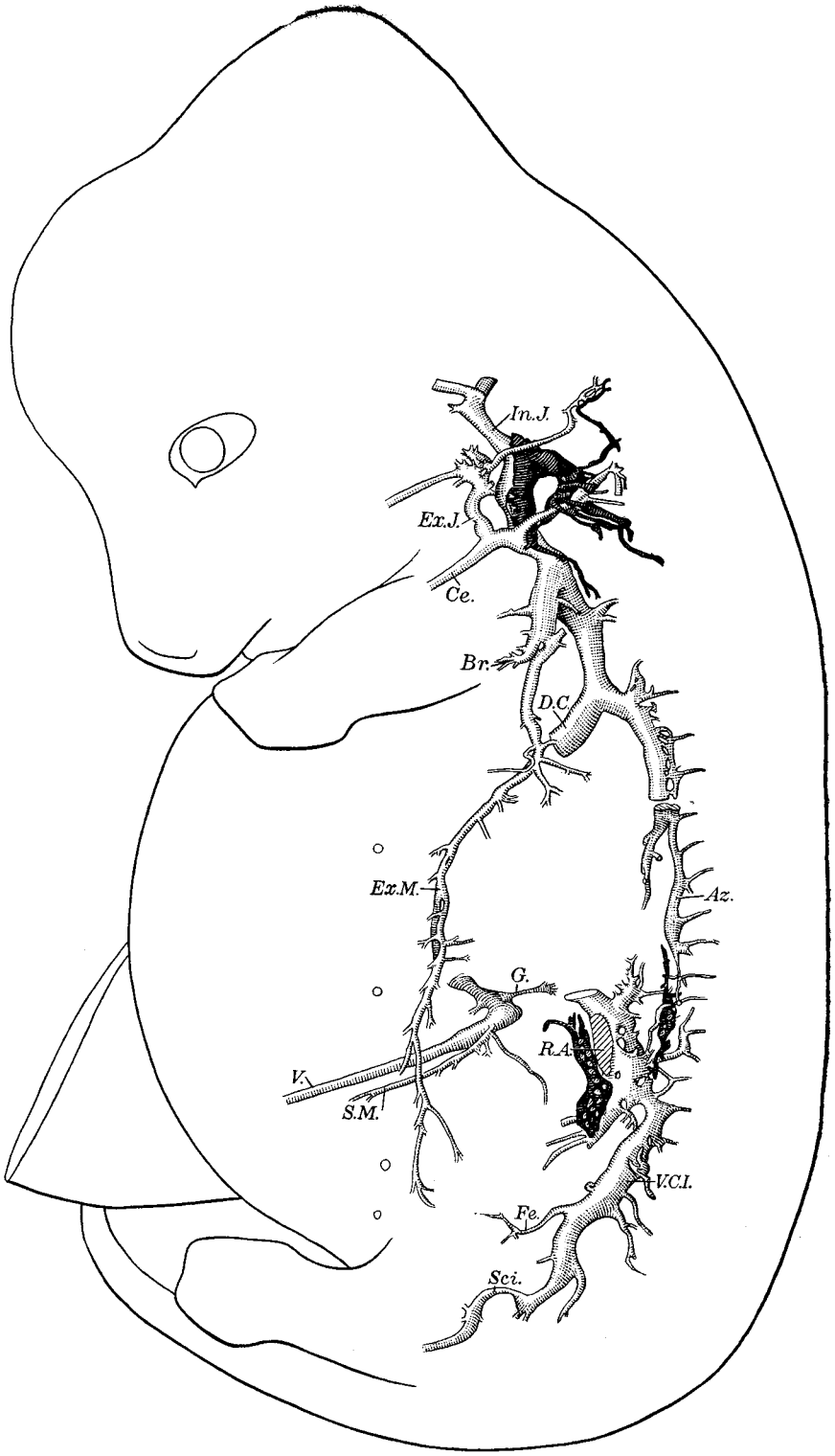

FIG. 5. Pig, 20 mm., H. E. C., Series 59, × 10 diams. The veins are: *Az.*, azygos; *Br.*, brachial; *Ce.*, cephalic; *D. C.*, duct of Cuvier; *Ex. J.*, external jugular; *Ex. M.*, external mammary; *Fe.*, femoral; *G.*, gastric; *In. J.*, internal jugular; *R. A.*, renal anastomosis of subcardinals;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

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

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

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

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

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

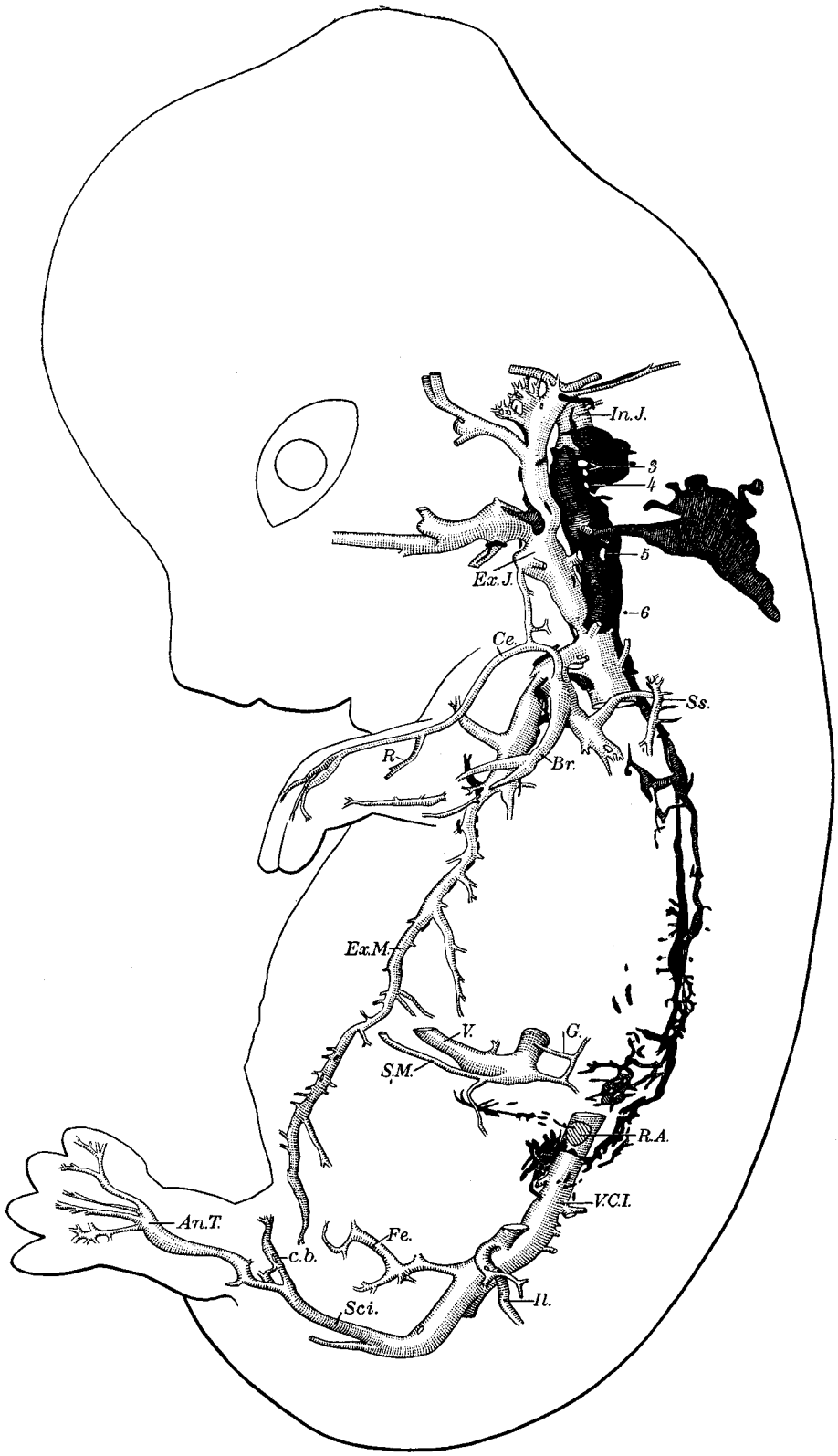

FIG. 7. Rabbit, 17 days, 21 mm., H. E. C., Series 738, × 10 diams. The veins not previously lettered in the rabbit figures are: *Il.*, ilio-lumbar; *Ss.*, subscapular; *R.*, radial; *Sci.*, sciatic.

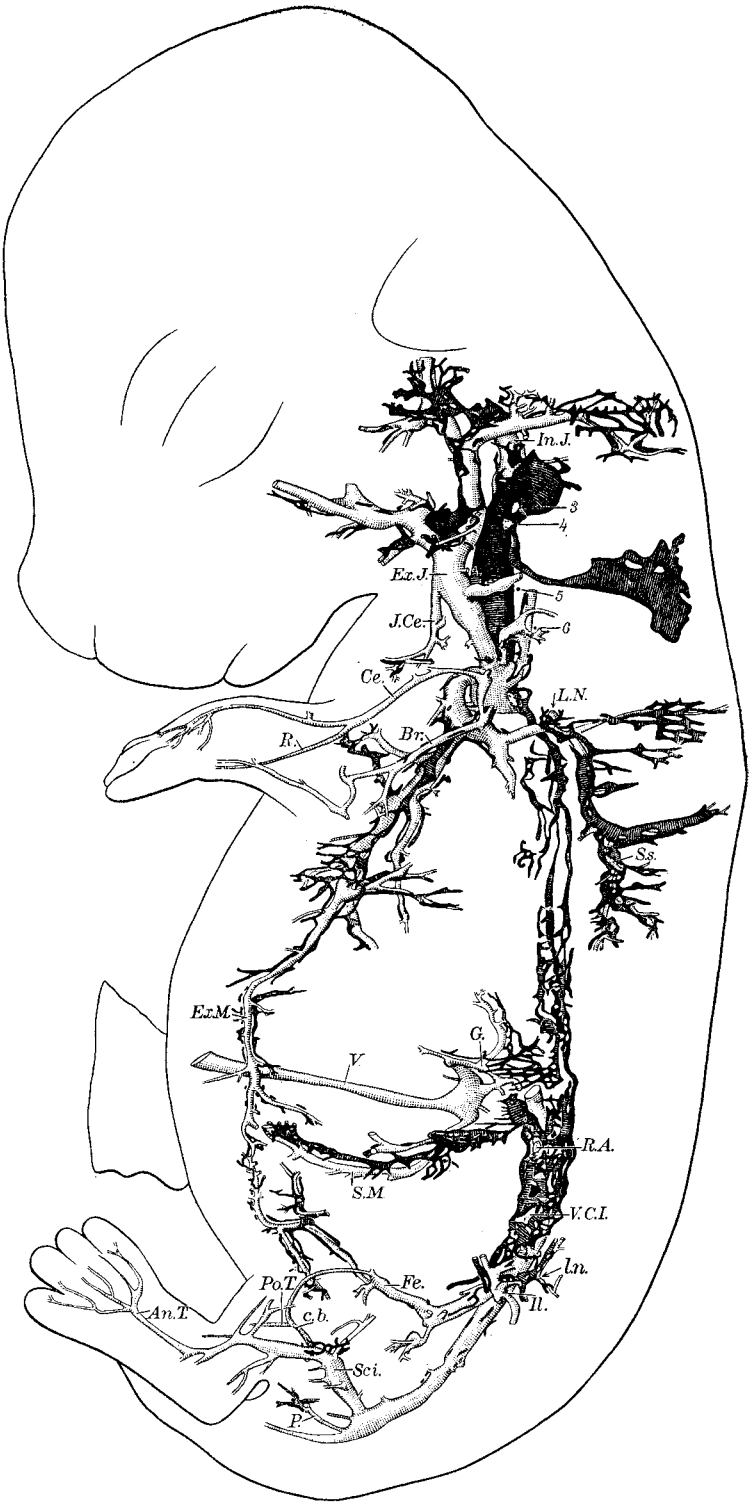

FIG. 8. Rabbit, 20 days, 29 mm., H. E. C., Series 170, × 6.9 diams. The first lymph nodes develop at *L. N.*, along the subscapular vein, *Ss.;* and at *l. n.*, along the ilio-lumbar vein, *Il.* The veins of the arm are: *Br.*, brachial; *Ce.*, cephalic; *J. Ce.*, jugulo-cephalic; *R.*, radial. Those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

The jugular sac on the left side, except for an extensive rupture, does not connect with the vein. On the right, a pore is found leading from the sac to the internal jugular vein near its union with the external, but this also may be artificial. Thus in all the series of rabbits no bilateral communication of the lymphatics and veins, in the position of the adult openings, could be found. The pores, sometimes detected in various positions, are not adequate to empty the large sacs, and may indeed be artifacts. Communication with the veins in these stages must be by osmosis, therefore, and the permanent outlets of the lymphatic system must develop later.

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

SUMMARY.

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

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

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

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

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

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

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