

THE DEVELOPMENT OF THE THORACIC DUCT IN THE PIG

OTTO F. KAMPMEIER

From the Laboratory of Comparative Anatomy, Princeton University

THIRTY-FIVE FIGURES (FIVE COLORED PLATES)¹

I. INTRODUCTION

At the present day the question of the origin of lymphatics has become one of the most interesting problems and is holding the attention of perhaps as great a number of investigators as any other problem in the field of embryology and anatomy. This is partly due to the fact that the lymphatic system as a whole is the last of the organ-systems to be taken up for more thorough investigation, and partly it is the result of the impetus given by modern physiology which has emphasized the question of the relationship between lymphatics and blood vessels and their functional significance in the economy of the organism. Although this problem has been attacked at various times in the history of anatomy, relatively few important advances have been made, and it is only recently during the last decade that it has been attacked with renewed vigor and has been pushed to the critical point of its solution. The question of the individuality of a lymphatic, especially, has developed a most animated controversy between two schools diametrically opposed in their contentions. One of these maintains that all lymphatic channels are a direct product of the venous system, and the other that they arise independently of it, and that if they do enter into certain structural venous relations during their genesis such relations are purely of a secondary character.

¹ Expense of illustrations partly borne by author.

In 1909 Sabin² published a paper on the origin of the lymphatic system in the pig which reinforced and extended the theory of Langer³ and Ranvier,⁴ that lymphatic vessels arise from the veins by a process of sprouting and centrifugal growth. Because her results and conclusions did not present fully all of the evidence or agree with the results of later investigations, Professor McClure suggested to the writer the expediency of repeating her work on the same kind of material, namely, pig embryos, but restricting this research to one lymphatic channel and concentrating upon it alone all attention. The thoracic duct was selected on account of its size, importance, and definite position in the body, thus serving better as a test or control than perhaps any other lymph channel.

For the completion of this work, carried on during the last two years, I am indebted to Professor McClure for his advice and valuable criticisms, and to Dr. A. G. Brown of Columbia University and to Mr. Charles F. Silvester for their hints in the preparation of the microphotographs. I acknowledge my gratitude to Professor Houser for permitting me to use the laboratory at the Iowa State University during the summer of 1910. My thanks are also due to Professor Sabin for the use of series 23a of the Johns Hopkins University Embryological Collection, and for the privilege of publishing any information or evidence which might be derived from this series.

II. MATERIAL AND TECHNIC

Most of the embryos used in this work were fixed in Zenker's fluid and the sections stained on the slide with Delafield's haematoxylin and orange-G. Sabin's series 23a was preserved in the same fixative but stained with Congo red. A few embryos were

² Florence R. Sabin: On the origin of the lymphatic system from the veins, and the development of the lymph hearts and thoracic duct in the pig. *Am. Jour. Anat.*, vol. 1, 1902, pp. 367-389.

³ C. Langer: Ueber das Lymphgefäß-systems des Frosches. *Sitzb. d. Akad. d. Wissensch.*, Bd., 57, 1, Abth., 1868.

⁴ L. Ranvier: Morphologie et developpement des vaisseaux lymphatiques chez les mammiferes. *Comptes Rendus*, 1895, 1896. *Archives d'Anatomie Microscopique*, tome 1, 1897.

fixed in picro-sublimate and chrom-aceto-formaldehyde, and several others were stained with borax carmine in toto and counterstained with picric acid or blue de Lyon. Picro-sublimate is very unsatisfactory in its preservation of tissue and should not be used. For excellency of fixation and differentiation of vascular structures the first method mentioned proved by far the best. Not only did the sections show a beautiful transparency of color and a strong contrast when this method was carefully followed in detail, but they also produced the most favorable microphotographs.

A few embryos were injected with India ink by the writer. Series 23a was prepared and injected by Professor Sabin and serves as an excellent critical stage in the development of the thoracic duct.

Table 1 which represents only a partial catalogue of the Princeton Collection of pig embryos enumerates the series studied and reconstructed. They are arranged and grouped according to the developmental status of the thoracic duct region in each, and therefore certain embryos precede others of slightly lesser length. On the whole, however, this system of gradation corresponds very closely to gradations by length, the little inconsistencies here and there being of trivial account when we consider that measurement can be at most of only approximate accuracy, and that fluctuations in growth are not at all infrequent.

The length of each embryo, excepting Sabin's series 23a, was obtained after fixation, when the danger of possible mutilation to the embryo is least, and represents the crown-rump measurement, viz., the distance between the crown of the head and the base of the tail. Sabin's series being measured before fixation is consequently longer; but when it is considered that the processes of fixation, hardening and dehydration reduce the absolute length of such an embryo by 1 or 2 mm., this discrepancy in size disappears and it is seen to fit in smoothly with related series.

All of the embryos were sectioned transversely to 15 and 20 micra in thickness. The significant sections of the series selected for reconstruction were carefully drawn with the aid of the Edinger drawing apparatus, the details then confirmed by the high power

of the microscope, and the wax models made after a slight modification of Born's method. In this way the regions of immediate interest in nine series were reproduced at a magnification of 100 diameters, excepting series 69 which was magnified to 50 diameters. Besides these wax-models, a number of graphic reconstructions

TABLE 1
List of material examined

SERIES NO.	LENGTH OF EMBRYOS	REMARKS
	<i>mm.</i>	
214	14	
215	14	
211	15	
210	15	
212	16	
151	16	
216	16	
217	16	
Unnumbered	16	Injected
150	18	Reconstructed in wax, $\times 100$
167	17	Reconstructed graphically, $\times 100$
141	17	
168	19	Reconstructed in wax, $\times 100$
101	19	
104	18	
194	20	Reconstructed in wax, $\times 100$
106	20	
102	20	
193	20	Reconstructed graphically, $\times 100$
186	21	
227	20	
Unnumbered	21	
23a	23	From the Johns Hopkins U. Emb. Coll.; injected; reconst. in wax, $\times 100$
103	21	
191	21	Reconstructed in wax, $\times 100$
105	22	Posterior region reconstructed in wax, $\times 100$
192	21.5	Reconstructed in wax, $\times 100$
Unnumbered	23	Injected
67	23	Posterior region reconstructed in wax, $\times 100$
Unnumbered	24	Injected
69	26	Anterior region reconstructed in wax, $\times 100$
Unnumbered	28	

were carefully made, but on account of the more restricted accuracy of this method and the probable errors in the expression of relations and proportions none were used as plate figures.

The series labelled 'unnumbered' represent incomplete series, embryos of which only the anterior two-thirds or middle regions were sectioned.

III. REVIEW OF LITERATURE

It is needless to survey all of the former investigations which have been concerned with the genesis of the lymphatic system; suffice it to say that the fundamental question in all of them has been: Do the lymphatics arise from the veins, or are they a direct product of the mesenchyme? Only the more recent literature bearing on this query and intimately related to the writer's own observations will be cited and discussed here.⁵

In 1902 Sabin published her first work on the development of the lymphatic system.⁶ By the method of injection she found that all lymph vessels, both peripheral and systemic, arise at four centers, two anterior and two posterior, and that they invade the skin as well as the deeper-lying regions of the embryo by a process of centrifugal growth. In other words, consecutive injected stages will show the channels springing apparently as a few simple sprouts from these points of radiation and then gradually growing longer and branching in an intricate manner until they have spread throughout the whole body. The two anterior centers, situated one on each side of the neck in the fork of the jugulars, and the posterior ones, inguinal in position, represent the locations of the anterior and posterior lymph hearts, respectively. With the aid of a series of clear diagrams she has mapped out the general course of lymphatic development. Unfortunately, how-

⁵ For a comprehensive list of the literature bearing on the development of the lymphatic system, the reader is referred to the following two papers: George S. Huntington: *Die Entwicklung des lymphatischen Systems der Vertebraten vom Standpunkte der Phylogenese des Gefäß-systems*. Anat. Anz., Bd. 39, 1911. Florence R. Sabin: A critical study of the evidence presented in several recent articles on the development of the lymphatic system. Anat. Rec., vol. 5, no. 9, 1911.

⁶ Florence R. Sabin: 1902; loc cit.

ever, she did not describe or figure the details or factors of this growth, except to say that the lymph vessels at first bud from the veins and thereby derive their endothelium from them and then continue to grow distally apparently by the proliferation of their cells. The sprouting and elongation of the thoracic duct is indicated in three diagrams constructed from embryos of 20, 27, and 30 mm. In the first of these schemes the right lymphatic and thoracic ducts are seen as two short caudal extensions from the jugular lymph sacs. In the second, they have become longer and the left one or thoracic duct has divided into two branches, one of which passes to the right side of the aorta as the rudiment of the right thoracic duct, and the other remains on the left side as the left duct. In the diagram of the 30 mm. embryo these two branches have extended far back and have established continuity with the cisterna chyli and the posterior lymph hearts, thus completing the thoracic duct system.

A few years later, in 1906, F. T. Lewis made public a short account of the development of the lymphatic system in rabbit embryos.⁷ His results are of great interest because they represent a new conception of the genesis of the lymphatics, being neither identical with the centrifugal growth theory of Sabin nor with the theory of their direct mesenchymal origin, but in a sense standing between these two. Furthermore, he was the first investigator to determine the principle involved in the formation of the lymph sacs and to point out certain definite events preparatory to the completion of the thoracic duct in the mammalian embryo. In this studies on the transformation of the venous system in the posterior regions of rabbit embryos, he noticed that portions of the subcardinal veins became isolated and seemingly converted into lymphatic vessels. He found more and more of these so-called detached 'lymphatics,' and led by this suggestion, he took up a more systematic investigation of the pathways of the larger systemic lymphatics of the body. He made a number of serial graphic reconstructions and thereby brought to light some very interesting results. He observed that the jugular lymph

⁷ Frederic T. Lewis: The development of the lymphatic system in rabbits. *Am. Jour. Anat.*, vol. 5, 1905, pp. 95-111.

hearts arise by the coalescence along the internal jugular vein of several venous outgrowths which become detached to form a large isolated sac. A similar process was observed in the posterior part of the body in the region of the subcardinal and mesenteric veins. He also discovered a chain of discontinuous 'lymphatic spaces' or endothelial-lined anlagen, apparently detached venous outgrowths, situated along the azygos veins and in the path of the future thoracic duct. From their position and consecutive arrangement he concluded that they fuse with one another and the jugular and mesenteric sacs and thus produce the continuity of the duct. To quote his own words: "The study of the specimens seems to show that the lymphatics along the aorta (thoracic ducts) are derived in part from the azygos veins; below from the subcardinal; and above from the jugular sacs." It is also of importance to state here that the 'lymphatic spaces' which he described are 'scarcely distinguishable from blood vessels.'

In 1907, shortly after the work of Lewis, Huntington and McClure made a more detailed and extensive study of the development of the jugular lymph sacs and confirmed in the main his results.⁸ They had formerly believed that the sacs arise by the formation, enlargement and fusion of perivascular spaces,⁹ but now, from the data of a large number of beautiful and accurate wax reconstructions of cat embryos, they established the opinion that they come from the precardinal and in part from the post-cardinal veins by the confluence of a series of outgrowths or derivatives which they called veno-lymphatics, as suggesting their venous origin and their subsequent transformation into a lymphatic structure. For a short time McClure also carried this conception to the developing thoracic duct,¹⁰ agreeing with Lewis in the formation of a chain of discontinuous anlagen along

⁸ George S. Huntington and Charles F. W. McClure: The anatomy and development of the jugular lymph sac in the domestic cat. *Anat. Rec.* vol. 2, 1908, pp. 1-18. *Am. Jour. Anat.*, vol. 10, no. 2, April, 1910, pp. 177-311.

⁹ George S. Huntington and Charles F. W. McClure: The development of the main lymph channels of the cat in their relation to the venous system. *Am. Jour. Anat.*, vol. 6, 1907. *Abstr. Anat. Rec.*, vol. 1, pp. 36-41.

¹⁰ Charles F. W. McClure: The development of the thoracic and right lymphatic ducts in the domestic cat. *Anat. Anz.*, Bd. 32, nos. 21 and 22, 1908, pp. 534.

the azygos veins and derived from them, but he later withdrew this view since it was based on the study of an insufficient number of critical stages.

Concerning all lymphatic vessels, not including the lymph hearts, Huntington¹¹ maintained the theory advanced jointly by McClure and himself in 1906, that lymphatics have their origin in the fusion of extra-intimal spaces which arise irregularly and disjointly along primitive temporary venous channels.¹² Thus he says:

The peripheral general lymphatic channels appear to be developed by confluence of spaces independent of the venous system, although closely associated with the same. The histological picture presented by them differs radically from that of the jugular veno-lymphatic derivatives. They begin as minute extravenuous vacuoles closely applied to the surface of the veins which they accompany. They enlarge as the lumen of the veins diminishes. They become confluent with each other but they never from their first inception contain red blood cells, nor do they, as far as I have been able to ascertain in numerous carefully studied series of excellent preservation and fixation, communicate with the blood channels.

In 1908, Sabin published a short paper¹³ in which she reviews the several positions held relative to the genesis of lymphatic channels and attempts to turn the evidence in favor of the centrifugal growth theory. Concerning Lewis' multiple anlagen she says:

Since these spaces are lined with a definite endothelium, they form a much more serious obstacle to the theory of growth of the lymphatics from the endothelium of the veins than the more indefinite spaces to be found in earlier embryos, and I cannot but think that if these multiple endothelial-lined isolated spaces do exist along the veins in the later stages, they would form serious evidence against the theory of the origin of the lymphatics from the veins. Or at least if the lymphatics, in their growth, do pick up isolated endothelial-lined spaces, we shall again be left without a clue as to the origin of the lymphatic system.

¹¹ George S. Huntington: The genetic interpretation of the development of the mammalian lymphatic system. *Anat. Rec.*, vol. 2, 1908, pp. 19-45.

¹² This theory was presented by Huntington and McClure before the Association of American Anatomists in 1906 and published as a preliminary account in the *Anatomical Record* no. 3 and in the *American Journal of Anatomy*, vol. 6, 1907.

¹³ Florence R. Sabin: Further evidence on the origin of the lymphatic endothelium from the endothelium of the blood vascular system. *Anat. Rec.*, vol. 2, 1908, pp. 46-54.

However, she firmly believes that the 'lymphatic anlagen' of Lewis appear isolated only in the study of serial sections, and that their continuity can be demonstrated by the method of injection. In other words, "in complete injections there are no vessels which have not received the injecting mass," but "in partial injections and uninjected specimens there are endothelial-lined vessels" which appear to be broken up into segments, so that continuity "can be traced only with difficulty or not at all." In this same article she admits the presence of true mesenchymal spaces "which undoubtedly contain lymph," but tacitly assumes that they "are to be excluded from the lymphatic system morphologically." They are isolated and cannot be injected and they do not possess a clearly defined intima.

A year later, in 1909, Sabin published her observations on the development of the lymphatic system in human embryos.¹⁴ In this work she reaches and emphasizes essentially the same points as in her previous investigations. In the case of the thoracic duct, however, she hesitates to take a definite position. She believes that it originates as outgrowths of the jugular lymph sac and cisterna chyli, but she states in this connection that 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." Further:

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 these veins, or does the duct grow from the two sacs, the cisterna chyli and the jugular one. For the 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 earlier stages the picture is always liable to be confused by Lewis' multiple anlagen.

¹⁴ Florence R. Sabin: On the development of the lymphatic system in human embryos, with a consideration of the morphology of the system as a whole. *Am. Jour. Anat.*, vol. 9, 1909, pp. 43-90.

In 1910 Huntington¹⁵ and McClure¹⁶ read two papers at the International Congress of Anatomists at Brussels and, on the basis of a study of cat embryos, presented striking evidence for the theory, that the lymph ducts of the body are developed by the confluence of mesenchymal spaces which are largely extra-intimal, that is, formed around the lumen of an embryonic venous channel which subsequently disappears completely. Furthermore, they drew the distinction very clearly that the lymphatic system of mammals may be divided into two morphological components: the lymph ducts which arise as indicated, and the lymph hearts which form the connecting segment between the systemic lymphatics and the veins and are transformed from a venous plexus derived from the veins in their respective regions. Concerning the former, which at present are of the greatest interest, Huntington says:

Die Lymphgefäße des ganzen Körpers entstehen durch den Zusammenfluss einer grossen Anzahl von Hohlräumen, welche sich intercellulär in Mesoderm entwickeln, in sehr genauer Anpassung an die Wand der Embryonalen venösen Bahnen und in ganz derselben Weise wie die ersten Anlagen des Blutgefäss-systems, aber unabhängig vom demselben. Das Endothel, welches diese Hohlräume die ersten lymphatischen Anlagen auskleidet, ist von Anfang an unabhängig vom Endothel der Blutbahnen und entwickelt sich mit dem ersten Auftauchen der lymphatischen Hohlräume aus den indifferenten Mesodermalzellen, welche diese Hohlräume begrenzen. Mit anderen Worten, das lymphatische Endothel hat dieselbe genetische Herkunft wie das Endothel der Blutgefäße, nämlich es besteht aus modifizierten Mesodermalzellen, welche in die Wandung der intercellulären Hohlräume eintreten. Die erste Stufe des histogenetischen Verlaufes ist ganz die gleiche, ob nun das resultierende Hohlraumssystem in der Folge der lymphatischen oder der hämalen Abteilung des Gefäss-systems zugeteilt wird. Es gibt demnach zwei Generationen der embryonalen vaskulären Endothelzelle, eine lymphatische und eine hämale. Beide entstehen auf gleiche Weise und infolge gleicher genetischer Einflüsse aus indifferenten Mesodermalzellen. Beide sind vom Anfang des Vorganges an unabhängig voneinander.

¹⁵ George S. Huntington: Ueber die Histogenese des lymphatischen Systems beim Säuger-embryo. Verhandl. d. Anat. Gesellsch., Bd. 24, 1910.

¹⁶ Charles F. W. McClure: The extra-intimal theory and the development of the mesenteric lymphatics in the domestic cat. Verhandl. d. Anat. Gesellsch., Bd. 24, 1910.

In order to emphasize the principle involved in the extra-intimal development of the mammalian lymphatics, Huntington selected the thoracic duct as an example for the reason that the histogenetic processes which enter into its inception and completion are clearly expressed and easily followed, and also because the duct retains a more definite and constant position relative to surrounding structures than perhaps any other lymph channel. He observed that the continuity of the thoracic ducts is realized by the confluence of a large number of spaces which have sprung from the mesenchyme immediately in contact with decadent venous channels. Their first appearance is as numerous intercellular isolated fissures which then coalesce to form larger spaces, and these in turn become confluent to produce the continuous vessels. Lined with undifferentiated tissue cells at their beginning, they gradually assume the flattened and delicate endothelium of the adult lymphatic.

McClure has described the same process of development in the formation of the mesenteric lymphatics of the cat.¹⁷ He showed that at a certain period, a plexus of veins situated in the dorsal mesentery becomes detached from the postcava and soon after manifests signs of atrophy. With the aid of several clear microphotographs he further pointed out that the mesenteric lymphatics follow topographically, or better, appropriate these abandoned venous channels by a process of extra-intimal replacement. The haemal endothelium collapses and large mesenchymal spaces appear around it. In this connection he states:

These lymph spaces which lie external to the intima of the veins gradually encroach upon the territory formerly occupied by the veins and finally fill it completely; the result being that the original intima of the vein, no longer serving in the capacity of lining a functional venous channel, gradually degenerates and disappears. Traces of this intima can often be observed, however, in older embryos, clinging to the walls of the lymph channels within which a new lymphatic intima has been established.

That these pictures are real, and not artifacts induced by poor preservation of tissue, is conclusively shown by the fact that they

¹⁷ Charles F. W. McClure: 1910; loc. cit.

occur only at a definite period and place, and only in connection with those venous channels which have become detached from the main venous trunks and no longer serve in the economy of the blood vascular system.

Last year there appeared in monograph form the first two parts of Huntington's investigations on the anatomy and development of the systemic lymphatic vessels in the cat.¹⁸ Besides its great detail, the work is profusely illustrated with convincing microphotographs and reconstructions and is a very positive and elaborate confirmation of the theory of the direct mesenchymal origin of all lymph ducts. Once more he emphasizes sharply the analogy between the blood vascular and lymphatic systems in their earliest anlagen, both beginning their history in a similar manner and in the same soil. The first blood vessels arise in and amongst the strands and 'blood islands' of the mesoderm as intercellular clefts and fissures which enlarge, elongate and flow together to create a network of intercommunicating channels. Their boundaries at first are the unspecialized and cuboidal mesodermal cells among which they lie. The fluid which fills their cavities and which is perhaps secreted by these cells is evidently under a certain pressure and exerts its influence in the modification of the immediate or limiting walls into a vascular endothelium. The cells by a mechanical adaptation to this pressure lose their cuboidal form and become flattened and scale-like. Likewise the lymphatic anlagen begin as intercellular spaces and enlarge, elongate and coalesce into continuous vessels, and like the intima of the blood vascular anlagen their intima is a differentiation of the cells among which they are formed.

After such general considerations, Huntington enters into a very complete description of the development of the thoracic duct. Because a résumé of this history, as determined by him, has already been given in the review of an earlier paper, it need not be repeated here. Suffice it to say that nowhere has he found the

¹⁸ George S. Huntington: The anatomy and development of the systemic lymphatic vessels in the domestic cat. Part I. The development of the systemic lymphatics in their relation to the blood vascular system. Part II. The development of the pre-azygos and azygos segments of the thoracic duct. *Memoirs of the Wistar Institute of Anatomy and Biology*, May, 1911.

slightest evidence for 'centrifugal growth' as the fundamental principle in the genesis of the thoracic duct, nor for its origin from multiple venous anlagen.

In her most recent paper Sabin¹⁹ takes a position plainly at variance with her earlier view of the development of the thoracic duct, in that she restricts to a considerable degree the importance of centrifugal growth by budding as the active principle or factor in its formation. After briefly mentioning the conditions found by her in two 23 mm. embryos and one measuring 25 mm., she says:

It is not possible to set limits to the transformation of veins into lymphatics making the cisterna chyli and thoracic duct, for by comparing the two specimens measuring 23 mm. it can be seen that vessels which are clearly branches of the azygos veins in the one specimen do not seem to connect with the vein in the other. The thoracic duct develops in part as a down growth of the jugular sac and in part, especially its dilated portion or cisterna chyli, as a direct transformation of the branches of the azygos veins.

This quotation would seem to indicate that she now believes that the longer part of the thoracic duct is produced as a caudal extension of the jugular lymph sac alone, and not, as she formerly held, from two growing sprouts which subsequently meet, one of them derived from the jugular sac, and the other from the cisterna chyli as an extension cephalad. Her failure to mention either the absence or presence of this last or second sprout, which she claimed to have found in her earlier investigations on the origin of the thoracic duct in human and pig embryos, confirms strongly enough her change of view in this respect.

IV. OBSERVATIONS AND DISCUSSION

It is clear now, that there are three distinct views in the field concerning the development of lymphatic vessels:

1. They spring from the veins at four centers of radiation and by continuous elongation, centrifugal growth and branching invade practically the entire body.

¹⁹ Florence R. Sabin: A critical study of the evidence presented in several recent articles on the development of the lymphatic system. *Anat. Rec.*, vol. 5, no. 9, 1911.

2. They are derived from the embryonic venous system either by a direct transformation of certain of its channels or by the fusion of multiple derivatives which have become detached from it.

3. They arise by the confluence of mesenchymal spaces, which in the mammalian embryo are frequently perivenous in formation.

After a thorough and prolonged study of an extensive series of pig embryos, the writer is forced by direct evidence to ally himself with the third position which holds that the thoracic duct has an independent origin, that it is not a product of the veins either by centrifugal growth or by the direct fusion and transformation of venous derivatives, and that its intima is a gradual differentiation from the mesenchymal reticulum. Essentially then he is in harmony with and can confirm Huntington's conclusions. Indeed so positive and convincing are the results and so perfect in their agreement among themselves that the possibility of doubting their accuracy would seem to be entirely excluded.

In further corroboration of this view is the fact that some of the individual stages of pig embryos show very clearly how the views of the venous origin of the thoracic duct sprang into existence and secured a strong foothold. During the formation of this duct the use of the injection method can produce conditions, which, disregarding all other details, would seem to corroborate the theory of its centrifugal growth. Again, in certain stages there are structures and data which would seem to furnish the necessary basis for the other view, that it arises directly from a transformed vein or its detached derivatives. But in both cases a comparative study of a sufficient number of closely graded series will prove that these appearances are due to the examination of an inadequate number of embryos, to the tyranny of one method of investigation, or to a faulty coördination of all the facts available. The appearance of centrifugal growth is plainly given by injection, but this method with all its advantages can only indicate the regions of completed channels, or the direction of their growth, and at best serve as a control by supplying negative or indirect evidence; it can never portray the actual genesis of a channel or reveal the histogenetic processes which are at work from the beginning. Furthermore, the fact that in certain stages there are venous channels which

have a course or position subsequently occupied by the thoracic duct does not imply or prove that the latter is a transformation of the former. Embryologists are learning that in order to select and understand all of the steps of embryonic changes, one embryo of each consecutive age will not suffice as was formerly the practice, but a number of embryos of the same stage are requisite. Variations do not begin with the finished organism, but they are potent throughout ontogeny from the beginning onward. Fluctuations of growth either of the whole or of parts are not infrequent, and should we base conclusions on a scanty number of embryos the chances are that they will be fragmentary or distorted. There are genetic changes of intrinsic importance, but so evanescent that we may not even catch a glimpse of them unless we have a series of embryos which approaches the ideal of complete continuity. To produce such a closely graded series may prolong the investigation and make the technic more tedious, but the end result will be more certain and will justify the labor expended. In the following descriptions of typical and consecutive stages of the thoracic duct history, and in the discussion of the data presented by them, these general considerations, as well as the various points suggested in the review of earlier investigations, will become more significant.

The fundamental genetic period of the duct, that is the time between the first appearance of its anlage and the acquisition of continuity throughout its entire extent, is of very short duration. Embryos of 18 or 19 to 23 or 24 mm., depending on individual variations, are the important ones and almost the only ones necessary for this study. In order to demonstrate more clearly, however, the relation which the development of the duct bears to the remainder of embryonic history, we must be aware of the factors and events that lead up to it or, in other words, believe it to be already potential in the period just preceding its actual inception and realization. With this supposition in mind we may artificially divide its history into three phases.

1. A veno-lymphatic phase, in which a system of provisional venous channels, or 'veno-lymphatics,' is laid down throughout the entire distance subsequently occupied by the thoracic duct.

2. A transition phase, characterized by the atrophy of these veno-lymphatics and the genesis of discontinuous lymphatic anlagen.

3. A lymphatic phase, in which continuity is established along the whole thoracic duct anlage and secondary growth processes bring about its completion.

Because these developmental changes proceed in a general antero-posterior direction, more than one phase may be present in the same embryo at the same time, although at different levels, but so definite is the succession of events that the above division into three phases will invariably suggest itself.

1. The veno-lymphatic phase (15-19 mm. pig embryos)

In the early embryonic history the venous plan of the thoracic region is composed of two strictly symmetrical and bilateral halves which are practically disconnected from each other except through the heart. Later by the formation of plexuses, anastomoses and fusions, certain channels acquire more of the blood current and thereby gain supremacy over others, which gradually dwindle in size and vanish and consequently give rise to the startling asymmetries of older embryonic stages and of the adult. At the time when the first of these profound transformations are initiated, a series of vessels are developed which function only temporarily in this scheme and then disappear completely. Reference is here made to the 'veno-lymphatics' which have their origin and consummation in those stages approximately between 15 and 19 mm. in length and are a part or product of the supra-cardinal or azygos system during its early transformations.

For want of a better descriptive term, the word 'veno-lymphatics' has been extensively used throughout this paper but nevertheless with some reluctance. A veno-lymphatic, as defined by Huntington and McClure in their work on the development of the jugular lymph sac in the cat, is a venous derivative which by confluence with other such channels is directly transformed into the lymphatic structure. Instead of restricting himself to this original meaning, the writer has employed this term in a somewhat different and a wider sense, as designating those temporary embryonic venous channels which occupy topographically the position of the future thoracic ducts, or other lymph ducts, but atrophy and disappear during the genetic period of these lymphatics. If this

distinction is clearly grasped all possible confusion will be avoided. There is a firm suspicion, however, that all veno-lymphatics vessels, whether apparently direct or indirect antecedents of some lymphatic, are fundamentally identical or homologous structures.

A brief account of the early history of the supracardinal or azygos system of veins will simplify the explanation of the source and character of the veno-lymphatics in the thoracic duct area and their grouping into three divisions. In 14 and 15 mm. embryos, the precardinal and jugular veins give rise, in the region of the anterior lymph sac, to a number of dorsal tributaries which are continued back to the posterior part of the body as two slender channels immediately above and parallel to the pre- and post-cardinal veins. On account of their position and subsequent history, these longitudinal channels may be called the supracardinal lines. Throughout their course they are joined to the pre- and postcardinals by numerous cross-anastomoses. They also possess branches which may be described as dorsal segmental veins, because they drain the regions of the back on each side of the vertebral column and spinal cord and appear to be arranged metamerically. The disposition and fate of the supracardinal lines in a later stage (19 mm. embryo, fig. 28) can be indicated as follows: Their precardinal division (A), that segment extending between the levels of the jugular lymph sacs and the Cuvierian ducts, is complicated into a plexus, some channels of which become veno-lymphatics (6a), to be considered presently, and others are absorbed by the dorsal branches (8) of the precardinal veins. Their middle or postcardinal division (B, 12d, 12ls), between the Cuvierian ducts and the anterior extent of the mesonephroi, fuses longitudinally with the postcardinal veins. The posterior division (C) furnishes the true supracardinal veins²⁰ (12d, 12s),

²⁰ George S. Huntington and Charles F. W. McClure: The development of the postcava and tributaries in the domestic cat. *Am. Jour. Anat.*, vol. 6, 1907, *Abstr. Anat. Rec.*, vol. 1: "A bilateral and originally symmetrical venous channel develops dorso-medial to the primitive postcardinal vein by longitudinal anastomoses between somatic postcardinal tributaries. This secondary vein channel forms what we have termed the supracardinal system of veins. It extends from the level at which the posterior limb veins open into the postcardinals to a point cephalad where it joins that portion of the postcardinal which alone persists to form the anterior end of the adult azygos."

which proceed caudally as two large and important vessels and only very much later take a further part in the production of the asymmetrical venous plan of the trunk, especially in their transformation with the postcardinals to form the azygos system of veins; this segment may therefore be termed the supracardinal division. Since the pre- and postcardinal divisions (A, B) of the longitudinal supracardinal lines disappear or lose their independence, either by being transformed into transient venous plexuses as in the first division or by fusing entirely (12ld, 12ls) with the postcardinals as in the second division, it is evident that the dorsal segmental tributaries (8), which in the beginning spring from these lines, must shift their roots so that they arise directly from the pre- and postcardinal veins in the two divisions mentioned. In the third or posterior division (C), however, these segmental tributaries (8) continue to return their blood to the supracardinals, for the latter exist as independent venous trunks.

Because the three divisions in the transformations of the supracardinal lines coincide perfectly with, or better map out, three well-defined regions in which the history of the thoracic duct is enacted, the terms, pre-, post-, and supracardinal divisions are fully as applicable here, the events in this history occurring immediately along the pre-, post-, and supracardinal veins, respectively.

(A) *Precardinal division.* In 17 to 19 mm. embryos the anterior segment of the left supracardinal line, originally a simple longitudinal channel as in the 15 mm. embryos, has been transformed into an intricate plexus the roots of which now appear as numerous dorsal tributaries of the internal and common jugular and precardinal veins. The branches of these tributaries extend dorsad and vascularize the areas on both sides of the sympathetic nerve trunk, but in number and complexity the internal branches exceed the external ones. As will become evident later, an important distinction is potentially present between these two sets of branches, and, although the lack of differentiation at this stage would not warrant the use of specific terms, in the light of future events they may be described as precardinal veno-lymphatics, lying internal and mesial (6a, figs. 1 and 28), and precardinal segmental veins (8), functionally related to the ter-

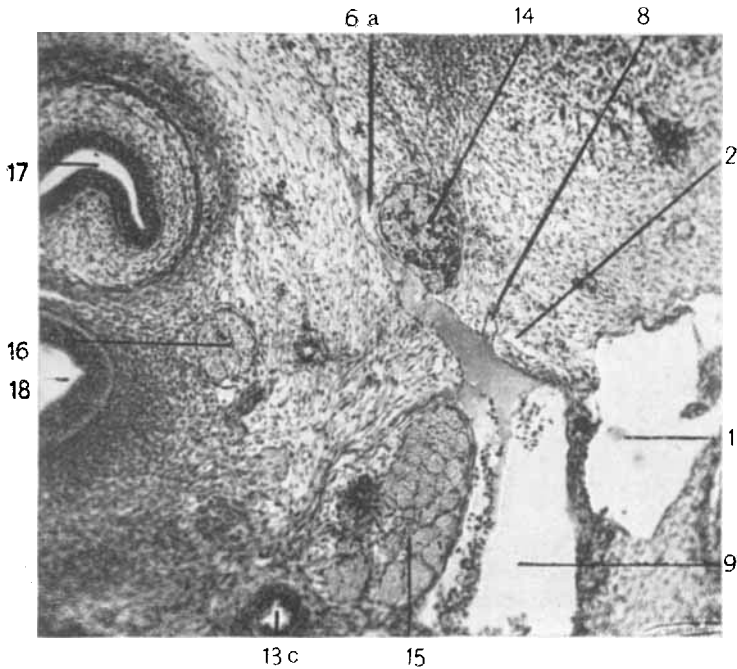


Fig. 1 Transverse section through the left jugular lymph sac region in a 19 mm. pig embryo (series 168, slide 16, section 16), $\times 100$. 1, lymph sac; 2, thoracic duct approach; 6a, precardinal veno-lymphatic; 8, root of a precardinal segmental tributary; 9, internal jugular vein; 13c, carotid artery; 14, sympathetic nerve trunk; 15, vagus; 16, recurrent laryngeal nerve; 17, oesophagus; 18, trachea. (Reconstruction, fig. 28.)

ritory lateral and dorsal to the sympathetic trunk. In figure 28, which represents a reconstruction of a 19 mm. embryo, these two kinds of tributaries (6a, 8) and their relations to the neighboring structures can be clearly distinguished.

Continued back from the terminals of the precardinal veno-lymphatics (6a, fig. 28) is a vessel which passes obliquely over the aorta and oesophagus to enter the right postcardinal vein at the level of the Cuvierian ducts (7, fig. 28). Although it is morphologically a part of the precardinal veno-lymphatics, it will often be treated separately and called the 'oblique vessel' on account of its diagonal course. The simplicity and size of this vessel varies with the individual, but it is a constant factor in all of the early

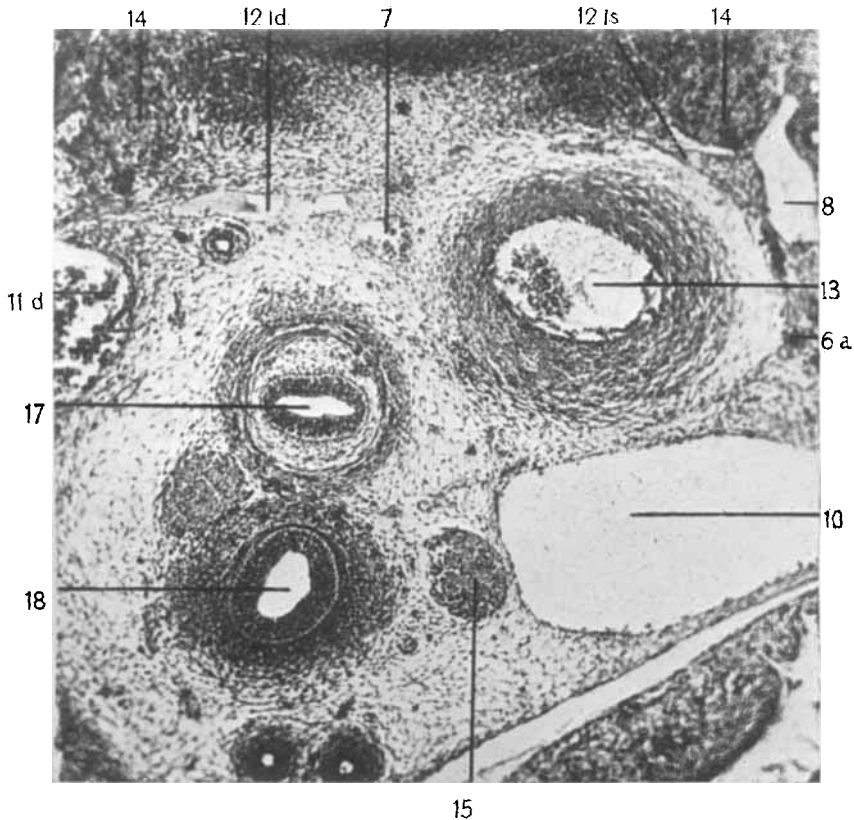


Fig. 2. Transverse section taken near the level of the left Cuvierian duct in a 17 mm. embryo (series 167, slide 15, section 22), $\times 100$. *6a* and *7* (oblique vessel), precardinal veno-lymphatics located in the positions of the later right and left thoracic ducts respectively; *8*, dorsal segmental vein; *10*, left precardinal; *11d*, right postcardinal; *12ld*, *12ls*, right and left supracardinal lines; *13*, aorta; *14*, sympathetic nerve trunk; *15*, vagus; *17*, oesophagus; *18*, trachea.

stages of the thoracic duct history. In figure 2,²¹ it (*7*) is illustrated in section, taken just in front of its junction with the middle segment of the right supracardinal line, which in this embryo (17 mm.) had not yet fused throughout its entire extent with the right postcardinal.

²¹ The image of the sections being reversed by the microscope lens, the structures of the right side of the embryos are seen on the left side in the microphotographs, and vice versa. However, in the dorsal views of the reconstructions and in figure 13 the right and left sides correspond with the respective sides of the page.

(B) *Postcardinal division.* Small venous derivatives are formed by a process analogous to fenestration along the mesial border of the postcardinal (6b, figs. 12, 28 and 32) of each side and apparently always in the line of fusion of this vein with the middle segment of the originally independent supracardinal channel, thus suggesting their derivation from the supracardinal system also in this division. During their development consecutive ages may be distinguished among them by the amount of individuality they manifest. Some are merely little bulging irregularities in the circumference of the parent vein, others describe the first step of separation by the presence of thin strands or partitions, and still others are complete throughout a number of sections but open to the veins at one or both ends. Being in the direct axis of the precardinal veno-lymphatics and homologous with them, these venous spurs or derivatives may be called the postcardinal veno-lymphatics. Ordinarily they are exhibited more distinctly on the right side. Later they constitute longer and shorter venules (6b, figs. 29, 32) parallel to the postcardinal, but they are never quite independent of the latter, remaining joined to it here and there until their reduction when they break up into degenerating segments and disappear in the mesenchyme. Such a final procedure will become clearer in the consideration of the second or transition phase.

(C) *Supracardinal division.* In the region of the mesonephroi, plexuses of vessels spring from the ventral aspect of the supracardinal veins, and in some embryos they become so extensive as to encompass the aorta almost completely. For this reason and the fact that they are the equivalents of the anterior veno-lymphatics in function, they were named the supracardinal peri-aortic veno-lymphatics. A further description of them would be superfluous considering their clearness in the accompanying microphotograph and reconstruction (6c, figs. 3 and 32).

Caudally, at the level of the superior mesenteric artery, subsidiary channels arise from the supracardinals ventro-medially, become more and more plexiform, and approach one another from both sides to anastomose in the area dorsad of the aorta (6c, fig. 4). Since eventually they will be concerned in the pro-

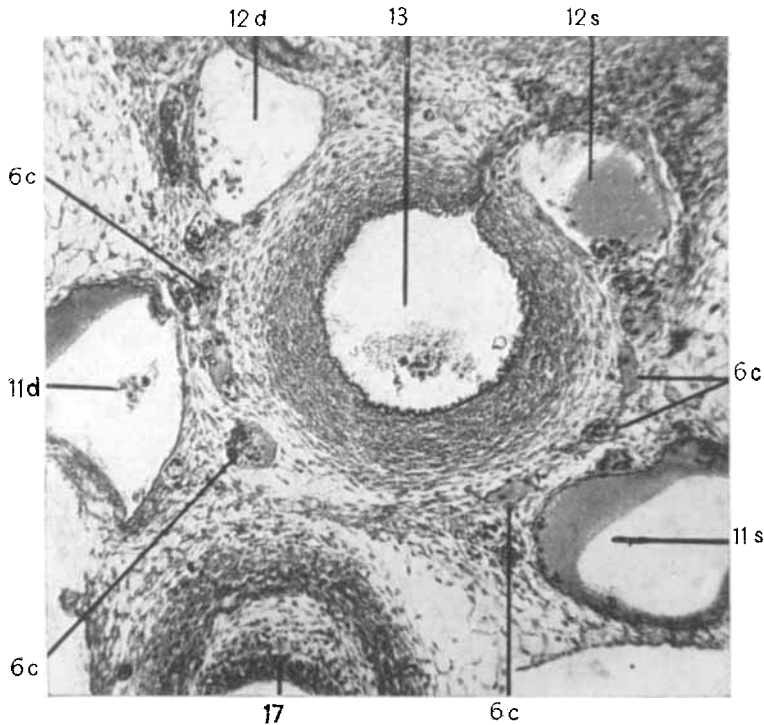


Fig. 3 Transverse section through the region just in front of the mesonephroi in a 19 mm. embryo (series 168, slide 23, section 17), $\times 150$. *6c*, supracardinal periaortic veno-lymphatics in the positions of the later right and left thoracic ducts; *11d*, *11s*, right and left postcardinals; *12d*, *12s*, right and left supracardinals; *13*, aorta; *17*, oesophagus. (Reconstructions, figs. 28 and 32.)

duction of the most posterior segment of the thoracic duct, or cisterna chyli, they may be labelled posterior supracardinal veno-lymphatics.

Situated topographically in the pathway of the future thoracic duct, all of the veno-lymphatics mentioned in the three divisions would be said to give rise to it directly, or to be transformed into it, were the second or transition phase of its history disregarded. This leads up to the suggestion that Lewis' and temporarily also McClure's multiple endothelial-lined anlagen derived from the veins along which they lie are nothing more and nothing less than the veno-lymphatics or precursors of the thoracic duct as deline-

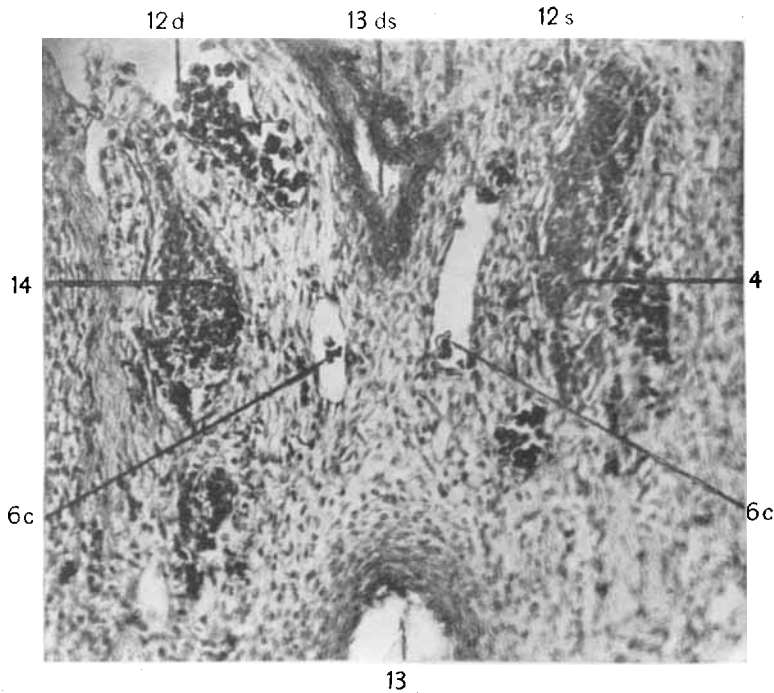


Fig. 4 Transverse section taken near the level of the superior mesenteric artery in a 20 mm. embryo (series 194, slide 40, section 16), $\times 200$. *6c*, posterior supra-cardinal vno-lymphatics, the precursors of the subsequent cisterna chyli; *12d*, *12s*, right and left supracardinals; *13*, aorta; *13ds*, dorsal segmental artery; *14*, sympathetic nerve trunks.

ated by the writer. According to the descriptions, their character and derivation in the rabbit and cat embryos accord accurately with their tendencies in the pig embryos and allow of no other conclusion than that they are homologous structures; for not only do they occupy identical positions but they also have their sources in the same venous trunks. This similarity is even more strikingly emphasized in somewhat older pig embryos where they are on the verge of evanescence and break up into isolated segments.

Until they have reached their culmination these vno-lymphatics can be followed and reconstructed with remarkable ease and accuracy. Their caliber or lumen is as uniform as a plexiform condition permits, and constrictions, irregular shrinkage, collapse,

or any other characteristic which might suggest artifacts due to the action of the preservative are nowhere in evidence. Their endothelium is tense and clear and the enclosed blood corpuscles take a clean and transparent stain.

During the veno-lymphatic phase the mesenchyme is evenly woven and fairly compact, and lymphatic anlagen, or conspicuous and discontinuous spaces, are not present throughout the entire thoracic duct area. Nor can vacuities, fissures, or rents be observed which might be ascribed to unequal fixation, or which differ in any way from the regular intercellular lacunae of the tissue reticulum.

2. The transition phase (19-22 mm. embryos)

Having arrived at the second phase of the thoracic duct history, we are confronted with the paramount point at issue, namely, the source and formation of lymphatic anlagen. A critical examination of the stages belonging to this period will disclose three facts of major importance which are impressed upon the observer firmly and constantly. In the first place the longer portion of the thoracic duct anlage arises discontinuously from mesenchymal lymphatic spaces, but it may present various aspects according to its genetic levels or to the degree of relation it bears to immediately surrounding structures, that is, it may be instituted either by extra-intimal spaces, or by spaces in the near environment of the veno-lymphatics but not in contact with them. Secondly, the wall of the entire thoracic duct is a differentiation in situ from the mesenchyme. Thirdly, the development of the thoracic duct proceeds in a general antero-posterior direction; for example, in series 194, a 20 mm. embryo, lymphatic development has made considerable progress in the anterior or precardinal division, has just been initiated in the middle or postcardinal division, and is totally lacking in the supracardinal division.

(A) *Precardinal division.* In the collection of pig embryos studied by the writer the first instances of incipient lymphatic anlagen are found in series 168 (19 mm.) along a limited extent of the precardinal veno-lymphatics as far back as the anterior half of the oblique vessel (fig. 28). Located in the path of the poten-

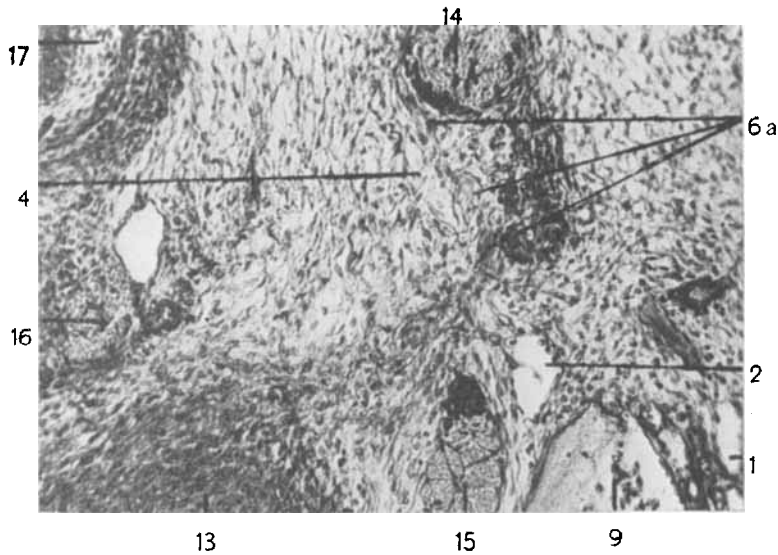


Fig. 5 Transverse section through the left lower cervical region in a 19 mm. pig embryo (series 168, slide 17, section 9), $\times 150$. 1, posterior tip of the jugular lymph sac; 2, thoracic duct approach; 4, lymphatic space formed against the intima of a precardinal veno-lymphatic, (6a); 9, internal jugular; 13, wall of the aorta; 14, sympathetic trunk; 15, vagus; 16, recurrent laryngeal nerve; 17, oesophagus. (Reconstruction, fig. 28.)

tial thoracic duct there is a distinct vacuolation of the tissue as shown in figure 6 (4), medial to a veno-lymphatic tributary (6a). Although exceedingly difficult to describe, these vacuoles or spaces are seen to stand out conspicuously, perhaps by the greater clearness of their cavities, even if their boundaries are ill-defined, and by their preponderance in size over the more regularly disposed openings of the ground substance. In a section taken further forward another lymphatic anlage occurs as a large lenticular space (4, fig. 5) against the intima of a retrogressive veno-lymphatic (6a), but it can be distinguished definitely only in two sections. In the same figure the thoracic duct approach (2) is indicated. This ends blindly but is followed shortly by large clear-cut spaces (4) which are in no way continuous with it (fig. 28). Similar and widely separated anlagen occur along the anterior or upper half of the oblique vessel (7) and immediately ventral to it.

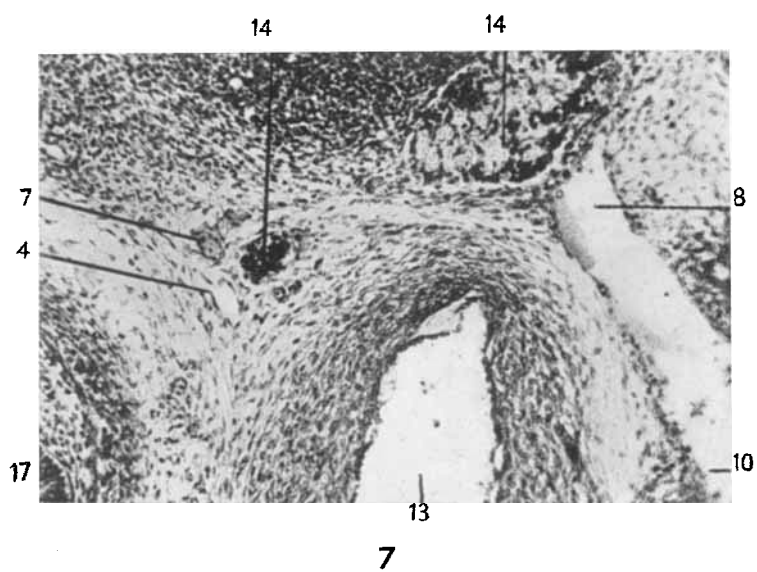
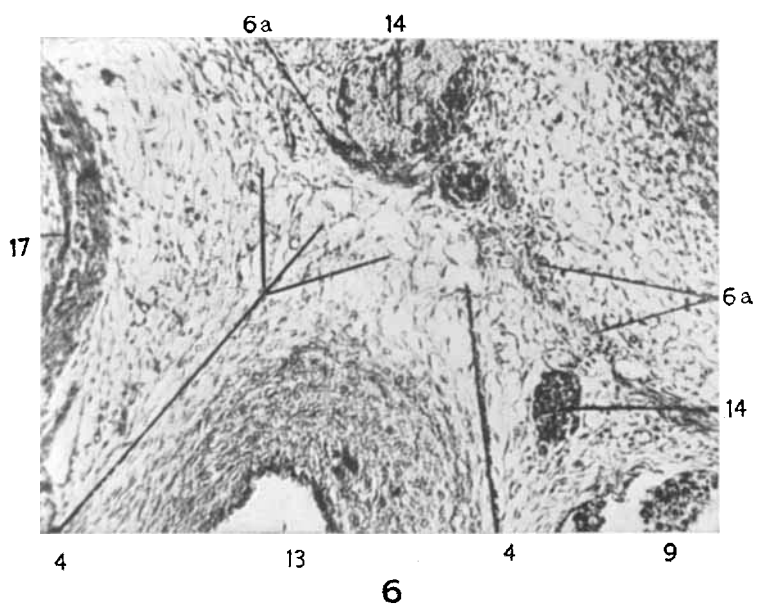
Those that were not hidden in the dorsal view of the reconstruction are illustrated in the drawing (4, fig. 28). These spaces are short capsule-shaped vesicles running through four or five sections and are quite distinct in outline, so that their extent and discontinuity can be easily determined. A transverse section of one of them is represented in figure 7 (4). The nicety with which it can be discriminated from the interstices of the surrounding mesenchyme and from the lumen of the neighboring venule which is filled with blood removes all doubt as to its reality and individuality.

On the evidence of the few microphotographs inserted here, the presence of the spaces pointed out as lymphatic anlagen can not be denied. In the embryo just described they exist only in the foremost region of the thoracic duct line; outside of this path there are no spaces which might invalidate the significance of these anlagen.

Extra-intimal replacement of evanescent venous channels, a method of lymphatic development just hinted at in figure 5 (4) finds a most convincing expression in series 194 (20 mm.), where the entire anterior precardinal veno-lymphatic plexus (6a) is being replaced by large perivenous spaces (3, fig. 29). The veno-lymphatics designated have been detached from their parent veins and thus abandoned by the systemic blood vascular circulation, and they now display successive steps towards complete collapse. The section represented in figure 8 is typical, for most of the other sections taken at random from this region offer equally decisive illustrations. Besides revealing the shriveled and discarded venous intima and its gradual disintegration in the

Fig. 6 Transverse section through the left lower cervical region in a 19 mm. pig embryo (series 168, slide 18, section 4), $\times 150$. 4, vacuolation of the mesenchyme in the formation of lymphatic spaces; 6a, precardinal veno-lymphatics, beginning to degenerate; 9, internal jugular; 13, aorta; 14, sympathetic nerve and branches; 17, oesophagus. (Reconstruction, fig. 28.)

Fig. 7 Transverse section through the left upper thoracic region in a 19 mm. pig embryo (series 168, slide 19 section 1), $\times 150$. 4, lymphatic space in the line of the future right thoracic duct; 7, oblique vessel; 8, dorsal segmental vein; 10, left precardinal; 13, aorta; 14, sympathetic nerve trunk and branch; 17, oesophagus. (Reconstruction, fig. 28.)

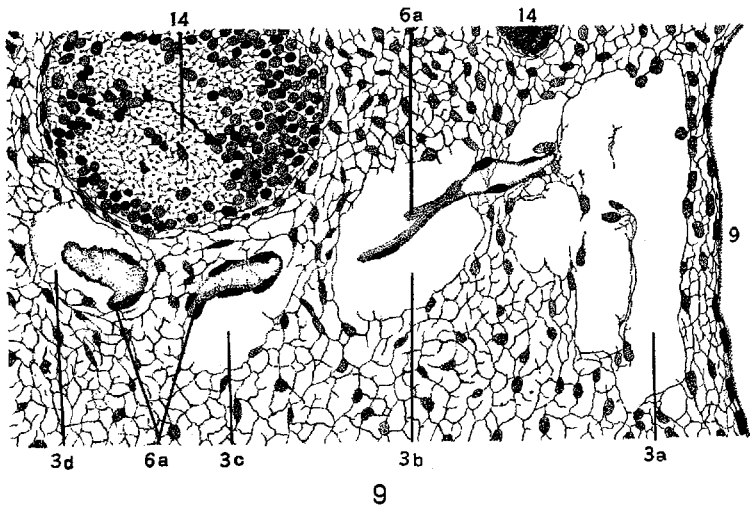
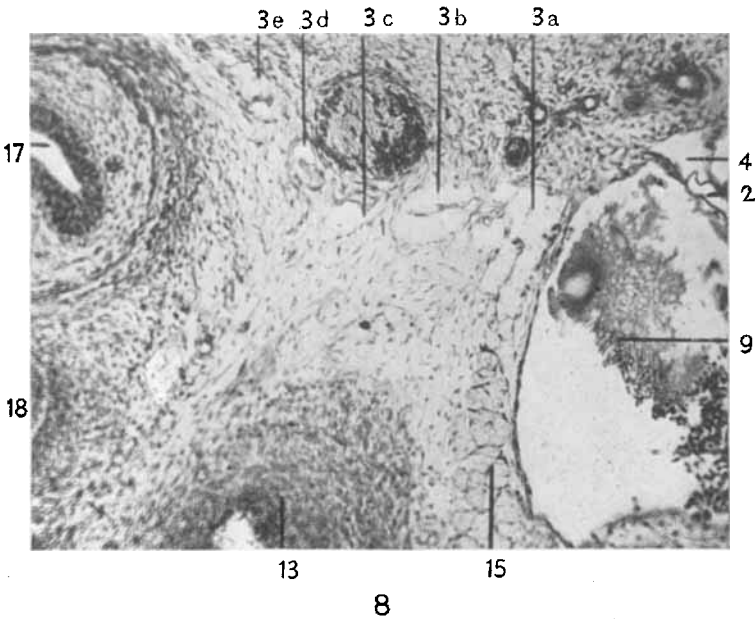


lumina of the replacing lymph vessels (*3a, b, c, d, e*), the photograph suggests the method by which the latter enlarge, as well as their mode of origin. The anlage at *3e* (fig. 8) presents an excellent initial stage in which the endothelium of the blood channel has receded from the original circumference and two small mesenchymal vacuoles have appeared one on each side of the points of weakness. On the other hand, the anlage at *3a*, the channel of the lymphatic plexus nearest to the lymph sac and internal jugular vein (*9*), is very large and irregular and has increased in size obviously by the coalescence of several closely-crowded spaces, as indicated by the extremely ragged periphery of its lumen and the remnants of tissue traversing it.

The lymphatic plexus just described is widely confluent with the jugular lymph sac (fig. 29) through the thoracic duct approach (*2*). The reader will recall that this structure, the approach, is a part of the sac and has its origin with it. In the preceding or veno-lymphatic phase it exists normally as one (fig. 28) or two, and sometimes three, short prolongations between the roots of the dorsal tributaries of the jugular vein. At the time when lymphatic spaces are appearing along the precardinal veno-lymphatics, the sharply defined venous endothelium of the approach (*2*, fig. 8) retracts from its former circumference, evidently as the result of a stagnation in its growth, and becomes surrounded by a clear and larger cavity (*4*) which is lined with ordinary unmodified mesenchymal cells, the progenitors of the

Fig. 8 Transverse section through the left lower cervical region in a 20 mm. pig embryo (series 194, slide 23, section 21), $\times 120$. *2*, thoracic duct approach, and its original venous intima replaced by a large space (*4*); *3a, b, c, d, e*, lymphatic plexus replacing as extra-intimal spaces the precardinal veno-lymphatic plexus; *9*, internal jugular; *13*, wall of the aorta; *15*, vagus; *17*, oesophagus; *18*, trachea. (Reconstruction, fig. 32.)

Fig. 9 An accurate camera lucida drawing of a highly magnified area of the section represented in figure 8, $\times 266$ (reduced from $\times 400$). *3a, b, c, d*, cross-sections of the extra-intimal lymphatic plexus replacing the anterior precardinal veno-lymphatics; the absence of any specialized endothelium in the wall of the lymphatic spaces is plainly evident; the strands of mesenchyme jutting into their cavities suggest the breaking down of contiguous spaces in the formation of the lymphatic plexus; *6a*, the collapsed venous intima of the abandoned veno-lymphatics; *9*, wall of the internal jugular; *14*, sympathetic nerve trunk and branch.



later more specialized lymphatic endothelium. Synchronously, the reorganized approach becomes confluent with the contiguous lymphatic spaces of the duct anlage by the breaking down of tissue partitions and septa between them. The vestiges of the old vascular intima may persist throughout a number of stages clinging to the wall of the new cavity, but it gradually fades and vanishes as the thoracic duct acquires more and more of its functional activity.

Histologically, all incipient lymphatic anlagen, whether they are spaces independent in position or spaces following, transforming and expanding the discarded pathways of redundant venous channels, are decidedly different from either an active vein or a mature lymphatic. They lack definition and possess vague and undifferentiated outlines; for the cells of their walls are not arranged in that end-to-end fashion so characteristic of vascular endothelia. Instead, many instances were observed under strong magnification where the tissue cells in their longest diameter stand perpendicular to the periphery of the anlagen and project far into the lumen with their cytoplasmic filaments, a condition unquestionably brought about by the addition or fusion of contiguous spaces. Figure 9, which is an accurate camera lucida drawing of a highly enlarged portion of the area pictured in figure 8, should be carefully examined as depicting clearly the features here mentioned. With the most critical observation one is not able to detect differences at this stage between those cells constituting the boundaries of the lymphatic anlagen (*3a, b, c, d*) and those of the mesenchymal reticulum, either in regard to their arrangement and shape or to their staining attributes.

While there is sufficient evidence for the atrophy of venolymphatics (*6a*, fig. 9) in their elimination from the blood stream and the recession of their intima, further evidence is revealed at this stage by their reaction to the stain. Treated with heamatoxylin and orange-G, the defunct intima takes an opaque brownish color as compared with the transparency of a functional vessel. Their lumina also contain the débris of blood cells. That these conditions are not induced by poor fixation is evinced by the normal appearance of the veins in the immediate vicinity. For

example, the endothelial lining of the jugular vein (9, fig. 9) stains clearly and is sharply defined, and there are no spaces external to it.

Extra-intimal replacement occurs only among those venous channels which are immediate antecedents of lymphatics in time and place. This is attested by the fact that other veins in their atrophy are not surrounded by spaces but disappear by the gradual reduction of their caliber, or by a process of constriction cutting the channel into segments which become smaller and smaller to form dense masses or islands of cells ultimately to be lost in the mesenchyme. As an instance of such a process may be described the reduction and dismemberment of a large portion of the plexuses uniting the original supracardinal lines (25, figs. 28, 29 and 30). The writer has often noticed these temporary venous plexuses in the various stages of degeneration. In 20 mm. pig embryos, for example, such retrogressive venous channels frequently reveal constrictions at irregular intervals along their course. In later stages they begin to break up into segments, which at first, however, are still connected with one another by densely staining cell-strands, the remains undoubtedly of the endothelial walls, and in this way indicate the pathways of the originally functional vessels. The segments at the beginning possess a distinct cavity or lumen, but subsequently the cavity becomes filled up with a solid cell mass which is apparently due to a proliferation of the former endothelial or lining cells. Such cell aggregations gradually vanish in the mesenchyme perhaps by the regression of their elements to undifferentiated tissue. In other words, the cells which at one time functioned as the limiting walls of a haemal vessel, after the elimination of such a vessel from the blood channel system, lose their specialized characteristics and possibly return to the mesenchyme by assuming the qualities and functions of the ordinary tissue cell. By comparing the reconstructions illustrated in figures 28 and 35, it is seen that the venous plexuses which are so profusely developed in the 19 mm. embryo have almost entirely disappeared in an embryo of somewhat greater length. In the stained sections of the latter, however, some of the dense cellular masses are still visible here and there in

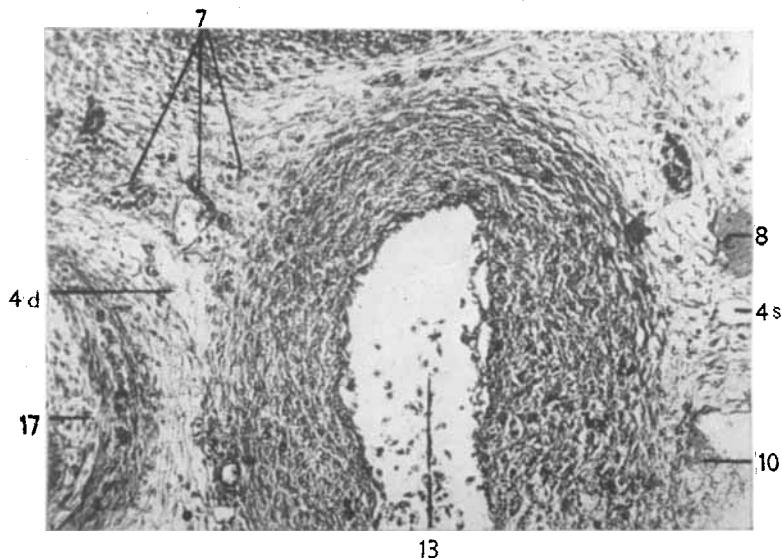


Fig. 10 Transverse section through the upper thoracic region in a 20 mm. pig. embryo (series 194, slide 26, section 21), $\times 150$. *4d*, potential lymphatic anlage in the right thoracic duct line; *4s*, lymph space in the left duct line; *7*, oblique vessel, here plexiform; *8*, dorsal segmental vein; *10*, left precardinal; *13*, aorta; *17*, oesophagus. (Reconstruction, fig. 29.)

the territory formerly traversed by the temporary venous plexuses.

In series 194 (20 mm. embryo) the atrophy of the oblique vessel (*7*) and its replacement by lymphatics has begun anteriorly, where it is supplanted to a greater or lesser degree by perivenous spaces (*4d*, fig. 29) which reflect the features of those further forward but are smaller and less conspicuous by reason of the greater simplicity of the vessel replaced. Along the second half of its course lymphatic anlagen are met with which are not formed in such intimate relations but lie ventral to and not closely in contact with it. Figure 10 illustrates a section from this region. The clear area (*4d*) subjacent to the plexiform oblique vessel (*7*) in the triangular or wedge-shaped territory between the aorta and the oesophagus unhesitatingly suggests a thoracic duct anlage in the making, as an examination of its character and a comparison with later stages seem to affirm. That this clear area is less com-

paet in texture than the tissue surrounding it is evident at a glance. Within it the cells are fewer in number, and the tissue fibrils, which appear to be more delicate than those of the mesenchymal reticulum elsewhere, enclose larger interstitial openings or tissue spaces. In longitudinal extent this potential duct-anlage, as we may call it, occurs along a considerable portion of the posterior half of the oblique vessel but varies from section to section in its definition. Often, distinct vacuoles appear suddenly in it, continue through several sections, and as suddenly disappear. Only these 'centers of space-formation,' however, can be reproduced in a model (fig. 29), the remainder of the anlage being as yet too indefinite to warrant reconstruction.

The atrophy of the oblique vessel in this specimen, series 194, serves also as a typical example of the atrophy of all temporary or redundant venous pathways, both of the veins which are the immediate antecedents of lymphatics and of those veins which are not so intimately associated with the development of a lymphatic channel. In the embryo from which figure 28 was drawn the oblique vessel (?) is still complete and continuous with the main venous trunks; in figure 29, on the other hand, it is seen to be broken up into irregular segments, some of which are replaced by extra-intimal spaces, and others gradually diminish in size and disappear in the mesenchyme adjacent to an incipient lymphatic anlage but with an appreciable amount of tissue between them. Thus it is evident that the vanishing segments of redundant venules and the growing segments of potential lymphatics may exist side by side in the same section. But now it may be asked, what distinguishes the one from the other, what basis is there for naming this one a lymphatic rudiment and that one a venous remnant, and how can both be followed to their ultimate fates without confusion? The distinction between these two vascular structures can easily be recognized beyond the possibility of a doubt. A lymphatic segment, here specifically a thoracic duct anlage, is invariably characterized by a very clear lumen and, if it is in the formative stage, by the absence of a clear-cut and specialized lining, as shown in the microphotographs already mentioned (figs. 6, 7, 8 and 10); whereas, the segments of a ven-

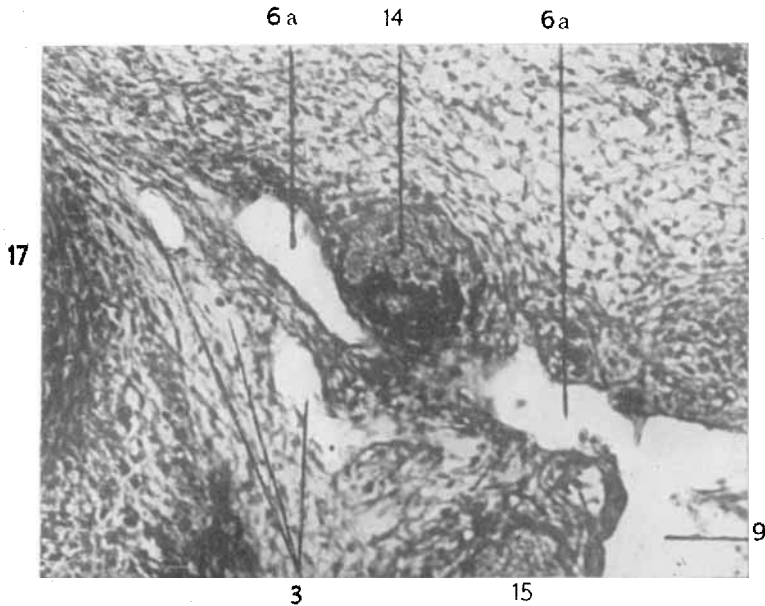


Fig. 11 Transverse section through the left lower cervical region in a 20 mm. pig embryo (series 193, slide 14, section 20), $\times 200$. This figure shows the anterior lymphatic plexus (3) of the thoracic duct not developed from extra-intimal spaces, but formed in the mesenchyme independently of the precardinal veno-lymphatics (6a); 9, internal jugular; 14, sympathetic nerve trunk; 15, vagus; 17, oesophagus. (Compare with fig. 8.)

ous channel undergoing atrophy almost constantly contain numerous blood corpuscles, or the deeply staining debris of cellular material (figs. 6, 7, 8 and 10), and without exception possess a sharply defined endothelium.

To strengthen the evidence of figures 6, 7 and 10, illustrating the formation of lymphatic spaces without the features of extra-intimal replacement, figure 11 from series 193, another 20 mm. embryo, may be introduced here as a very definite specific case to show conclusively that such replacement of venous channels is not inevitably a requisite in the genesis of lymphatics, and that its absence is quite as common as its occurrence. In this embryo the most anterior portion of the thoracic duct anlage (3) has not been modelled, so to speak, upon the anterior precardinal veno-

lymphatic plexus (6a), but has formed a plexus independently of it in a position closely parallel and medial to it. Histogenetically, however, this lymphatic plexus is the same as that of the preceding embryo (series 194, figs. 8, 29), but the redundant venous lines of which it seems a shadow picture are still quite regular, although occasional constrictions do suggest their decline and subsequent atrophy.

The foregoing descriptions of the relation between the development of lymphatic anlagen and the degeneration of the veno-lymphatics determine clearly that this relationship possesses only secondary significance. These two processes are necessary events in the embryonic history, and if they occur simultaneously and the abandoned venous derivatives occupy a position identical with that of the potential thoracic duct, then the anlagen of the latter will follow the path of least resistance or, better, follow a hydrostatic tendency and collect around their weakened intima and cause its collapse. If, however, these venous lines do not lie in the pathway of the duct, or if their degeneration is slightly retarded so that they are still joined to the systemic blood circulation, and are under the influence of its pressure and their intima is still tense, then the lymphatic anlagen will arise independent of any contact with them.

Fluctuations in the amount of progress attained by the right and left branches of the duct at any given moment during the critical stages of their development are not infrequent; indeed there appears to be a reciprocal action, for when one is large and long, or well represented in the number of its anlagen, the other is only scantily represented. All transition stages show these variations to a greater or lesser degree, but especially favorable examples are series 103 and 191 (21 mm. embryos), which can be regarded as complements of each other, the former being prominently dextral, and the latter sinistral in lymphatic growth. In series 103 the right limb of the duct extends as an unbroken channel far back into the postcardinal division of the thoracic region, but the left limb is just visible in its earliest rudiments as a few minute and isolated spaces. In series 191, on the contrary, we meet with a complete reversal of conditions so that the descrip-

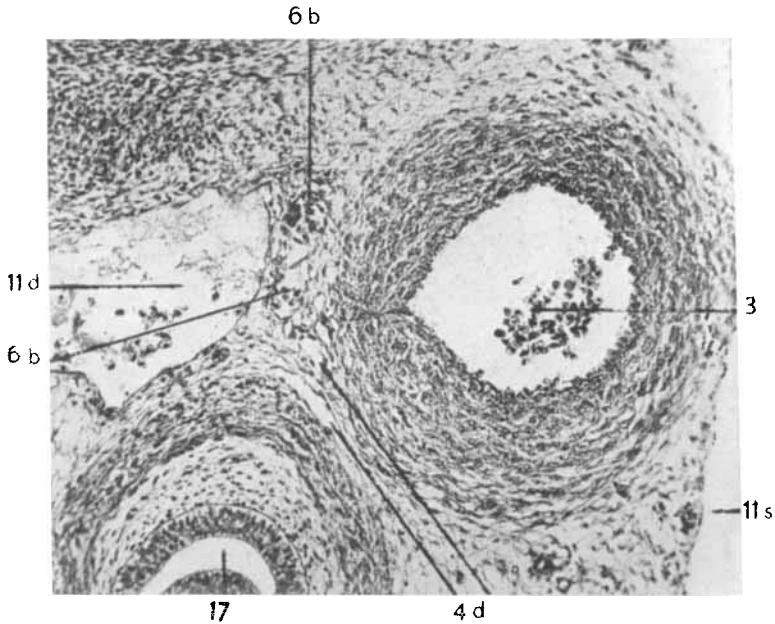


Fig. 12 Transverse section taken immediately posterior to the left Cuvierian duct in a 20 mm. pig embryo (series 194, slide 29, section 4), $\times 150$. *4d*, isolated lymphatic spaces in the right thoracic duct line; *6b*, postcardinal veno-lymphatics; *11d*, *11s*, right and left postcardinals; *13*, aorta; *17*, oesophagus. (Reconstruction, fig. 29.)

tion of the right thoracic duct limb of series 103 applies with almost equal force to the left limb of this embryo, and vice versa.

(B) *Postcardinal division.* In the region of the postcardinal veno-lymphatic channels the discontinuity of incipient lymphatic Anlagen and their origin in situ from the mesenchyme can be plainly demonstrated. In a destined course and at intervals, though not metamerical in sequence, spaces and fissures are present in the evenly meshed tissue along the channels mentioned. For instance, they can not be mistaken in figure 12 (series 194–20 mm.), where two of them are shown as clear crevice-like spaces (*4d*) quite sharply defined, while several others are invading the environs of the veno-lymphatics (*6b*) and are beginning to enclose one of their smaller branches. In other sections this process of circumclusion has proceeded further, but in every case venous

can readily be distinguished from non-venous, the first by the presence of blood cells and heavier walls, and the second by clear cavities and more delicate walls.

In series 23a (23 mm. before fixation), an embryo somewhat older than series 194, the discontinuous lymphatic spaces of the postcardinal division are much larger and more conspicuous in the figures. Series 23a is from the Johns Hopkins University Embryological Collection and was injected and prepared by Professor Sabin. The fixation and preservation of its tissue is excellent, and the injection was successfully carried out and is as perfect as a developing lymphatic channel permits. It was sent to the Princeton Laboratory as a crucial stage in favor of the 'centrifugal growth' theory of the origin of the thoracic duct, and therefore the evidence derived from it will seem more significant perhaps than that derived from any other series described. For this reason it will here be dealt with in greater detail.²²

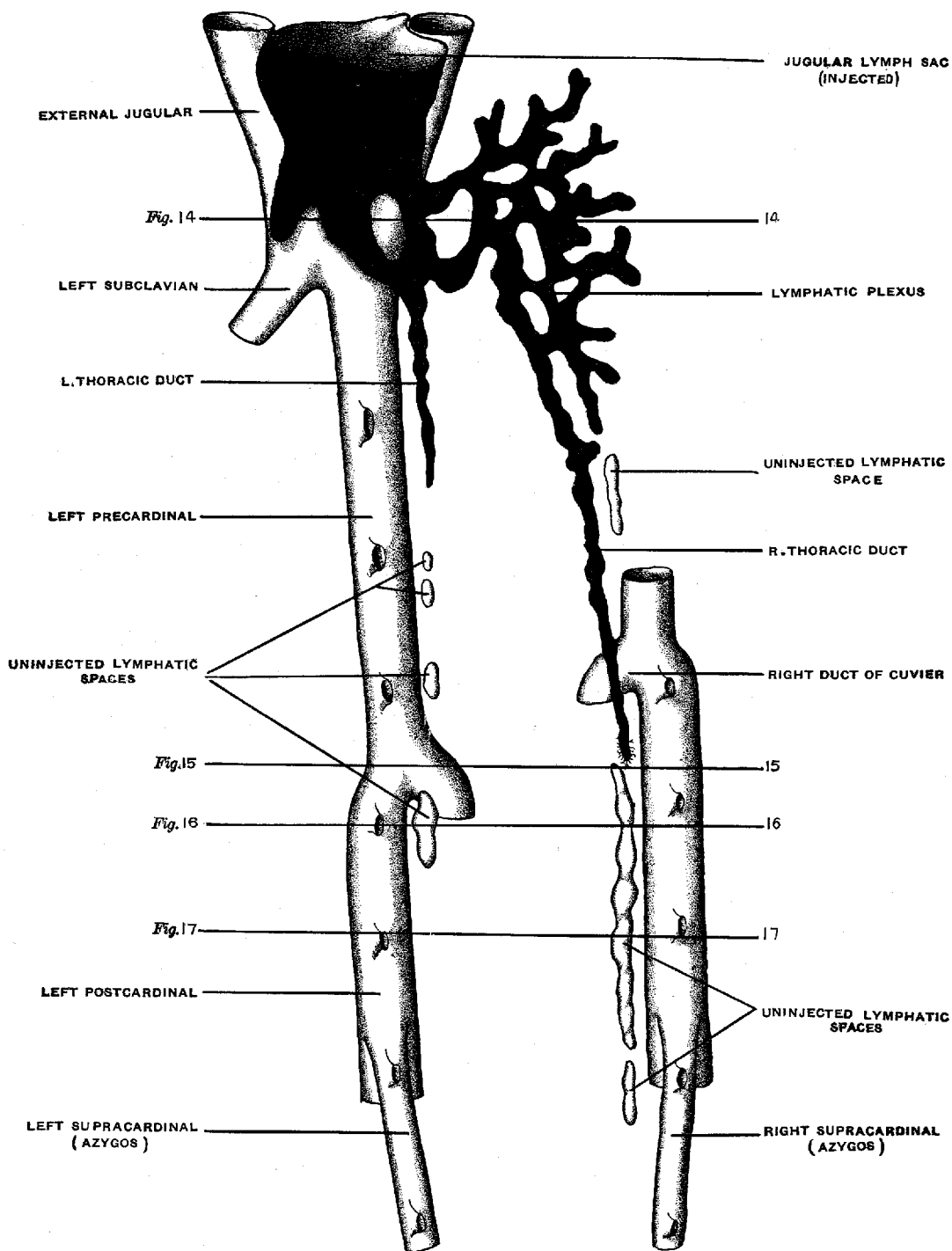
The right and left limbs of the thoracic duct anlage which are joined to the jugular lymph sac are continuous channels as far back as the points X, X on the drawing, figure 30, and being injected are shown in black on the diagram, figure 13. The foremost portion of the embryonic duct is in the form of a broad and extensive lymphatic plexus, a typical section of which is reproduced in figure 14 to illustrate the large size of the channels (*β*) and the extravasations or leakage (*Ex*) of the injection mass into the surrounding mesenchyme. To consider the right limb of the duct first, the injected vessel, or vessel confluent with the lymph sac, extends unbrokenly backward and dextrad towards the right postcardinal vein as a slender channel, subjacent to the oblique vessel (*7*, fig. 30) and at intervals applied to its wall, and termi-

²² The description of series 23a was presented by the writer before the last or 28th session of the American Association of Anatomists (1911) and published in the June number of the *Anatomical Record*, vol. 6, no. 5, 1912, as a part of a preliminary paper, entitled *The value of the injection method in the study of lymphatic development*. In this connection it is also well to call attention to Professor McClure's article, *A few remarks relative to Mr. Kampmeier's paper on the value of the injection method, etc.*, which appeared in the same number of the *Anatomical Record*, and which is a critical analysis of some of the papers published by Professor Sabin and Dr. Eliot Clark on the development of the lymphatic system.

nates just below the level of the right Cuvierian duct at *X*. There is also a long and spindle-shaped space which lies lateral to it and in the pathway of a tributary of the anterior lymphatic plexus were it prolonged downward, as suggested in figures 13 and 30

Passing to the postcardinal division (B, fig. 30) of this embryo, we meet with the most decisive evidence in favor of the non-venous origin of the thoracic duct, namely, a clear case of discontinuity in its anlage than which nothing could be more conclusive. Immediately following the injected portion of the right duct (*5d*) is a long fusiform mesenchymal space (*4d*), but in no way connected with it, as exemplified by the drawings, figures 13, 30 and 31 (ventral view), and the microphotographs, figures 15, 16 and 17 which represent transverse sections taken at this level. Especially the ventral view of the reconstruction (fig. 31) illustrates the abrupt break (*X*) in the duct anlage, the position of the terminal portion of the injected channel (*5d*), and the independent fusiform space (*4d*) and its longitudinal extent. The injected channel ends obscurely in a 'mossy' area produced by slight extravasations, the position of which is indicated at *X* in figure 15 just ventral to the broad lumen of the anterior tip of the independent space (*4d*). That there is absolutely no open communication between these two segments of the duct-anlage is strikingly confirmed by both observation and experiment. In the first place the most critical examination with the high powers of the microscope was not able to detect continuity, and secondly, not a particle of the injection mass was found to have entered the cavity of the blind fusiform space (*4d*), although the pressure of the injection was sufficiently great to produce the extravasations referred to above.

Fig. 13 A simplified or schematic drawing of an accurate reconstruction of the thoracic duct region in series 23a (Johns Hopkins University Embryological Collection) represented in figure 33. The lymph sac and the injected portion of the thoracic duct anlage were drawn in black; the uninjected lymph spaces are discontinuous but are located in the axes of the injected channels and consequently in the paths of the future complete thoracic ducts. The cross lines indicate the levels at which figures 14, 15, 16 and 17 were taken.



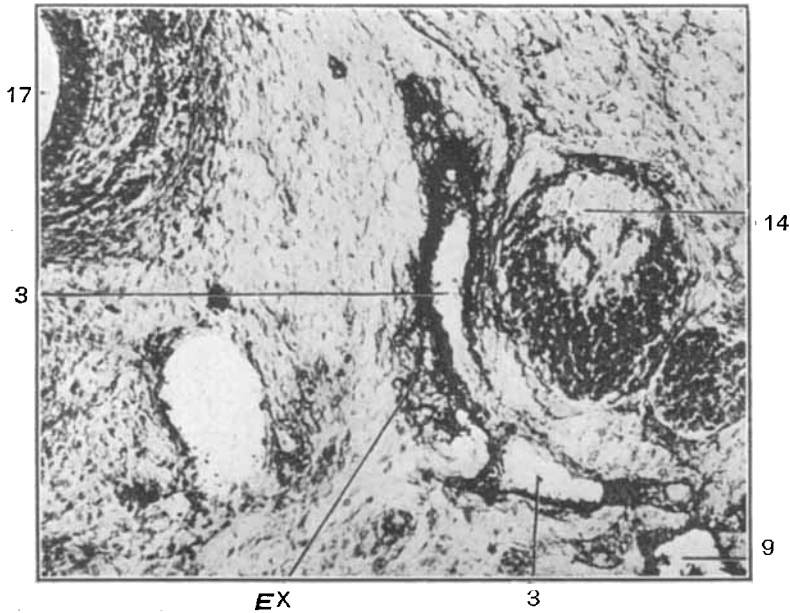


Fig. 14 Transverse section through the left lower cervical region in a 23 mm-pig embryo (series 23a, J.H.E.C., slide 21, section 16), $\times 200$. 3, anterior lymphatic plexus of the thoracic duct injected; *Ex*, extravasations of the injection substance into the surrounding mesenchyme; 9, internal jugular; 14, sympathetic nerve trunk; 17, oesophagus. (Reconstruction, fig. 30.)

The discontinuous fusiform lymphatic space (*4d*) is of considerable length, capable of being followed through thirty-seven sections (thickness of sections: 20 micra), and it is variable in diameter (figs. 31, 15, 16 and 17), at times being very broad and at other times narrow and not so sharply demarcated from the intercellular lacunae of the mesenchyme surrounding it. In form it is very irregular, and its lumen is often bridged by tissue strands of greater or lesser thickness which give to it a multilocular appearance as shown in cross-section in figures 16 and 17 (*4d*). This condition and the fact that it is bounded by ordinary mesenchymal cells supply strong proof against its venous origin. The difference between its lining and that of the neighboring venules (25) and veins is strikingly expressed even in figure 15, in which its boundary is quite regular and clear-cut but the greater delicacy

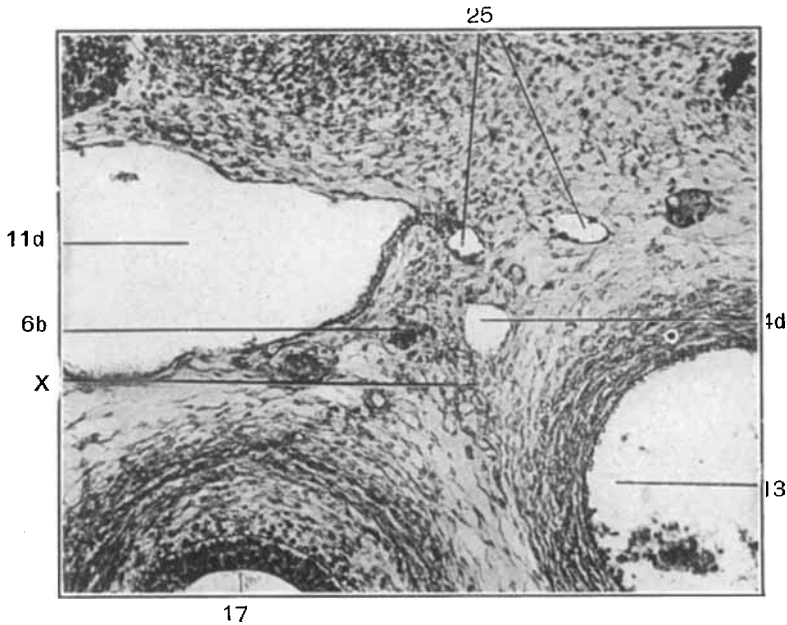


Fig. 15 Transverse section taken shortly beyond the right Cuvierian duct in a 23 mm. pig embryo (series 23a, J.H.E.C., slide 26, section 10), $\times 200$. *4d*, anterior tip of the long fusiform lymphatic space in the right thoracic duct line; *X*, position of extravasated particles from the injected portion of the right thoracic duct anlage; *6b*, postcardinal veno-lymphatic; *11d*, right postcardinal; *13*, aorta; *17*, oesophagus; *25*, venules or branches of the postcardinal. The more delicate lining of the lymphatic space as compared with that of the veins and venules can be clearly distinguished in the figure. (Reconstruction, fig. 30.)

of its wall can be distinguished without the least difficulty. Figure 17, again, illustrates the occasional circumclusion of the precardinal veno-lymphatics (*6b*) by this space and draws more plainly, perhaps, the distinction between lymphatics and venous channels, where the latter are replete with blood and possess sharply defined boundaries as compared with the often ill-defined outlines of the lymphatic space. Caudally this long space after a course which can be easily pursued through thirty-seven sections, as already stated, becomes more indistinct until it vanishes by the loss of its cavity in the confusion of the interstices of the tissue reticulum, but after a number of sections it is followed by a second space, which, though shorter and simpler (figs. 13 and 31),

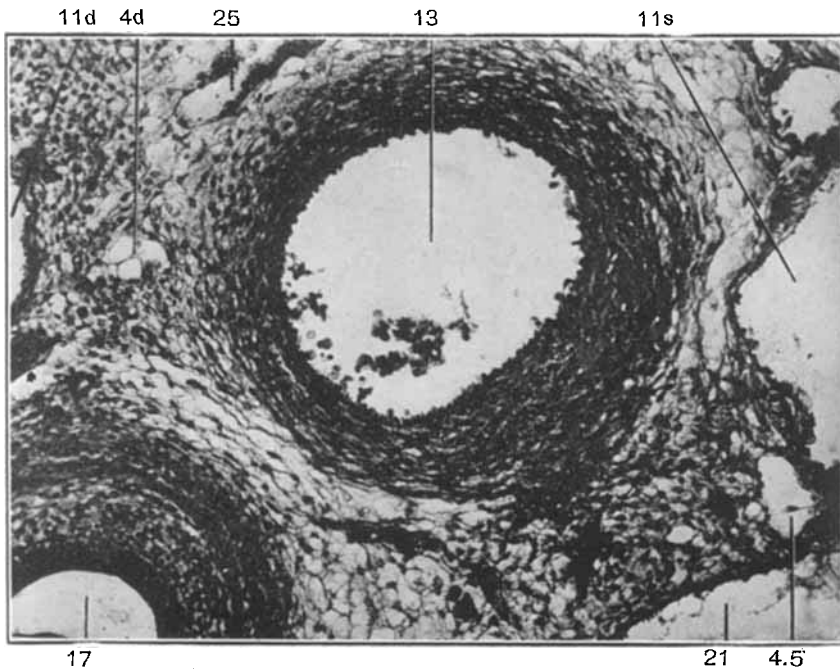


Fig. 16 Transverse section taken a few sections beyond the one represented by the preceding figure from a 23 mm. pig embryo (series 23a, J.II.E.C., slide 26, section 12), $\times 200$. 4d, long fusiform space in the right thoracic duct line, and mesenchymal bridges traversing its lumen; 4s, lymphatic space at the level of the left Cuvierian duct (21) and in the pathway of the left thoracic duct; 11d, 11s, right and left postcardinals; 13, aorta; 17, esophagus; 25, branch of the postcardinals. (Reconstruction, fig. 30.)

exhibits the same peculiarities of character. This again is followed by tissue which is still undifferentiated but coarsely reticulate and persistently suggests the potentiality of further lymphatic anlagen. Both of the spaces described and figured are situated in the axis of the injected channel and consequently in the axis of the ultimately complete thoracic duct.

On the left side in series 23a the principle of lymphatic development is the same and is expressed fully as well as on the right side. The injected segment of the left thoracic duct limb (5s, figs. 30 and 13) is much shorter than that of the right, but it is slender and often it can only be traced by a 'mossy' path due to

slight extravasations. At intervals beyond the farthest extent to which the injection mass has penetrated (*X*, fig. 30), and located in a line destined to become the pathway of the future thoracic duct, are a number of small blind mesenchymal vacuoles (*4s*), the largest one of which extends through eight sections at the level of the Cuvierian duct. Being hidden by the veins in a dorsal view of the reconstruction, their positions are indicated on the drawing by dotted circles (*4s*, fig. 30; see also fig. 13). The conspicuous size of the lumen of the last space (*4s*) and the mesenchymal strand bisecting it, as illustrated in the microphotograph, figure 16, require no further comment.

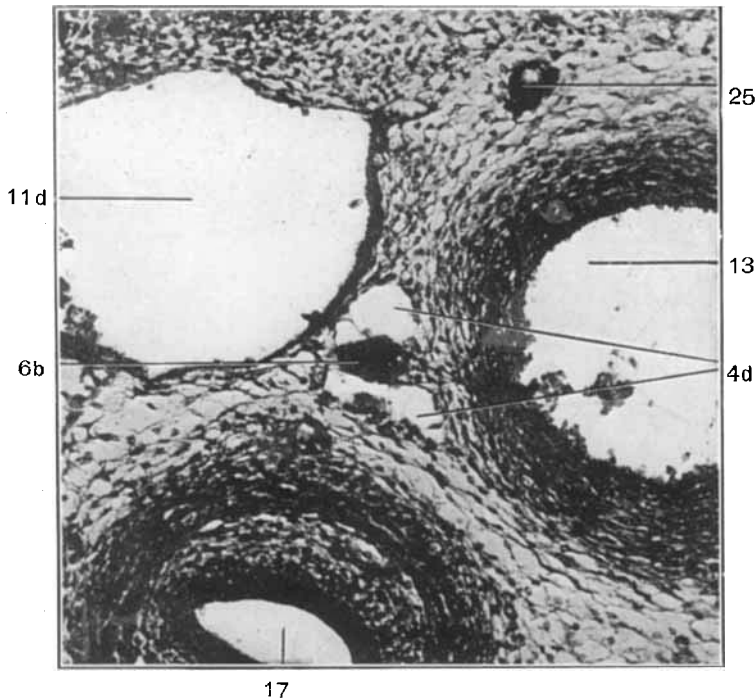


Fig. 17 Transverse section through the thoracic region in a 23 mm. pig embryo (series 23a, J.H.E.C., slide 27, section 18), $\times 200$. *4d*, long fusiform lymphatic space in the right thoracic duct line, and tissue bridges traversing its lumen; *6b*, postcardinal veno-lymphatic surrounded by this space; *11d*, right postcardinal; *13*, aorta; *17*, oesophagus; *25*, venule, branch of the postcardinal. (Reconstruction, fig. 30.)

In series 192, a 21.5 mm. embryo slightly older than the preceding embryo 23a, the right thoracic duct anlage (5*d*) extends as a continuous channel back to the point *X*, as indicated on figure 35. On comparing this with figure 30, it can be observed that the longer portion of the postcardinal division of the right anlage, which in Sabin's series 23a exists in the form of the long fusiform space described above, has established connection with the pre-cardinal thoracic duct segment, and in this way it has increased considerably the length of the channel joined to the jugular lymph sac. Like the fusiform space of that embryo, the postcardinal thoracic duct division of series 192 is irregularly beaded or varicose, the constrictions or nodes suggesting more recent fusion between successive internodes. That this suggestion is a fair one is substantiated by the fact that toward the region of the periaortic veno-lymphatics, or beyond the point *X* (fig. 35) where the continuous anlage ends blindly, it is followed at intervals by a few large mesenchymal vacuoles (4*d*) between which no communication is as yet noticeable save through the indifferent tissue network.

The continuous portion of the left thoracic duct anlage in series 192 is perhaps no longer than in Sabin's series 23a, but the blind lymphatic spaces (4*s*, fig. 35) following it are far more extensive in length, especially those in the region of the left Cuvierian duct. Here there are two long spindle-shaped spaces parallel to each other, the shorter one of them being that portion of the anlage of the future mediastinal lymphatic vessel situated near the point of its subsequent junction with the other, or longer space, which represents an anlage of the left thoracic duct. In a 22 mm. embryo, a slightly older stage, all of the blind spaces of the pre- and post-cardinal divisions have become confluent to form the uninterrupted duct and its mediastinal tributary.

(C) *Supracardinal division.* In the third division of the thoracic duct area there may be recognized an anterior and a posterior half, those regions, respectively, in which the duct-anlage during its initial development is associated with the periaortic (6*c*, fig. 3) and the posterior supracardinal (6*c*, fig. 4) veno-lymphatics.

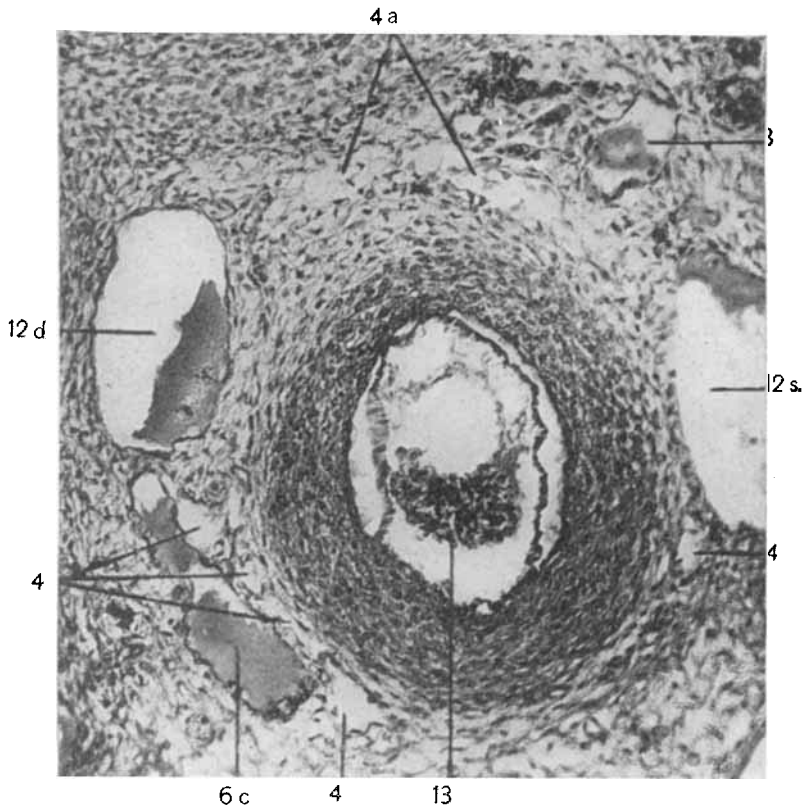


Fig. 18 Transverse section through the region of the mesonephroi in a 21 mm. pig embryo (series 103, slide 32, section 9), $\times 200$. 4, lymphatic spaces or discontinuous thoracic duct anlagen forming near or against the periaortic veno-lymphatics (6c); 4a, potential lymphatic spaces; 8, dorsal segmental vein; 12d, 12s, right and left supra-cardinal veins; 13, aorta.

In series 103 (21 mm.) is foreshadowed the decadence of the periaortic veno-lymphatics and the transference of supremacy to their successors. Frequently throughout their course the veno-lymphatic channels have lost their former fullness and their endothelium has been thrown into a slightly wavy and uneven contour, this being especially true of that side of the plexus facing the aorta where these areas of weakness are more abundant and accentuated. Coexistent with this condition is an incipient vacuolation

of the mesenchyme by which large and small fissures and spaces (4, fig. 18) arise irregularly and indiscriminately along the channels designated. Sometimes these spaces (4) cling closely to the receding walls of the venules (6c), and at other times they are separate with a perceptible amount of tissue intervening, but they are always discontinuous and non-venous in character. Like the more anterior lymphatic anlagen in their inception, no visible difference either of form or arrangement can be discerned between the cells which comprise their circumference and the cells of the intricate meshwork of the mesenchyme. Strands and

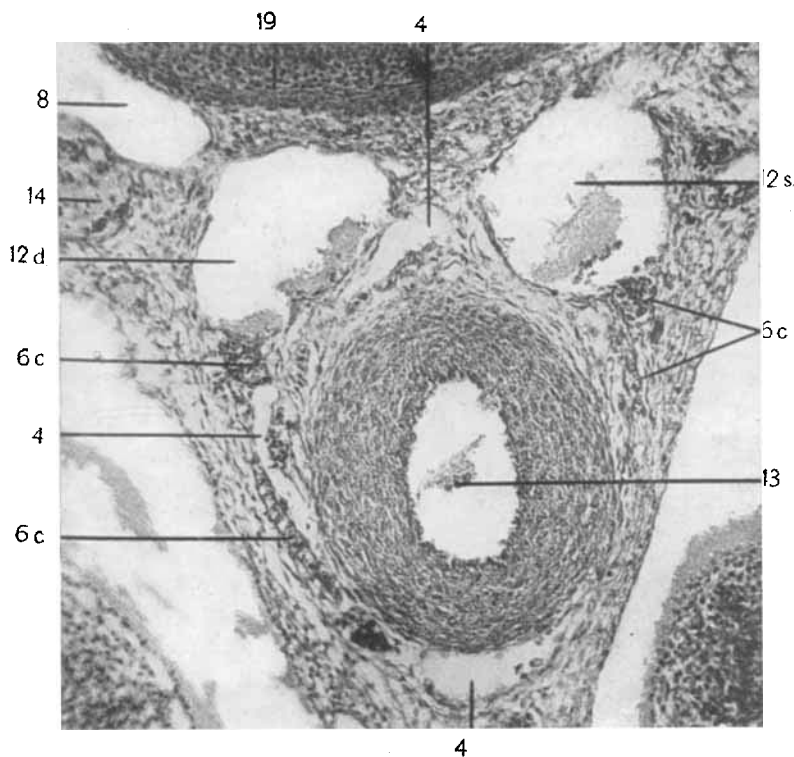


Fig. 19 Transverse section immediately in front of the mesonephroi in a 22 mm. pig embryo (series 105, slide 36, section 1), $\times 150$. 4, lymphatic spaces replacing the periaortic veno-lymphatics (6c); 8, dorsal segmental vein; 12d, 12s, right and left supracardinal veins; 13, aorta; 14, sympathetic nerve trunk; 19, embryonic vertebral column. (Reconstruction, fig. 33).

fibrils or their spur-like remnants often bridge the spaces or project into them and seem to suggest the existence of stresses and strains, as well as their direction, in the production of the anlagen by the breaking down of barriers and the fusion of interstitial spaces.

A later stage in the transformation of the periaortic region is offered by series 105, a 22 mm. embryo; but a minute account is hardly necessary considering the clearness of the appended figures which are self-explanatory and almost sufficient in themselves. Figure 19 is from a section taken just in front of the mesonephroi and is representative of the conditions active along the whole range of the periaortic plexus. The veno-lymphatics (6c) have lost most of their connections with the supracardinal veins and throughout the greater part of their course present a shrunken cavity filled with the deeply staining debris of blood cells. The lymphatics (4) which enmesh them are either broad spaces and have obliterated the venous core almost completely, or small crevices hugging one side of a vessel which has just begun to manifest degeneration. They are irregular in arrangement but they may always be distinguished by their clear lumina and unspecialized walls. A segment of the proximal portion of the supracardinal division in a 22 mm. embryo was reconstructed, a drawing of which is reproduced in figure 33, illustrating in three dimensions the conditions described.

The cisterna chyli is the outcome of a number of changes which proceed in very rapid succession and at the beginning often occur simultaneously: the detachment of the posterior supracardinal veno-lymphatics from their venous trunks; the condensation of these abandoned channels progressively toward the production of a plexiform or multilocular channel; the recession of their intima; the breaking down of broad partitions of tissue between them; the expansion of the resultant cavity by the addition of spaces from the mesenchyme; the simplification of its lumen and the acquisition of a lymphatic endothelium. In other words, a large part of the cavity of the cisterna chyli is derived from the combined cavities of preëxisting venous channels, but its wall is newly differentiated from the mesenchyme. A consideration of

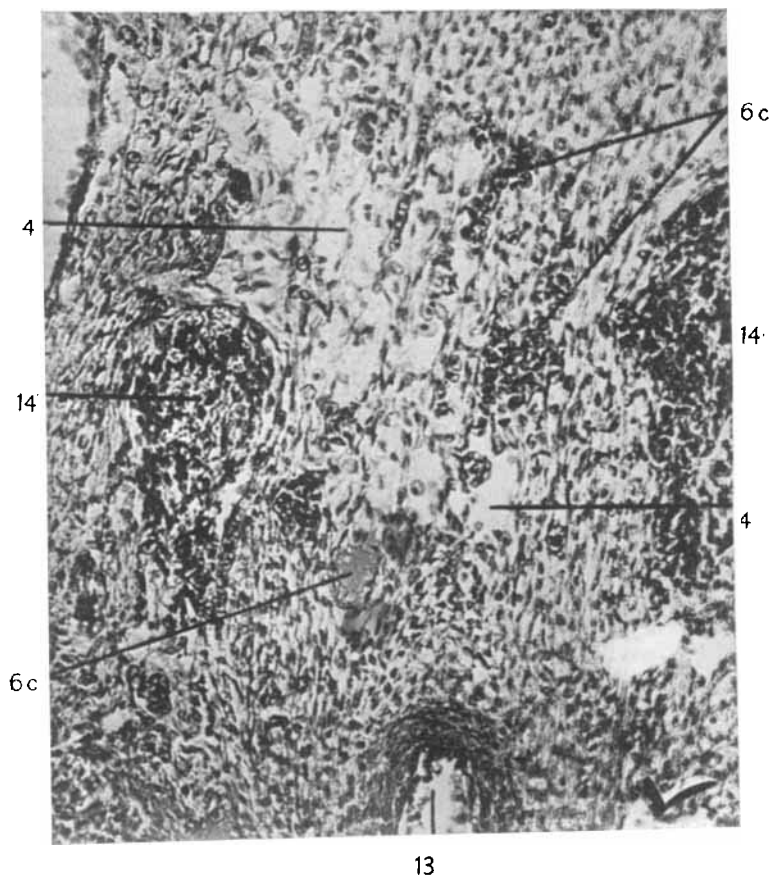


Fig. 20 Transverse section through the region of the future cisterna chyli in a 21 mm. pig embryo (series 103, slide 39, section 12), $\times 200$. 4, clear areas representing the formation of lymphatic spaces around the posterior supracardinal veno-lymphatics (6c); 13, aorta; 14, sympathetic nerve trunk.

the features revealed in 21, 22 and 23 mm. embryos will give support to these observations.

While the veno-lymphatics of the cisterna chyli region are anastomosing extensively in the median line dorsal to the aorta, they are beginning to lose their connections with the supracardinals of both sides so that they come to exist in the form of a condensed and abandoned multilocular venous plexus. Coincident with this process, the mesenchyme located between the plexiform channels

often become less compact, vesicles and fissures arise in it, and the venous intima, contiguous to these rarefied tissue-areas, retracts and breaks down; thus initiating continuity between the originally distinct channels. A section of such an incipient stage is illustrated in figure 20 which shows very clearly the vesiculated mesenchyme (4) in and amongst the veno-lymphatic plexus (6c). Typical examples of extra-intimal replacement also become very abundant and are significant as confirming the evidence already given for the reorganization of the intima whenever a lymphatic channel appropriates the pathways of redundant veins or venules. A photograph (fig. 21) of such a condition in a 22 mm. embryo describes more plainly than an extended narrative the important features in the transition period of the cisterna chyli. The compartments or loculi of the potential cisterna are traversed by distinct, delicate and devious lines which upon closer examination are found to be composed of compressed or scale-like cells, placed end to end, and to represent the discarded endothelium of the former venous derivatives (6c). This is shown when the endothelium is folded either forward or backward and it can be found occasionally lining a sharply defined cavity containing blood and then again to be pushed far into the lumen apparently by the pressure of the fluid within large mesenchymal spaces (4) on its external surface. At still other places where fusion of several parallel channels has occurred simultaneously, this evanescent venous intima is visible in cross-section as torn fibrils pendant from the irregular and frayed walls, or lying isolated in the lumen of the new or compound cavity. Besides these vestiges of the venous intima there are broader and thicker shreds of tissue, which are composed of a mass of ordinary mesenchymal cells jutting into the cavity and which indicate therefore the position of former boundaries between separate channels. Examination of the sections will also show distinctly that the outlines of the perivenous spaces are ill-defined and radically unlike those of the venous channels which they surround. In the confines of the transitional cisterna-anlage the irregular elliptical or cuboidal mesenchymal cell is the prevailing type and exists in strong contrast with the flattened and dense endothelial cell of a normal

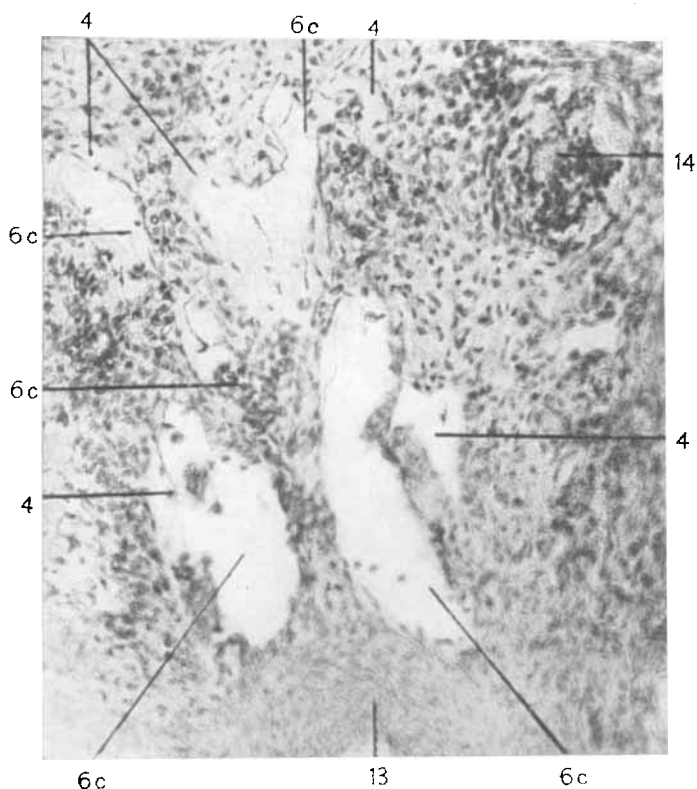


Fig. 21 Transverse section through the cisterna chyli region in a 22mm. pig embryo (series 105, slide 42, section 8), $\times 200$. 4, anlage of the cisterna chyli showing extra-intimal replacement of the supracardinal veno-lymphatics (6c); 14, sympathetic nerve trunk; 13, wall of the aorta.

vein or of the rejected and defunct intima lying in the lumen of this embryonic lymphatic.

The enlargement and perfection of the developing cisterna chyli, as well as of the other segments of the thoracic duct, will be considered in the treatment of the succeeding and final phase.

3. *The lymphatic phase (22-28 et seq. embryos)*

At the period when lymphatic spaces are appearing around the periaortic veno-lymphatics and are growing in volume, the thoracic duct anlage has already become a continuous structure in the

pre- and postcardinal divisions. Almost concurrently the changes producing the cisterna chyli and its connection with the posterior and mesenteric lymph sacs have been active, so that the segment of the duct anlage in the territory of the periaortic vessels, at the level of the mesonephroi, is the last one to acquire continuity among the lymphatic spaces and to make the thoracic duct an unbroken tube from one extremity to the other. The reason that those portions of the duct nearest to the lymph sacs are developed first, that the vacuolation of the mesenchyme, the formation of isolated spaces, and their confluence proceeds in a general centrifugal direction is not far to seek, being probably inherent in the explanation which would make the accelerating pressure of the lymph stream towards its points of entry to the veins sufficient to account for, or at least sufficient to furnish the stimulus for, the progressive occurrence of such phenomena.

The elongation of lymphatic spaces and their fusion finally into a continuous channel, as well as the growth of their cavities in diameter, is accomplished by the same process which gave origin to them, namely, by the disintegration of tissue fibrils and the concentric addition of spaces. Figure 22 represents a typical transverse section from the precardinal division of an early lymphatic stage and illustrates very plainly how the increment in size of the thoracic duct anlage is effected. An accurate camera lucida drawing, figure 23, of a portion of the same section is also inserted here to bring out more distinctly some of the details which may be obscure in the microphotograph due to the differences in focus. Both of these figures demonstrate that the duct (5*d*) at this embryonic period enlarges by a process of growth not from within outward but from without inward, by the admission of adjacent mesenchymal spaces to its channel. The strands intersecting the lumen are therefore indicative of successive lines of fusion or a measure of its gradual growth. From the facts just stated and the exceedingly indefinite boundaries of the anlage, we should expect the absence at this time of any kind of demarcation membrane between the cavity and the interstices or lacunae of the surrounding tissue. That such is really the case is borne out by experiment. Both the veins and the thoracic duct of an

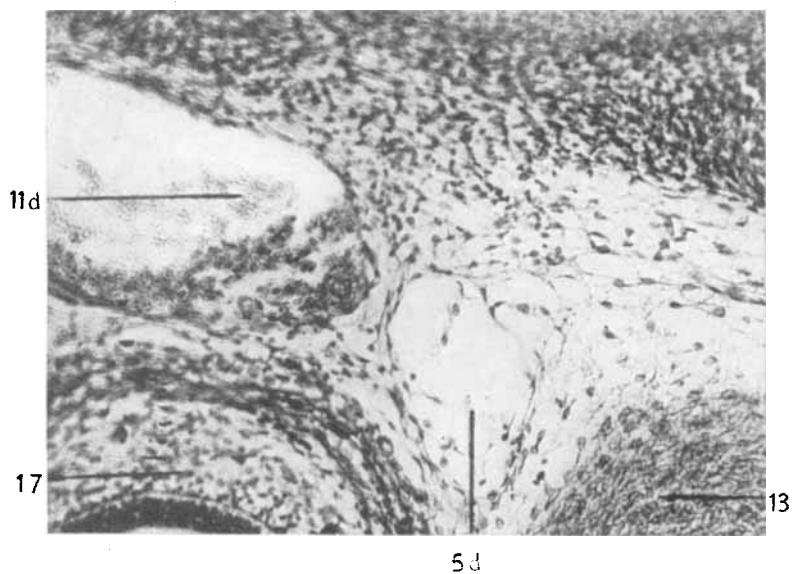
early lymphatic stage (23 mm. embryo) were injected and therefore are favorable for a comparison of their reactions to the injection mass. The veins (*11d*, *11s*, *25*, fig. 24), possessing perfect endothelial walls, did not admit of any extravasations or even of blurred outlines. In the case of the thoracic duct (*5d*), on the contrary, the injection mass (*Ex*) passed freely from the lumen into the surrounding tissue reticulum, as pictured clearly in figure 24, showing the absence of any definite wall at this early embryonic period.

Figure 25 is from the lower postcardinal division in a 26 mm. embryo and reveals essentially the same features as portrayed in figure 22 but shows even better perhaps the multilocular character of the thoracic duct anlage. The formation of spaces from the mesenchyme and their addition to the anlage is very clearly expressed on the right side in the wedge-shaped territory (*5d*) between the oesophagus (*17*) and aorta (*13*), and also on the left side (*5s*) immediately ventral to the left postcardinal vein (*11s*).

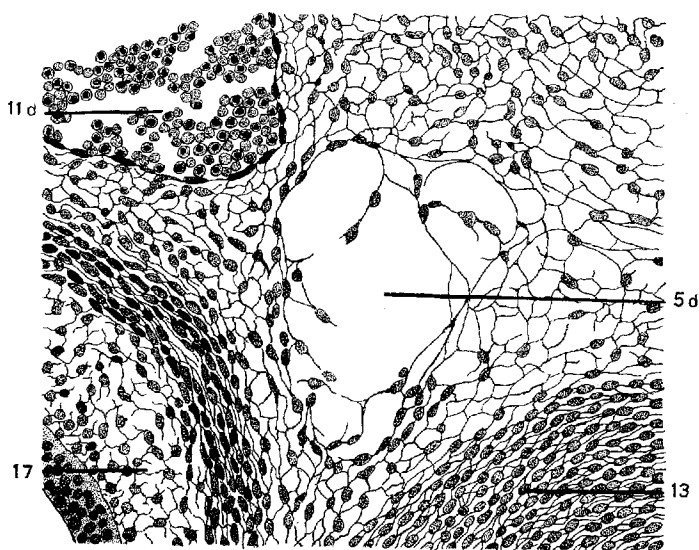
A later stage (23 mm.) in the development of the cisterna chyli (*5*) is shown in figure 26. The vestiges of the antecedent veno-lymphatics have completely disappeared, and only occasional trabeculae indicate the originally extensive tissue partitions between the early rudiments of the cisterna. What is of greater significance, however, is the ragged outline of the cavity, the absence of any specialized endothelium, and the addition of small mesenchymal spaces (*4*) to its lumen, upholding therefore in every respect the writer's contention that the cisterna chyli, concordant with the anterior divisions of the thoracic duct, is primarily and fundamentally a product of mesenchymal differentiation.

Fig. 22 Transverse section through the upper thoracic region in a 21.5 mm. pig embryo (series 192, slide 21, section 11), $\times 200$. *5d*, right thoracic duct anlage showing its enlargement by the addition of adjacent mesenchymal spaces; *11d*, right postcardinal; *13*, wall of the aorta; *17*, oesophagus; (compare with fig. 23).

Fig. 23 An accurate camera lucida drawing of a highly magnified area represented in fig. 22, $\times 266$ (reduced from $\times 400$). *5d*, right thoracic duct anlage showing its concentric growth from enlarged tissue spaces and the absence of a specialized intima; *11d*, right postcardinal vein filled with blood and possessing a well-defined endothelial lining; *13*, wall of the aorta; *17*, oesophagus.



22



23

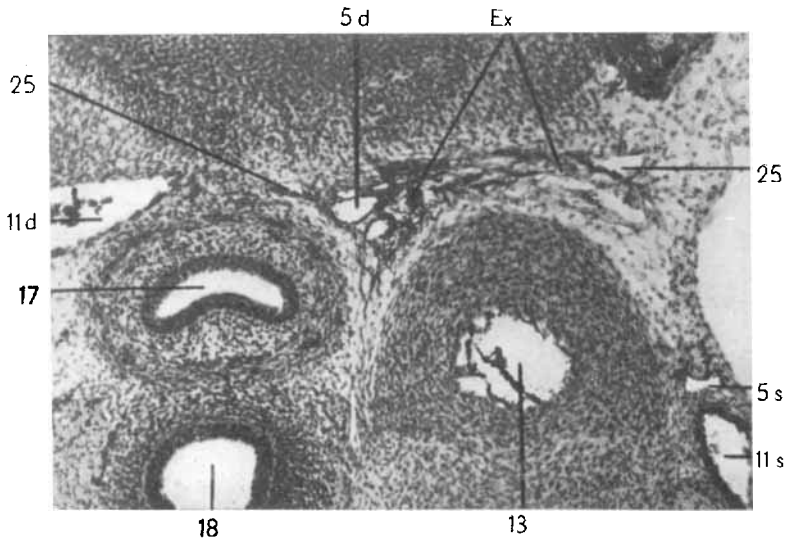


Fig. 24 Transverse section through the upper thoracic region in a 23 mm. pig embryo (injected series, slide 15, section 11), $\times 100$. *5d*, right thoracic duct anlage injected; *Ex*, extravasations of the injection mass from the right duct-anlage into the loose surrounding mesenchyme; *5s*, left thoracic duct anlage; *11d*, *11s*, right and left postcardinals injected; *13*, aorta; *17*, oesophagus; *25*, branches of the postcardinals injected.

As shown in figures 22, 23, 25 and 26, at the beginning of the lymphatic phase the thoracic duct in transverse section resembles a condensed plexus and may be said to be at the height of its complexity. For from now on there is a gradual reduction of this condition until the two limbs of the thoracic duct exist normally as single simple channels. By the breaking down of the abundant tissue bridges which had divided its channel into a labyrinth of loculi, the ducts assume more and more the appearance of unobstructed tubes. In figure 27, taken from a 26 mm. embryo, the outlines of the ducts (*5d*, *5s*) are assured, and only the vestiges of former septa and trabeculae are still visible here and there in the form of few small tissue spurs and filaments projecting into the cavities.

The most startling change, however, occurs in the precardinal division in the region of the jugular lymph sac. The reader will

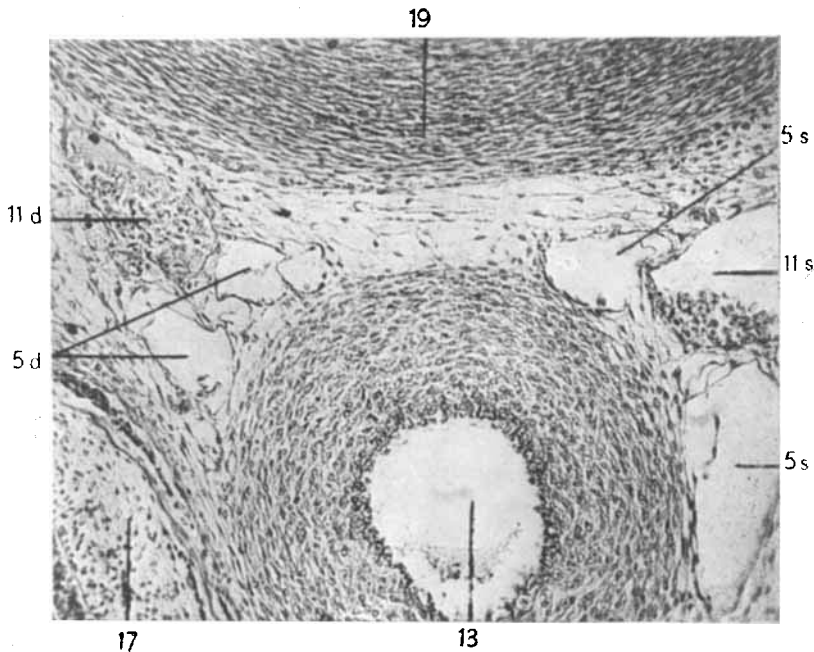


Fig. 25 Transverse section through the postcardinal region in a 26 mm. pig embryo (series 69, slide 44, section 11), $\times 150$. *5d*, *5s*, right and left thoracic ducts showing the manner of their growth by the addition of spaces from the mesenchyme; *11d*, *11s*, right and left postcardinals; *13*, aorta; *17*, oesophagus; *19*, embryonic vertebral column.

recall that in the later transition stages the most anterior portion of the thoracic duct anlage is characterized by an extensive and complicated lymphatic plexus, especially well developed in Sabin's series 23a (3, fig. 30). During the lymphatic phase such a plexus is completely reduced and converted into a simple channel (22–26 mm. embryos) whose fork or division into the right and left limbs of the duct becomes shifted far back of the lymph sac. Series 192 (3, fig. 35) presents an intermediate stage in which the original plexiform condition is still suggested but has been almost entirely obliterated by the transverse fusion and consequent reduction of the number of interanastomosing channels.

No matter whether the thoracic duct anlagen arise as extra-intimal spaces or entirely apart from the veno-lymphatics, dur-

ing their genesis their walls are composed of the undifferentiated mesenchymal cells (figs. 6, 9, 18, etc). We have also seen the method of concentric addition of tissue spaces by which the anlagen enlarge during the later transition and early lymphatic stages, implying thus a continual shifting of their boundaries so that an intima may be said to be temporarily established, replaced, and reorganized a number of times (figs. 22, 25, etc.) Ultimately, however, we can speak of a definite and permanent endothelium

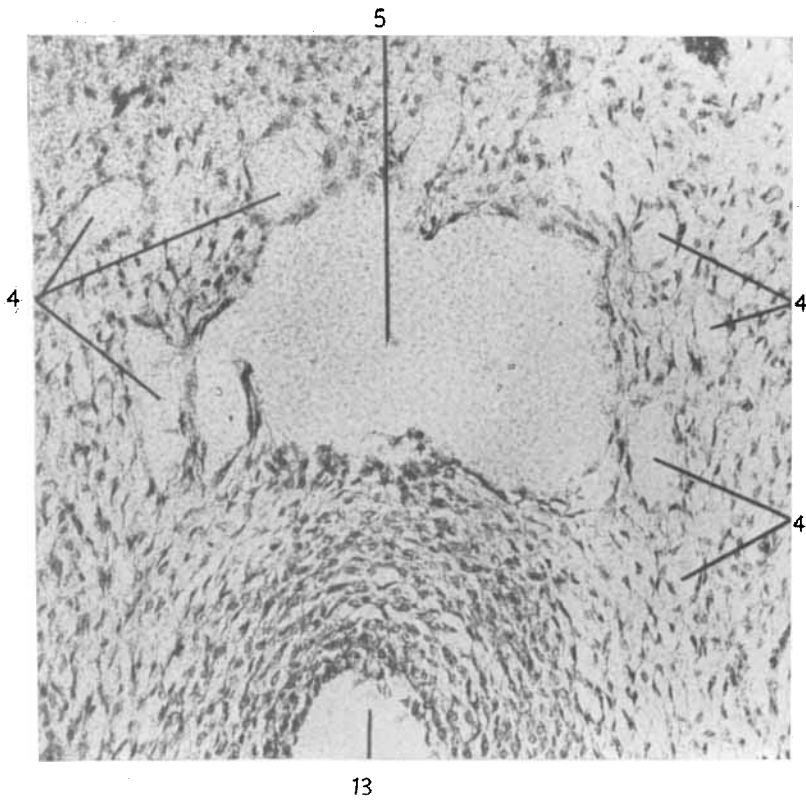


Fig. 26 Transverse section through the region of the cisterna chyli in a 23 mm. pig embryo (series 67, slide 43, section 14), $\times 150$. 5, anlage of the cisterna chyli showing the indefinite and ragged outline of its cavity, the absence of a specialized intima, and the addition of small spaces (4) from the mesenchyme; the vestiges of the former veno-lymphatics have completely disappeared; 13, aorta; 14, sympathetic nerve trunk.

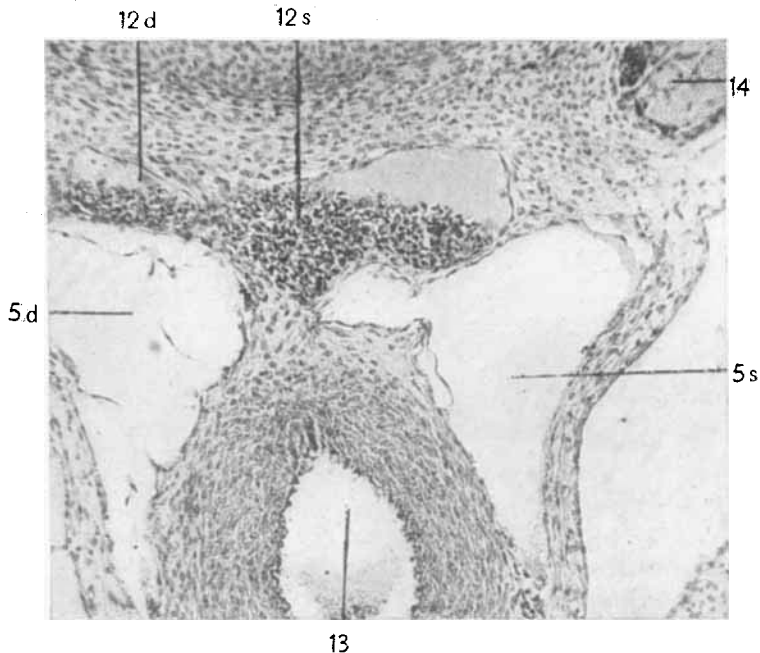


Fig. 27 Transverse section through the anterior supracardinal region in a 26 mm. pig embryo (series 69, slide 53, section 10), $\times 150$. *5d*, *5s*, right and left thoracic ducts; *12d*, *12s*, right and left supracardinal veins; *13*, aorta; *14*, sympathetic nerve trunk.

only after the thoracic duct has lost its multilocular and plexiform character, and its channel approaches more nearly to a clear-cut and simple tube (*5s*, fig. 27). The manner in which the mesenchymal cell is transformed into an endothelial cell occurs undoubtedly, as Huntington has suggested, by a mechanical adaptation to the pressure of the fluid within the lymphatic cavity. This view is entirely in harmony with the conditions observed in the extra-embryonic area of the chick²³ where the

²³ At the last session of the American Association of Anatomists, December 27, 1911, at Princeton, N. J., John E. McWhorter and Allen O. Whipple of the College of Physicians and Surgeons, Columbia University, presented a report on the development of the blastoderm of the chick in vitro. This report appeared as a short preliminary paper with twelve figures in the *Anatomical Record*, vol. 6, no. 3, March, 1912. These investigators, on the basis of a study of the living growing

earliest anlagen of the blood vessels arise between the mesodermal cell-strands as isolated spaces and fissures, which at first are bounded by ordinary cuboidal cells but later acquire the characteristic vascular endothelium by the modification of these cells. Being plastic, the cells lining either a haemal or a lymphatic anlage must be regarded as obeying the internal pressure of the cavity and becoming more and more flattened and endothelial-like as the pressure of the fluid or plasma increases.

Although this work deals primarily with the source of the thoracic duct, attention was not confined to it exclusively but also considered briefly two other lymph ducts, the mediastinal channel draining the mediastinum and its organs, and the right lymphatic duct, which in earlier stages of phylogenesis undoubtedly composed a part of the thoracic duct system but now ordinarily remains independent of it and receives tributaries only from the cephalic, cervical and upper thoracic regions of the right side. In development these channels repeat in all details the history of the thoracic duct, arising as isolated mesenchymal or perivenous spaces which subsequently become confluent.

After the above description of the development of the thoracic duct and a consideration of the evidence presented, attention may be directed towards several criticisms advanced recently by those investigators opposed to the view of the direct mesenchymal origin of lymphatic vessels. These opponents would dismiss as artifacts all of the 'lymphatic anlagen' described by the writer. Sabin, referring to the figures of extra-intimal replacement discovered by Huntington and McClure in their investigations on the genesis of the lymphatic system in the cat, maintains that "they are all in the center of the embryo where the fixing fluid pene-

chick embryo, brought forth conclusive evidence that the haemal channel system of the extra-embryonic area is developed from isolated spaces, which arise blindly in the undifferentiated mesenchyme and which subsequently change their shape by expansion and elongation and become confluent with other such spaces to produce the complicated blood plexuses in the area designated. The "spaces are frequently bounded by a mere line, more or less refractile in character. In others the lumen is lined with rounded or oval cells which later become fusiform and flattened.

trates last;”²⁴ and concerning all ‘mesenchymal spaces,’ Clark says: “They occur most often around blood vessels, and are almost certainly to be interpreted as shrinkage spaces, or spaces caused by the retraction of the mesenchyme processes made possible by slight rents produced in the preparation of the sections.”²⁵ That these objections are wholly without foundation, at least in the case of the developing thoracic duct in the pig, is conclusively shown by the following observations: In the first place, the mesenchymal spaces termed lymphatic anlagen spring into existence at a definite period of embryonic history and invariably in a definite position. The embryos grouped under the veno-lymphatic phase are without an exception the younger embryos, and, although they were preserved in the same fixatives and the preparation of the sections followed the same methods as those employed for the specimens of the second phase, they do not show or even suggest instances of such anlagen. Secondly, in all of the earlier stages of the transition phase these lymphatic spaces can only be observed in the precardinal division, namely, in the territory of the most anterior segment of the thoracic duct which is formed first; in the postcardinal and supracardinal divisions the mesenchyme is still uniform, and the veno-lymphatics are functional and joined to the parent veins. In the later stages of this phase the last two segments repeat the history of the first. Thirdly, all of the extra-intimal spaces in the thoracic region of the writer’s specimens occur only in connection with those venules which have been severed from their venous trunks and which lie topographically in the pathway of the potential duct. As far as the writer was able to determine, other abandoned veins existing in the same general areas and in the ‘center of the body,’ but not antecedent in position to the duct or to any of its tributaries, never manifest extra-intimal figures in their atrophy. All of the functional veins possess a normal and distended endothelium.

²⁴ Florence R. Sabin: A critical study of the evidence presented in several recent articles on the development of the lymphatic system. *Anat. Rec.*, vol. 5, no. 9, 1911.

²⁵ Elliot R. Clark: An examination of the methods used in the study of the development of the lymphatic system. *Anat. Rec.*, vol. 5, no. 8, 1911.

Fourthly, these mesenchymal perivascular spaces may have fused into a profuse plexus and become widely open to the jugular lymph sac as in the case of series 194 (fig. 29), but they, as yet, do not show a well-defined or specialized wall (figs. 8 and 9). In the fifth place, all of the discontinuous mesenchymal spaces follow one another in a succession practically undeviating which represents an outline or fragmentary picture of the future duct. Outside of this line there are no lymphatic spaces. Sixthly, in the third or lymphatic phase, when continuity of the duct and its branches has been established, no perivenous or other isolated vacuities can be discovered. If the discontinuous lymphatic anlagen were artifacts we should expect to find the largest number of them in this last phase because the diameter and the bulk of the embryos are greater, and therefore the longer time required for the fixing fluid to penetrate to their centers would make possible greater unevenness of fixation and consequently greater shrinkage.

Because the elongation of the thoracic duct is effected by a progressive summation or centripetal addition of large mesenchymal spaces to that part of the anlage already confluent with the lymph sacs, the injection of successive transition stages up to the time when continuity has been acquired throughout its entire course will show a gradual increase in the length to which the injection mass has penetrated; but the study of serial sections will also reveal anlagen which lie beyond the farthest point of the injection and are inaccessible to it on account of their discontinuity, or because they have not as yet become confluent with the anlage into which the injecta were introduced.

This leads to a second contention of Sabin, namely, that the study of serial sections alone is inadequate, and that continuity of the apparently discontinuous lymphatic anlagen can be demonstrated by complete injection. A more radical refutation of this argument than that furnished by her own series 23a is scarcely possible. The abrupt break between the precardinal injected segment of the right thoracic duct anlage in this embryo and the postcardinal uninjected segment (fig. 31) bears out in a striking manner the evidence derived from the writer's series. Notwith-

standing the inability of the eye to discover a connection between these two segments with the aid of high magnifications, it might be urged by those prejudiced that the injection may have been only a partial one. But this objection becomes groundless when the reader recalls that the pressure of the injecting fluid was of sufficient force to produce extravasations, which, as Clark maintains, signify an excess of pressure in filling the cavity completely; for he says, "With too great pressure there is produced a mossy appearance around the capillary (lymph), as has been pointed out by Hoyer, due evidently to forcing the injection mass through the lymphatic wall." If an opening had been present between these two anlagen the injecting substance would certainly have obeyed the direction of least resistance and passed into the second one. Nor is the objection valid which would exclude this large blind fusiform space from taking any significant part in the production of the thoracic duct; for not only is the distinct character and position of this space contrary to such a view but also the fact that the left side discloses similar spaces located in the identical line of the future left duct. Somewhat later embryonic stages make these observations conclusive; for example, in series 192 the post-cardinal segment of the right duct duplicates or agrees in all of its features with that of series 23a, except for its continuity with the anterior or precardinal segment (figs. 30, 31 and 35) and consequently with the jugular lymph sac. Moreover, during the progress of his investigation the writer has tentatively assumed the possibility of a centrifugal growing of thoracic duct buds through the large mesenchymal spindle spaces situated only in the thoracic duct pathway, and he has searched for such hypothetical sprouts but has not succeeded in finding a trace of evidence in their favor.

Sabin's and Clark's contention that discontinuities in a lymphatic channel are due to artifacts, resulting during fixation from the unequal shrinkage here and there of its caliber, is easily controverted by the observed facts. In the case of the developing thoracic duct such discontinuities only occur in the stages of the transition phase, in those embryos measuring approximately between 20 and 23 mm. The discontinuous segments or anlagen begin as minute mesenchymal vacuoles which gradually enlarge

and elongate with the increasing age of these embryos; in other words, in a 22 mm. embryo the blind segments of the duct anlage will be much longer and more conspicuous than in a 20 mm. embryo for instance. Further, there is a positive regularity in the progressive reduction of the number of these blind lymphatic anlagen in a general antero-posterior direction by their addition to the continuous anlage, which, as a consequence, gradually becomes elongated. Were these lymphatic spaces artifacts, or segments cut off from a continuous channel by shrinkage, then the determinate sequence of genetic changes pointed out in the descriptions of the individual stages could not exist, and we should find them in slightly older embryos or in those portions of the duct-anlage definitely known to be complete, for the same methods of technic should produce similar effects. The embryonic thoracic ducts when fully formed and indeed all lymphatic vessels possess a varicose channel constricted and dilated alternately into irregular nodes and internodes. Such a condition, however, is not brought about by fixation but is a characteristic peculiar to a lymph vessel and obviously harks back to the period when it was composed of a varying number of irregular fusiform or oblong mesenchymal spaces succeeding one another with distinct interruptions. Accordingly, the nodes or constrictions of a thoracic duct just completed would indicate the areas of final fusion between consecutive anlagen.

It should be emphasized here that Sabin and Clark base their criticisms chiefly upon the latter's investigations on the development of the lymphatic capillaries in the tail fin of the larval frog. The fallibility of their argument becomes therefore further evident when we find them comparing the reaction to the fixatives of these terminal lymphatics with that of other lymph channels, especially the larger ducts and trunks; for, although the principle of development probably is the same in both cases, the details of their behavior during the preparation of the sections may be quite different. It would be just as logical to describe a large systemic artery or vein entirely in terms of their terminal arterioles or venules. The writer will not deny that careless or imperfect fixation may cause the delicate capillaries of the fin of a tadpole

to shrink into seemingly isolated segments so that they can be pursued only with great difficulty, as described by Clark, but he does deny, supported by the decisive evidence of the injected series 23a and reinforced by all of the transition stages, that the discontinuous anlagen observed by him and invariably found to be concomitants in the formation of a large lymphatic trunk like the thoracic duct are artifacts, produced by the preserving or fixing reagents.

V. RÉSUMÉ OF OBSERVATIONS AND CONCLUSIONS

1. Derived from the supracardinal or azygos system of veins, a series of venous channels, called veno-lymphatics, are formed in the pathway finally occupied by the thoracic duct, and at the culmination of their development they exist as plexuses of vessels abundantly connected with the parent veins.

2. The actual genesis of the thoracic duct is initiated by the appearance of blind mesenchymal lymphatic spaces either around or not immediately in contact with the venous derivatives, or veno-lymphatics, which become detached from their venous trunks and break up into degenerating segments. The lymphatic spaces or anlagen arise by the local disintegration of the fibrils of the tissue reticulum and the fusion of the interstitial lacunae, and they enlarge and elongate in a similar manner. If they are of the nature of extra-intimal spaces the endothelium of the evanescent abandoned veno-lymphatics, which they replace, collapses as the result perhaps of the increasing influence of the lymph pressure on its external surface after its release from the blood pressure. During their inception and growth the walls of the discontinuous thoracic duct anlagen are composed of the ordinary unmodified mesenchymal cells. That such lymphatic anlagen are not artifacts is shown by their definite position and period of formation and the determinate sequence from their first appearance as mesenchymal vacuoles, through the phase of their growth and elongation, to their final fusion into a continuous channel. In the production of the most posterior portion of the thoracic duct, or cisterna chyli, veno-lymphatic channels by fusion with

one another give rise to the larger part, perhaps, of its cavity; but at the same time their endothelium recedes and degenerates, and the cisterna-anlage increases in size by the addition of spaces from the mesenchyme, so that, like the more anterior segments of the thoracic duct anlage, it is bounded by ordinary embryonic tissue cells during this early developmental period.

3. The elongation and final continuity of the thoracic duct anlage is effected by the progressive confluence of discontinuous fusiform lymphatic spaces in a general centrifugal direction, probably determined by the impulse of the lymph flow towards the radiation centers or lymph sacs. Injected specimens of the early lymphatic stages certify the reality of blind uninjectible anlagen beyond the farthest points to which the injecta have penetrated, demonstrating that discontinuities in a developing lymphatic channel are not 'appearances' found only by the study of uninjected embryos. Not a shadow of evidence was discovered in favor of the theory which maintains the centrifugal growth of the duct by budding from the lymph sacs or the derivation of the lymphatic endothelium from the veins. During the period of its initial growth the thoracic duct increases in diameter by the concentric addition of enlarged and immediately surrounding tissue spaces to its lumen. The intima of the thoracic duct is a differentiation in situ of mesenchymal cells as an adaptation probably to the pressure of the lymph flow within the cavity.

PLATES

PLATE 1

EXPLANATION OF FIGURE

28 Reconstruction of the vascular channels of the lower cervical and thoracic regions in a 19 mm. pig embryo (series 168, slides 16-24 inclusive), $\times 50$. Dorsal view. Arrows indicate the levels at which the microphotographs were taken. Cross lines, not labelled, indicate the extent of the divisions, A, B, and C.

- | | |
|--|--|
| A, precardinal division | 12ls, left supracardinal line, plexiform |
| B, postcardinal division | 13, aorta |
| C, supracardinal division | 13a, aortic arch |
| 1, left jugular lymph sac | 13c, left carotid artery |
| 2, thoracic duct approach | 13ds, dorsal segmental arteries |
| 4, lymphatic spaces, incipient thoracic duct anlagen | 13s, left subelavian artery and branches |
| 6a, precardinal veno-lymphatics | 14, left sympathetic nerve trunk |
| 6b, postcardinal veno-lymphatics | 15, vagus |
| 6c, supracardinal veno-lymphatics | 16, recurrent laryngeal nerve, and accompanying vein |
| 7, oblique vessel | 17, oesophagus |
| 8, dorsal segmental veins of the pre-, post-, and supracardinals, respectively | 20, mesonephroi |
| 9, internal jugular vein | 21, left Cuvierian duct |
| 10, left precardinal vein | 22, left subelavian vein |
| 11d, 11s, right and left postcardinal veins | 23, cephalic vein |
| 12d, 12s, right and left supracardinal veins | 24, external jugular vein |
| 12ld, right supracardinal line beginning to fuse with the right postcardinal | 25, venous plexus between supracardinal lines |
| | 26, subelavian approach of jugular lymph sac |
| | N5, fifth spinal nerve |

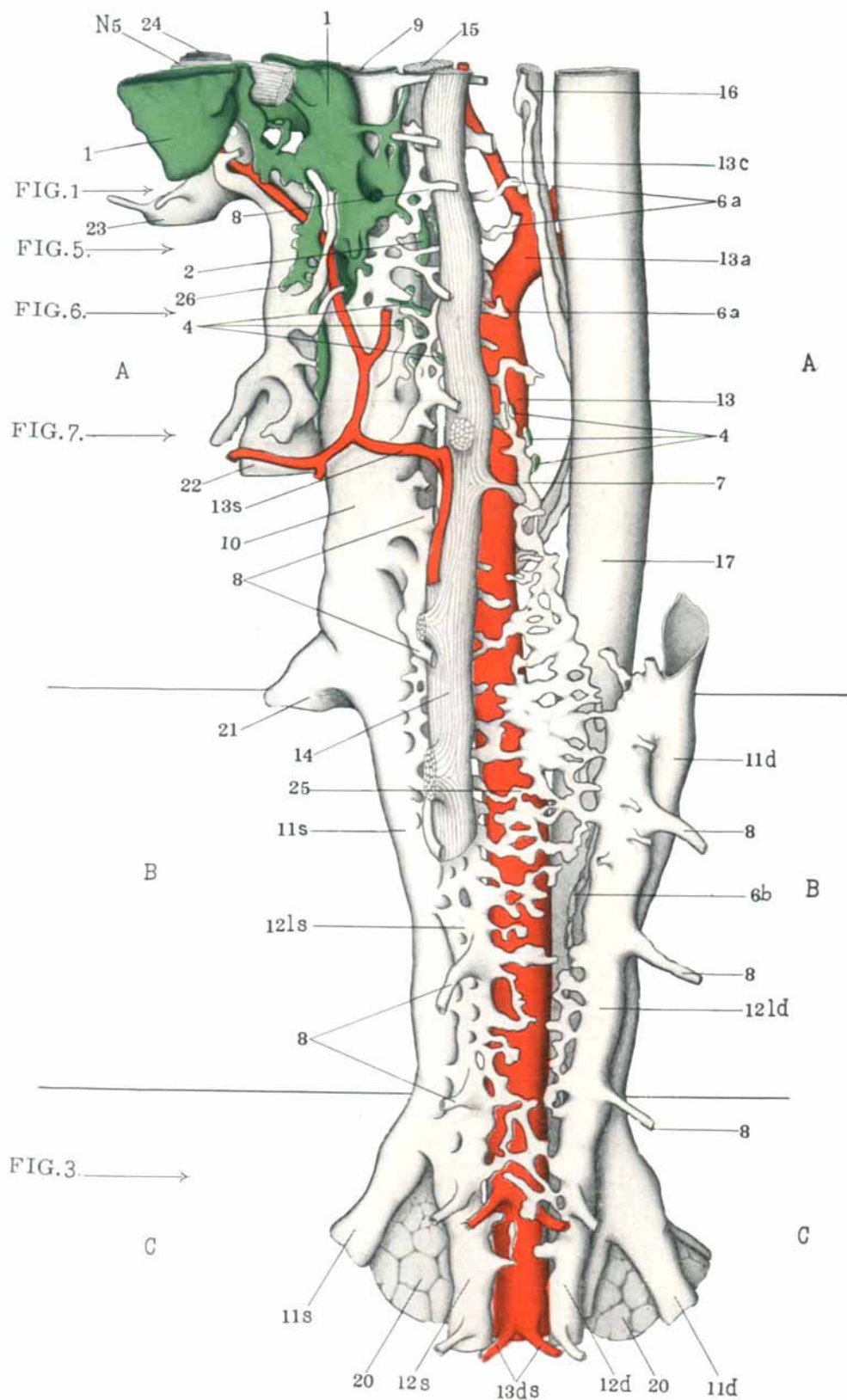


PLATE 2

EXPLANATION OF FIGURE

29 Reconstruction of the vascular channels of the lower cervical and thoracic regions in a 20 mm. pig embryo (series 194, slides 22-31 inclusive), $\times 50$. Sinistro-dorsal view.

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|--|---|
| A, precardinal division | 12 <i>d</i> , 12 <i>s</i> , right and left supracardinal veins |
| B, postcardinal division | 12 <i>ls</i> , left supracardinal line; right line has completely fused with the right postcardinal |
| C, supracardinal division | 13, aorta |
| 1, left jugular lymph sac | 13 <i>b</i> , ductus arteriosus Botalli |
| 2, thoracic duct approach | 13 <i>s</i> , left subclavian artery and branches |
| 3, anterior lymphatic plexus or subsequent common trunk of the right and left thoracic ducts replacing the precardinal veno-lymphatics | 14, left sympathetic nerve trunk |
| 4 <i>d</i> , lymphatic spaces in the right thoracic duct line replacing the oblique vessel | 15, vagus |
| 4 <i>s</i> , lymphatic spaces in the left thoracic duct line | 16, recurrent laryngeal nerve |
| 6 <i>a</i> , precardinal veno-lymphatics | 17, oesophagus |
| 7, oblique vessel, degenerating and breaking up into segments | 21, left Cuvierian duct |
| 6 <i>b</i> , postcardinal veno-lymphatics | 22, left subclavian vein |
| 6 <i>c</i> , supracardinal veno-lymphatics | 23, cephalic vein |
| 8, dorsal segmental veins of the pre-, post-, and supracardinals | 24, external jugular vein |
| 9, internal jugular vein | 25, degenerating remnants of the former venous plexus between the supracardinal lines. |
| 10, left precardinal vein | 26, subclavian approach of the jugular lymph sac |
| 12 <i>d</i> , 11 <i>s</i> , right and left postcardinal veins | N5, fifth spinal nerve |

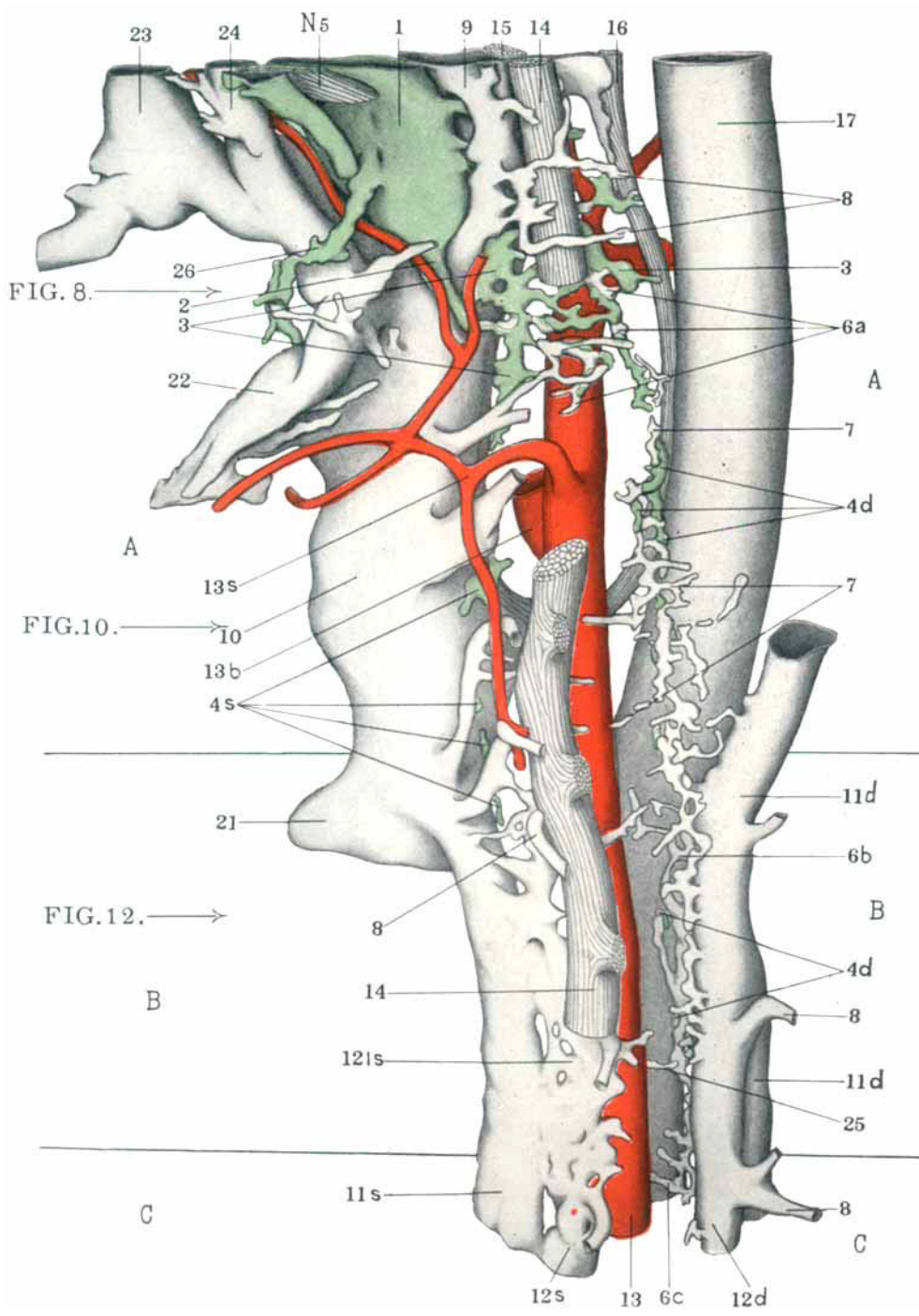


PLATE 3

EXPLANATION OF FIGURE

30 Reconstruction of the lower cervical and thoracic regions in a 23 mm. pig embryo (injected series 23a, Johns Hopkins University Embryological Collection, slides 21-30 inclusive), $\times 50$. Dorsal view, slightly from the left.

- | | |
|---|---|
| <p>A, precardinal division
 B, postcardinal division
 C, supracardinal division
 1, left jugular lymph sac
 3, anterior lymphatic plexus or subsequent common trunk of the right and left thoracic ducts.
 5<i>d</i>, 5<i>s</i>, continuous and injected portions of the right and left thoracic duct anlagen
 X, X, extent of the continuity of 5<i>d</i> and 5<i>s</i>, and the farthest points to which the injection mass has penetrated
 4<i>d</i>, long and short lymphatic spaces in the axis of the injected anlage and in the path of the future right thoracic duct
 4<i>s</i>, lymphatic spaces in the left thoracic duct line hidden by the veins, but indicated by the dotted circles
 7, oblique vessel, degenerating anteriorly
 8, dorsal segmental veins of the pre-, post, and supracardinals
 9, internal jugular vein</p> | <p>10, left precardinal vein
 11<i>d</i>, 11<i>s</i>, right and left postcardinal veins
 12<i>ls</i>, left supracardinal line, plexiform; the right line has fused with the right postcardinal vein
 12<i>d</i>, 12<i>s</i>, right and left supracardinal veins
 13, aorta
 13<i>s</i>, left subclavian artery and branches
 14, left sympathetic nerve trunk
 15, vagus
 16, recurrent laryngeal nerve, and accompanying vein
 17, oesophagus
 20, anterior tip of left mesonophros
 21, left Cuvierian duct
 22, left subclavian vein
 23, cephalic vein
 24, external jugular vein
 25, degenerating segments of the former extensive plexus between the supracardinal lines
 26, subclavian approach of the jugular lymph sac</p> |
|---|---|

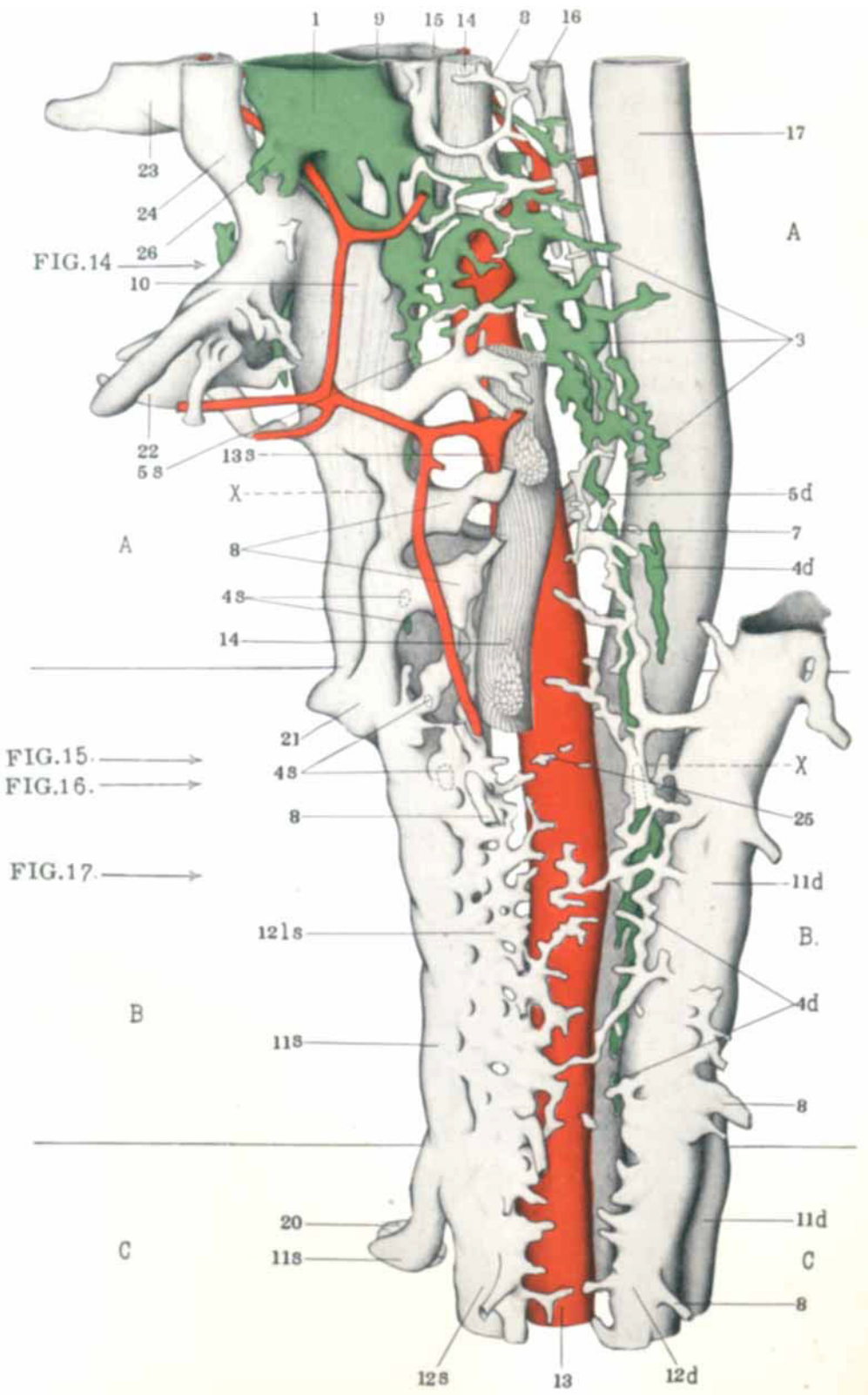


PLATE 4

EXPLANATION OF FIGURE

31 Ventral view of the lower half and right side of the reconstruction represented in figure 30 (23 mm. pig. embryo, series 23a, J.H.E.C., from section 12, slide 25 to slide 30 inclusive), $\times 50$.

- | | |
|---|---|
| <p><i>5d</i>, extremity of the injected portion of the right thoracic duct</p> <p><i>X</i>, blind end of <i>5d</i> and the farthest extent to which the injection mass has penetrated</p> <p><i>4d</i>, discontinuous and uninjected lymphatic spaces in the right thoracic duct line</p> | <p><i>6b</i>, postcardinal veno-lymphatics</p> <p><i>7</i>, posterior portion of the oblique vessel and its junction with the postcardinal</p> <p><i>8</i>, dorsal segmental veins</p> <p><i>11d</i>, right postcardinal vein</p> <p><i>12d</i>, right supracardinal vein</p> <p><i>13</i>, aorta</p> |
|---|---|

32 Sinistro-ventral view of the lower third of the reconstruction shown in figure 31 (19 mm. pig embryo, series 168, slides 22-24 inclusive), $\times 50$.

- | | |
|--|--|
| <p><i>6c</i>, periaortic supracardinal veno-lymphatics</p> <p><i>11s</i>, left postcardinal vein</p> | <p><i>12s</i>, left supracardinal vein</p> <p><i>20</i>, right mesonephros</p> |
|--|--|

33 Reconstruction of a segment of the anterior supracardinal division or region of the periaortic veno-lymphatics in a 22 mm. pig embryo (series 105, slides 34-36 inclusive), $\times 50$. Dextro-ventral view.

4d, *4s*, lymphatic spaces replacing the degenerating veno-lymphatics (*6c*). Other explanations the same as above.

34 Reconstruction of the same region, represented in the preceding figure, in a 23 mm. pig embryo (series 67, slides 36-37 inclusive), $\times 50$. Sinistro-dorsal view.

5, right and left thoracic duct; veno-lymphatics have been completely replaced. Other explanations the same as above.

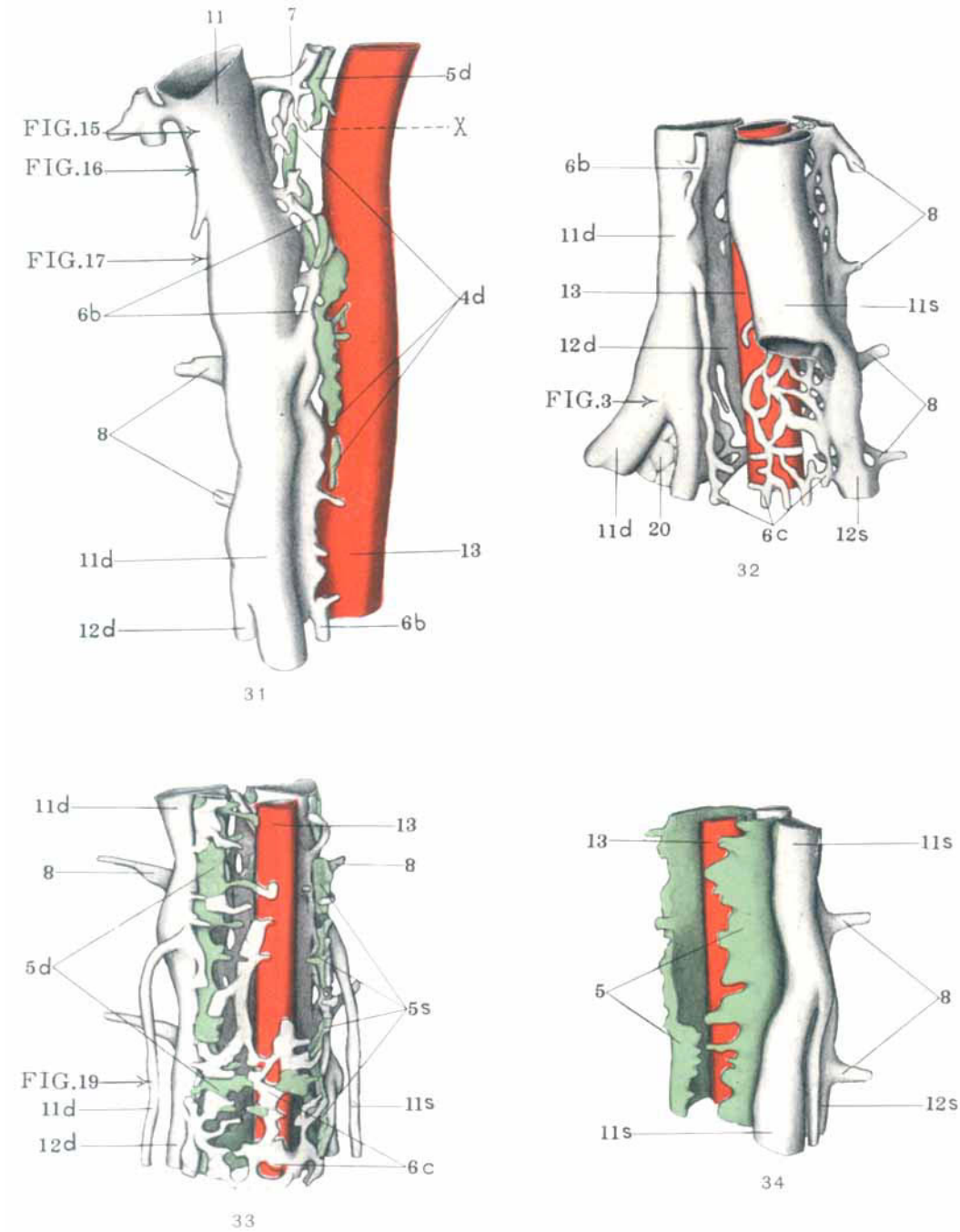


PLATE 5

EXPLANATION OF FIGURE

35 Reconstruction of the vascular channels of the lower cervical and thoracic regions in a 21.5 mm. pig embryo (series 192, slides 16-24 inclusive), $\times 50$. Sinistro-dorsal view.

- | | |
|--|--|
| A, precardinal division | 11d, 11s, right and left postcardinal veins |
| B, postcardinal division | 12ls, plexiform remnants of the left supra-cardinal line |
| 1, left jugular lymph sac | 13, aorta |
| 2, thoracic duct approach | 13b, ductus arteriosus Botalli |
| 3, anterior lymphatic plexus or subsequent common trunk of the right and left thoracic ducts | 13d, right subclavian artery |
| 4d, 4s, discontinuous thoracic ductanlagen | 13s, left subclavian artery and branches |
| 5d, 5s, right and left thoracic ductanlagen continuous with lymph sac | 14, left sympathetic nerve trunk |
| X, X, extent of continuity in the ductanlagen connected with the lymph sac | 15, vagus |
| 7, spur or vestige of the former oblique vessel | 16, recurrent laryngeal nerve |
| 8, dorsal segmental veins | 17, oesophagus |
| 9, internal jugular vein | 18, trachea |
| 10, left precardinal vein | 21, left Cuvierian duct |
| | 22, left subclavian vein |
| | 23, cephalic vein |
| | 24, external jugular vein |
| | 26, subclavian approach of the jugular lymph sac |

