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The Development of the Jugular Lymph Sacs in the Light of Vestigial, Provisional and Definitive Phases of Morphogenesis¹

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Looking at the roster of the American Association of Anatomists of 50 years ago, we count very few members who are living today; hence, only they have first-hand knowledge of the vehement dispute on the genesis of the lymphatic system that then agitated and took possession of the annual meetings for several years. Indeed, so utterly did it dominate them that other members not involved in it voiced the threat of secession. To understand how the mere thought of such a drastic protest could arise, we need but to be reminded that half a century ago the gathering was still small enough for the reading of all papers in one common or joint program, in contrast with the three or more specialized sessions running concurrently as at the meetings now.

Stripping the controversy to its core, the main issue was whether lymphatic endothelium had its source from the embryonic venous system or whether it sprang directly from mesenchyme. To name Florence R. Sabin, Frederic T. Lewis, George S. Huntington, Charles F. W. McClure, William F. Allen, Eliot R. and Eleanor L. Clark, Adam M. Miller, Henry McE. Knowler, without augmenting the list by mentioning their pupils, or by enumerating other anatomists of stature who were drawn into the debate inadvertently, brings to mind its magnitude and flavor. Over it all hovered the influence of Franklin P. Mall, the nestor, if not the "father," of Anatomy as a science in America, who, though he did not enter into the polemics, seemed to lend the guiding spirit to the proceedings. For nearly a decade this scientific battle drove the opponents to an intense preoccupation with the problem, resulting in the publication of a hundred

interesting papers, many of them voluminous. A curious aspect of it was that it remained almost wholly confined to America and that the fruit it bore constitutes an outstanding or unique contribution by American anatomists to biological science. Nevertheless, the complaint of some European workers was justified that during the same period their own observations in the field of lymphatic vasculogenesis, some of them with prior claims, were given slight attention, if not ignored, by their American confreres.

Despite the strenuous effort expended on the question, the conflict of conclusions has stayed unresolved. The pertinent chapters in some of today's anatomical and embryological textbooks suggest almost as fixed an adherence to one or the other of the opposing views as 50 years ago. Thus, the challenge stands to turn to the attack again, though from another point of vantage; for the writer it holds an added incentive, since this is seasoned with more than a pinch of poignancy in his recollection of the clash of those times. The motive becomes the more compelling in the realization that the persisting disagreement stems not from a dearth of data, but in large measure from the omission of the exponents in disregarding the biological fact that the developing embryo represents a succession of provisional stages—a process so self-evident to the embryologist as to be overlooked by him in the interpretation of morphological events. Its cognizance and the steps it marks in the development of the lymphatic system provide,

¹ Photographs prepared with the assistance of E. N. Hamilton, director of the Audio-Visual Department of the College of Medical Evangelists, Loma Linda and Los Angeles, California.

therefore, the decisive intent and the crux of the present discussion. The chick material which gave the final impetus to this paper was prepared, studied and evaluated by the writer about 20 years ago. The daily round of academic duties, along with a diversion of interest to other research, ostensibly was the reason for the long delay in publishing the findings; actually it was an underlying hesitation born of fear that the very simplicity of the explanations they offered might mask pitfalls of error—a caution, so tersely put by Whitehead's aphorism: "Seek simplicity; and distrust it."

Obviously, from what has been said, the limitation of space will not permit reference to all those papers which could afford a desirable background. The ones alluded to are employed chiefly to direct the drift of thought beyond the contemporary status of the subject to an idea that has remained in neglect or eclipse.

In 1896, Fr. Saxer, working in Professor Marchand's laboratory at the University of Marburg, was lured, from investigations on the changes in the mesenteric lymph nodes in man during typhoid fever, to focus on the formation of normal nodes. In the course of his studies he observed in the neck of a 2.5 cm bovine embryo on both sides a "cystic cavity," lined with flattened endothelium, from which an oblique communication joined the adjacent venous trunk. Saxer had discovered the "jugular lymph sac," the structure that has bulked so large in every discussion on the origin and development of the vertebrate lymphatic system, from the time when Sabin in 1901 spoke of it as a "lymph heart" to the present day when we see more clearly its phylogenetic significance.

We cannot say to what degree, if at all, the old and long since rejected idea of the open continuity of lymph radicles with intercellular spaces in connective tissue influenced the thinking of Albrecht Budge, who in 1881 published the first embryological work on the lymphatic system—if we exclude the observations of earlier investigators on the growth of lymph capillaries in the transparent tail of the tadpole. Budge died (1885) before he had completed his studies for a larger treatise on

the lymphatics in the chick embryo; his notes and figures were brought together and edited by Professor Wilhelm His and printed in 1887. We now know that the channels which Budge had injected between ectoderm and entoderm of the area vasculosa, and which he believed to comprise a "primary lymphatic system," were nothing more than the forming extra-embryonic coelom. What he designated as the "secondary lymphatic system," foremost in it the thoracic duct, arose dorsal to the aorta either from the pleuroperitoneal cavities or from spaces in the tissue.

That the concept of "open mouths" of lymph channels was at the bottom of the view of their origin from transformed connective tissue spaces, expressed as early as 1836 by Gilbert Breschet in Paris and by other anatomists later, is more than likely. This theory of lymphatic genesis was formulated plainly in 1894 by G. Lovell Gulland, physician at the Royal Infirmary in Edinburgh, in a paper dealing primarily with the development of lymph nodes. In summary, he says: "It is interesting to note the difference in the mechanism of development between lymph vessels and blood vessels. The former arise by dilation of pre-existing spaces, while the latter (apart from their very first origin) begin as solid buds from pre-existing capillaries, which only become hollow at a later date."

To get at the root of the other theory of lymphatic genesis, namely the outgrowth of the lymph trunks from the venous system, we must go back a hundred years to the discovery of endothelium and to the observations on the formation of both blood and lymph capillaries by sprouting. The noted histologist Louis Ranvier in 1895, shortly before his retirement from a long and active career at the College de France, observed this mode of extension of lymph capillaries in the skin of pig fetuses, thus confirming the work of German investigators several decades earlier on the development of such vessels in the tadpole's tail. On the ground of these observations and, it must be said, on the barest of evidence otherwise, Ranvier briefly announced the hypothesis that the lymphatic system, which he regarded as an immense vascular gland pouring into the

venous system its secretion, the lymph, had its inception as an offshoot from the veins and spread from center to periphery by budding. Throughout his work, Ranvier tried to heed the warning he voiced in one of his short papers on the lymphatics: "En Anatomie, rien ne peut prévaloir contre l'observation direct des faits et, lorsque l'on s'en écarte, quelles que soient les autorités sur lesquelles on s'appuie, on a bien des chances de tomber dans l'erreur," but in this case he did not test his hypothesis sufficiently. Being unable to inject the lymphatics in pig embryos before the 10-cm stage, a time when the embryology of the system may be considered to have been realized, as was shown subsequently, he assumed that it began to develop then.

In 1900, Luigi Sala, of the University of Rome, described the development of the posterior lymph hearts and the thoracic duct of the chick in a paper that would have received recognition much earlier than it did had embryologists known what to do with his seemingly ambiguous report. He portrayed the paired thoracic duct as arising from isolated spaces amongst compact mesenchymal cords and masses which appeared along the aorta from the neck to the level of the coeliac artery in the abdomen. Twelve years later, Adam Miller ('13) demonstrated the meaning of those mesenchymal aggregates as of hemopoietic nature, their cells being released into the forming thoracic duct which, as soon as it had become a continuous channel and its communication with the veins by way of the jugular lymph sac had been established, evacuated them into the blood stream. Sala's account of the posterior lymph hearts in 6- to 7-day chick embryos also led to confusion and misunderstanding since he spoke of the progressive excavation of little mesenchymal spaces or fissures which early connect with lateral diverticuli of the first 5 coccygeal veins of the same side. A few lines further he remarked that these spaces might be looked upon as "ramifications of the veins themselves." The work of later investigators, primarily that of Randolph West ('14), on the development of the posterior lymph hearts in the chick, have placed such misleading statements into the proper perspective. Additional clarifi-

cation is gained by a comparison with the vestigial and provisional stages of the jugular lymph sacs, these structures emerging at a much earlier point along the mainstream of evolutionary history than their posterior counterparts.

More forcible than the work of Ranvier in persuading the minds towards the espousal of the hypothesis of lymphatic genesis by outgrowth from the veins was the famous paper "Lecithoblast und Angioblast" by Wilhelm His in 1900. As far back as 1868 His had reinvestigated the blood islands to ascertain their exact derivation. Meanwhile, the observations of histologists on the growth of blood and lymph capillaries by sprouting induced him to propose the theory that the blood vessels of the extra-embryonic membranes invade the embryo, that these ingrowing cords of endothelium gradually spread through the body, and that from them the aorta, the heart anlage, the veins, etc. ultimately are formed. The theory, thus sketched, said that the fundamental morphology of the vascular system rests on the specificity of the endothelium or, in the words of His ('00): "Zu den am frühesten sich sondernden Gewebsanlagen gehört der Gefässkeim oder Angioblast. Seine Sonderung erfolgt sehr scharf, und sein Wachstum geht nach durchaus eigenthümlichen Gesetzen vor sich." The differentiation of the two tissues, endothelium and mesenchyme, occurs very early, he thought, so that the angioblasts once generated give rise to all the vascular endothelium of the body. This concept of His, though disproved, at least in part, some years later when with the use of experimental methods it was found that the large intra-embryonic hemal trunks began *in situ* from at first discontinuous anlagen or "blood islands" in the mesenchyme, was productive, because of its provocative nature, of a great amount of research, particularly in experimental embryology.

In America, the doctrine of the early specificity of endothelium was to receive its staunchest support in the anatomical laboratory of Johns Hopkins University at Baltimore. Professor Mall, recognizing the meager knowledge—the inconclusiveness of the differing observations of Budge and Ranvier—on the origin of the lymphatic

division of the vascular system, inspired his talented pupil, Miss Sabin, to apply herself to the problem. She commenced by injecting India ink at definite points into the subcutaneous tissue of pig embryos in a series of progressively older stages. In the course of such trials and the results noted, she arrived at the conclusion that all lymph vessels bud from the veins at 4 primary centers and then spread into the skin and into the deeper-lying regions by action of centrifugal growth. In the publication in 1902 of her work she says: "It has now been shown that the lymphatic system in the embryo pig begins as two blind ducts which bud off from the veins in the neck. At the very start the openings of these ducts into the veins are guarded by valves formed by the direction which the endothelial bud takes as it grows from the vein. In the ducts themselves there are no valves at first. From these two buds and later from two similar buds in the inguinal region ducts grow toward the skin and widen out to form 4 sacs or lymph hearts and from these sacs the lymphatics grow to the skin and cover its surface. At the same time there is a growth of ducts along the dorsal line following the aorta to make a thoracic duct from which the lymphatics grow to the various organs. Thus the ducts of the lymphatic system gradually invade the body, . . ." Sabin illustrated her conception with a few diagrams which, because they were the first figures to delineate the propagation of the lymphatic system, and because they were simple and hence easily understood, soon found entrance to anatomical and embryological books the world over, and have been reprinted many times despite the fact that not long after they were first published other observations disclosed information considerably at variance with them.

In Italy, an extensive work by G. Favaro, issued in 1905, on the vessels in the tail region of fishes, described paired "vasa intermedia" lying between the caudal artery and vein and connected with both. He held that they were formed from many serial evaginations of the vein, that they fused with one another lengthwise, that they served both a hemal and a lymphatic function, and that in reality they repre-

sented the subvertebral lymphatics or thoracic ducts. In the same year ('05), Frederic T. Lewis, at Harvard University, from a study of the lymphatic system in rabbit embryos, concluded similarly that it sprang from the veins not by centrifugal outgrowth and extension but by confluence of multiple venous anlagen. In the hind regions of the embryos he noticed that parts of the subcardinal veins seemed to become isolated and transformed into lymph vessels. Led by this conjecture, he began a more systematic investigation of the pathways of the larger lymph channels of the body. He saw that the lymph sac on each side in the cervical region arose along the corresponding internal jugular vein apparently by the union of several venous outgrowths which then broke from the parent vein to compose a large chamber. Later, this sac rejoined the venous system permanently. He also saw a chain of endothelial-lined spaces which looked to be detached outpocketings of the azygos veins in the path of the potential thoracic duct. Because of this location and their serial succession, he supposed that they coalesced with one another and with the mesenteric and jugular lymph sacs and thereby created the continuous conduit. The lymphatic anlagen could hardly be distinguished from blood vessels and he ventured the guess that lymphatics grow by the regular addition of spaces cut off from them.

In 1907, Professors Huntington and McClure, of Columbia and Princeton Universities, respectively, withdrew their former opinion deriving the jugular lymph sacs from perivascular mesenchymal spaces, when after a more intensive study of the development of these sacs in the cat they found that their findings confirmed those of Lewis. Their formidable and profusely illustrated monograph, published three years later ('10), had as one of its chief objectives to depict the embryology of those lymph sacs in a representative mammal in such detail that it would supply the basis for comparison in like studies not only in other mammals but in amniotes generally. With figures of numerous wax-plate reconstructions of the relevant areas in consecutive stages of cat embryos, they portrayed the derivation of the jugular

lymph sacs from the precardial (internal jugular) veins and in part from the postcardinal by the coalescence of a series of outgrowths or diverticuli, which they called "veno-lymphatics" to denote their venous origin and their conversion into lymphatics. At first, McClure extended this explanation to embrace also the mode of formation of the thoracic duct, so indicating his agreement with Lewis. But he soon retreated from this stand as resting on scanty evidence, and returned to the theory affirmed by Huntington from the outset that, except for the jugular lymph sacs, all other lymph trunks come into being by the confluence of extra-intimal spaces, which initially appear as small, discontinuous vacuoles just outside the endothelium of temporary and redundant embryonic veins. Huntington and McClure were emphatic in their conclusions that the terminal segments of the lymphatic system, here the jugular lymph sacs, spring from the veins, and that all lymph channels beyond are produced directly from mesenchyme.

Sabin ('08, '09), too, after renewed study of the lymphatic system in both pig and human embryos, modified somewhat her original concept, insofar as she now concurred with the finding of Lewis that the blood-vascular plexus which is to become the jugular lymph sac transiently severs its connections with the internal jugular vein and then joins this vein again by the acquisition of its lasting outlet. In principle, however, she held fast to the proposition that all lymph channels, peripheral and deep, arise by budding from the veins, if not directly then from their derivatives, the lymph sacs. She admitted the existence of "mesenchymal spaces" which, she said, "undoubtedly contain lymph," but which "were morphologically excluded from the lymph system because they could not be injected and because they did not possess a well-defined intima." She believed that the thoracic duct originated by the union of sprouts projected from the jugular lymph sac in the neck and from the venous plexus representing the developing cisterna chyli in the abdomen. Yet she was hesitant in this matter, since she confessed that the genesis of the thoracic duct was the most difficult part of the lymphatic

system to comprehend because of "our inability to inject it in its early stages."

In the annual session of the American Association of Anatomists in 1910, at Cornell University (Ithaca, New York), Sabin reiterated her view of the venous origin of the thoracic duct as a shoot growing from the forming jugular lymph sac towards the abdominal region. At the height of the ensuing discussion McClure asked her whether she would willingly loan him from her collection an injected and serially-sectioned pig embryo chosen as a crucial stage when the duct had not yet completed its entire course. She assented graciously, and soon thereafter sent him a well-injected and sectioned 23-mm embryo, granting at the same time permission to publish any observations that might be deduced from it. The specimen was turned over for study to the present writer who, in the autumn of 1909, had begun, in Professor McClure's laboratory of comparative anatomy, an inquiry into the beginnings of the thoracic duct in the pig embryo.

Kampmeier reported his findings briefly at the anatomical meetings convened at Princeton University in 1911, and more amply in his doctoral dissertation published in the following year. They substantiated the idea of the inception of the thoracic duct from blind mesenchymal spaces that spring up either around degenerating venous channels or in their vicinity but independent of them. The duct becomes continuous and lengthens not by centrifugal sprouting but by the centripetal addition of these discrete anlagen. In the pig embryo, loaned by Sabin, such distinct and uninjected rudiments were visible at intervals beyond the reach of the injected upper stretch of the thoracic duct in the path subsequently taken by it. Though the specimen was judged as exemplifying a fully achieved injection, a supposition which the extravasations—betokening a more than adequate pressure during the injection—at the farthest tip of the continuous and filled portion of that vessel seemed to prove, Sabin in a later critique ('11) implied a denial of this evidence when she declared that the non-existence of solid endothelial extensions between the successive and apparently interrupted rudiments has yet to be shown. Be that as it

may, these lymphatic spaces, both in Sabin's specimen and in Kampmeier's material, revealed a local reduction of tissue fabric during their expansion, fusion and prolongation, and the differentiation of their bordering cells into endothelium. Where the anlagen exist as extraintimal spaces, usurping the route of vanishing veins, the endothelial lining of the latter is seen collapsed in the lymphatic lumen, perhaps because of the decreasing blood pressure within them and the increasing lymph pressure upon them. Similarly, mesenchymal spaces impinging upon and replacing the receding channels of an antecedent venous plexus in the territory later occupied by the cysterna chyli initiate this abdominal division of the thoracic duct. At the time, Kampmeier felt no insistence to examine also the mode of origin of the uppermost or cervical division of the duct, because so confident and unanimous were all investigators in their statements on the derivation of the jugular lymph sacs from the veins.

That the jugular lymph sacs in the bird begin like those in the mammal was seen by Mierzejewski ('09) in Henryk Hoyer's laboratory at the University of Cracow. Near the junction of the subclavian, vertebral and jugular veins in the chick embryo he noted a subsidiary network of vessels which, by enlargement and then by further condensation, produces a sponge-like ("schwammartig") organ, comparable to the posterior lymph hearts in the same embryo. That Mierzejewski had missed a second step in its formation was pointed out by Adam Miller ('12). After telling how the "veno-lymphatic" rudiments of the future jugular lymph sac were in connection with a dorsolateral set of tributaries of the pre- and the postcardinal veins at their common cardinal (Cuvierian) confluence, in contradistinction to a more medial set—the potential dorsal somatic or intersegmental veins, Miller described the detachment of the first set from the parent veins and their progressive amalgamation to create what he termed a "pre-lymphatic sac." It becomes the jugular lymph sac as it acquires the permanent venous tap with the precardinal (internal jugular) vein on its dorsomesial side at the level of the subclavian vein. In the early embryonic life

of the bird there is thus an exact repetition of what occurs in the mammalian animal as related previously by Lewis in rabbit embryos and by Huntington and McClure in cat embryos, and as acknowledged later by Sabin in pig and human embryos.

Eleanor L. Clark, in 1912, stated correctly that the use of the term "sac" for the "jugular lymph plexus" in the chick is a misnomer, since this complex of channels never becomes the more or less uniform chamber characteristic in the mammal. More explicitly, she leveled a sharp criticism at Miller's account when she placed his reconstructions and her injections of the same developmental stages side by side to expose the fallibility of the former method. She restated the earlier contention of Sabin, Eliot R. Clark and others that vessels which in living embryos and in injected ones are seen to be unbroken may appear in sectioned embryos as isolated segments, consequently explaining the discrepancies or contradictions in the views concerning lymphatic development. Her own description of the jugular lymph plexus can be epitomized as follows: Soon after outgrowth from the veins, it becomes a close-meshed and intricate net, as displayed in her cleared specimens and illustrated here in figure 2. In all the chicks injected between 5 and 8 days of incubation she could demonstrate its connection with the venous system. In the younger stages the connections are numerous, but afterwards their number is reduced to one of two which she presumes to be its definitive vent. She seriously questions, therefore, the statements alleging the transitory separation of the sac from the veins and its subsequent reunion with them. During the formative stages of the jugular lymph plexus, its channels are filled with stagnant blood, this having backed up into them from the veins. It is during this "non-functioning" period, lasting from 24 to 30 hours, that the sprouting from the plexus and the rapid distribution of the peripheral lymphatics occur.

Because the genetic history of the amphibians' anterior lymph hearts, the equivalent of the jugular lymph sacs of amniote vertebrates, bears directly on the present discussion and illuminates it, we must allude to it. From necessity we approach

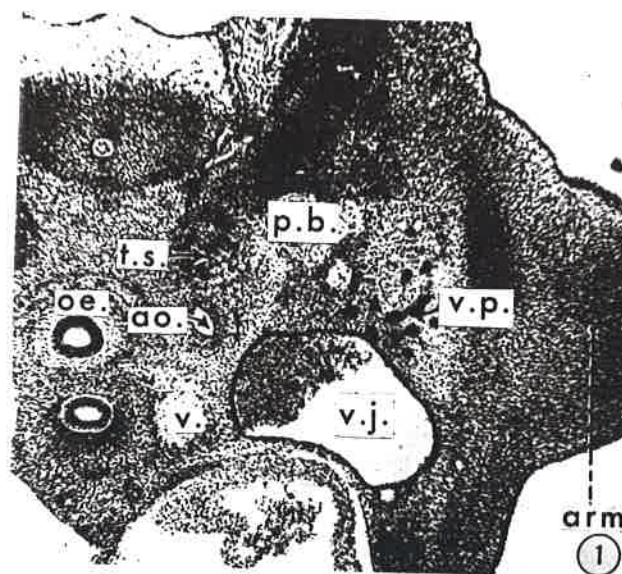


Fig. 1 (Reprinted from Morph. Jahrb., 1931, 67: p. 178; Otto F. Kampmeier). Transverse section of a 9.1-mm human embryo at the level of the upper limb bud (Arm). $\times 64$. Abbreviations: v.j., left internal jugular vein; v.p., venous plexus (black), forerunner of the jugular lymph sac; ao., aorta; oe., esophagus; v., left vagus; t.s., left sympathetic trunk; p.b., plexus brachialis.

it by a roundabout way. The immediate topographical relations of these hearts to the common cardinal confluences (ducts of Cuvier) and to the head kidneys, or pronephroi, are definite landmarks when we search for their homologues both in fishes and in higher vertebrates. In young tadpoles (for example, 4–10 mm embryos of *Bufo vulgaris*) the pronephros of each side is situated in the crotch of the pre- and postcardinal veins where these converge to the common cardinal vein. These veins combine in a plexiform manner, producing a net of sinusoids which surround the pronephric tubules and intertwine with them, and which composite is known to comparative morphologists as the "pronephric sinus." When the proximal end of the postcardinal breaks away from the common cardinal vein during the succeeding stages up to the metamorphosis of the tadpole, the pronephric sinus in consequence composes the base of the precardinal (internal jugular) vein where this

meets the precava (anterior vena cava), the earlier common cardinal vein (Kampmeier, '20). Meanwhile, the subclavian vein becoming established and confluent with the internal jugular at this point, the pronephric sinus, along with the degenerating pronephric tubules, actually comes to lie at the "jugulosubclavian angle"—a term, so familiar in human anatomy. That there is a remarkable stability in such phylogenetic clues is manifested again in an incidental observation, hitherto unpublished, made by the writer some years ago; in a 9.5-mm human embryo—a developmental stage when all vestiges of pronephric tubules have long been lost, except rarely as this instance shows, he found on the left side at the level of the 7th cervical spinal ganglion a coiled tubule of that kind lying next to the common cardinal vein and opening into the cephalic tip of the coelomic (pleuroperitoneal) cavity. In the chick embryo the longer persistence of the vestigial pronephric tubules is a constant feature (cf. figs. 5, 6 and 17, p).

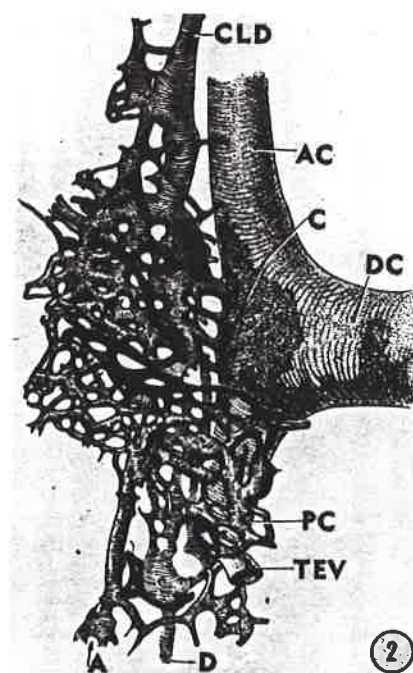


Fig. 2 (Reprinted from Anat. Rec., 1912, 6, p. 263; Eleanor L. Clark). Lateral view of the right jugular lymph plexus (injected with dilute India ink) in a chick embryo incubated 5 days and 20 hours. $\times 50$. Clark's explanations are: A, vessel connecting the superficial plexus, into which the injections were made, with the jugular lymph plexus; A.C., anterior cardinal vein; P.C., posterior cardinal vein; D.C., duct of Cuvier; C, (5) communications between lymph plexus and vein; through these connections the latter received some ink granules; D, deep lymphatic; T.E.V., thoraco-epigastric vein; C.L.D., cervical lymphatic duct.

The development bilaterally of the anterior lymph heart in the tadpole (4–10 mm toad embryos) takes place adjacent to the pronephric sinus. The first three dorsal intersegmental veins, with which the first lymphatics to be evident are associated, are tributaries of the pronephric sinus, an arrangement resembling that in avian and mammalian embryos in whom the first 5 or 6 dorsal intersegmental veins drain into the internal jugular and common cardinal veins. Knowler ('08, '39),

Hoyer ('05, '08, '34) and Kampmeier ('22) independently demonstrated the genesis of the anterior lymph hearts in different anuran species from a rather circumscribed and simple venous plexus on the lateral side of the third dorsal intersegmental vein and in continuity with it, so that the outlet of this vein into the pronephric sinus is at the same time the exit of the heart anlage. Both Hoyer and Knowler described the beginning of the jugular and the lateral line lymphatics as sprouts growing respectively cephalad and caudad from the developing heart. According to Kampmeier, this organ, as it consolidates from the plexus into a unitary and globular chamber and its walls thicken with the onset of differentiation of muscle cells, temporarily separates both from the forming afferent lymphatics and from the venous system, and then, shortly after, reunites with them, as valves arise in the portals of inflow and outflow and prepare it for effective pulsation. Kampmeier ('25) found an identical sequence of events in the development of the posterior lymph hearts, except for the one extraordinary difference that they have their beginning not from a hemal but from a lymphatic plexus. In groping for the meaning of this—at first thought—irreconcilable genetic diversity, he pointed again to the differences in time of origin between that of the anterior hearts in a 4-mm (*Bufo*) tadpole and that of the posterior hearts in a 11-mm one. The implied assumption of a notable advance in tissue differentiation in the interim, causing like but later structures in the anteroposterior gradient to begin differently, was emphasized with more assurance recently (Kampmeier, '58). Other perplexing observations of similar nature will be dealt with in a book in preparation on the comparative morphology and evolution of the lymphatic system.

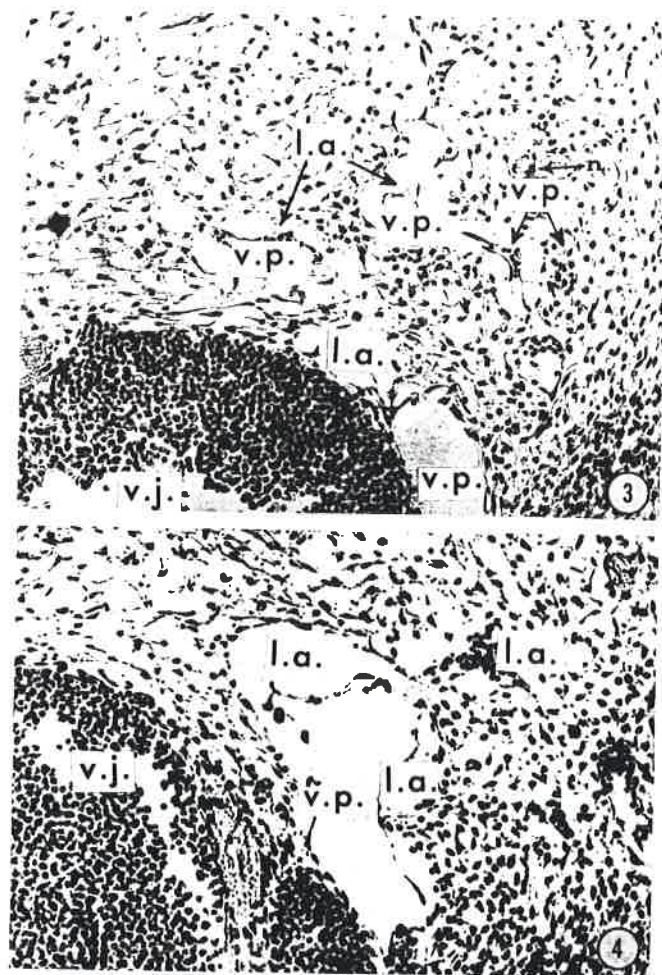
Knowler ('39) shortly before his death published an extensive and richly illustrated monograph on the development of the vascular system of frog tadpoles, as set forth by the injection method; much of this treatise is a critique of Kampmeier's findings. Particularly, he averred that none of his material lent support to the idea of a transitory isolation of the lymph heart synchronizing with the formation of its

first valves. He was, therefore, in entire accord with Eleanor L. Clark who, as said before, could not at any time detect a total detachment of the jugular lymph plexus from the veins in her injected specimens of chick embryos. On the other hand, on the topic of the mode of origin of the posterior lymph hearts in the tadpole, Knowler conceded the correctness of Kampmeier's observations as against the erroneous conclusions of Hoyer and his pupils who thought they could see these organs come into being, like their anterior fellows, by splitting from the veins. It may be surmised that this school of observers was misled by virtue of an implicit faith in the utmost harmony of behavior of intimately related biological processes—here, the generation of all lymph hearts. Such conflicting or paradoxical observations may also pass as a reply in part to Eliot R. Clark, who has been a long-standing and the most severe critic of the present writer's papers on lymphatic vasculogenesis, and who again not long ago ('40) flayed their apparent inconsistencies.

The memorable controversy on the genesis of the lymphatic system had subsided by the middle of the second decade of this century, and since then only sporadic though noteworthy papers, among them, for example, that of Knowler just mentioned, have barely kept it from being wholly forgotten. By then anatomists and embryologists everywhere had settled down to the avowal of the preponderant deduction that such parts of the lymphatic system as the lymph sacs, which are in immediate connection with the veins, spring from veins. Concerning the genesis of all lymph trunks beyond, notably the subvertebral lymphatics (thoracic ducts), the opinions remained as widely divided as ever. Consequently, the writer's astonishment can be imagined when a casual examination and then a more attentive study (Kampmeier, '31) of the forming lymphatic system in man brought to light that the jugular lymph sacs arise in precisely the same way as the thoracic duct, from mesenchymal anlagen replacing decadent and vanishing veins. What all investigators (Sabin, Lewis, Huntington, McClure, the Clarks, Mierzejewski, Hoyer, etc.) had regarded as the venous anlage of the sac

was but an elementary, provisional or, may we say, a vestigial substratum or forerunner of it. Just as the developing thoracic duct, continuous throughout its length in 25- to 30-mm human embryos, shows within its lumen the shrunken endothelial shells of discarded veins (cf. microphotographs, Kampmeier, '31) and even the traces of their former connections with the functioning venous trunk (azygos) nearby, so likewise the mesenchymal spaces that create the jugular lymph sac, invest and absorb the channels of the preceding venous plexus, thereby taking over their place (figs. 3 and 4). The objections that such configurations might be artifacts, that is, shrinkage products of fixation, were refuted point by point by the writer earlier ('12) and need not be repeated here. The latent, if not complete, detachment of the degenerating venous plexus from the parent veins probably occurs in human embryos of 16 to 20 mm length. The final, competent opening of the jugular lymph sac into the bloodstream is not obtained, it seems, until the sac has gained its greatest extent, proportionately, in 30 mm embryos; its expansive growth ceases at this period with the acquisition of its valvular aperture.

The radical departure from the then universally accepted view of the venous offspring of the jugular lymph sacs has gone virtually unnoticed by American anatomists. Its exposition in a foreign journal, and the greatly diminished curiosity, at the time, in the problem of lymphatic vasculogenesis, perhaps militated against the force of the new evidence. The data, with reference to the human organism, had just been assembled, when the writer was invited to submit a paper to the "Festschrift" honoring Professor Ernst Göppert of the University of Marburg; thus, it happened that the manuscript was rewritten in German for publication ('31) in the *Morphologisches Jahrbuch*, of which that eminent comparative anatomist was editor. About 10 years later, a revival of interest in the embryology of the lymphatic system was stimulated by the excellent and exhaustive monograph of Arnold A. Zimmermann ('40) on the origin and development of that system in the opossum. It corroborated



Figs. 3 and 4 (Reprinted from *Morph. Jahrb.*, 1931, 67: pp. 179 and 180; Otto F. Kampmeier). Transections through the area of the forming left jugular lymph sac in a 14-mm human embryo. $\times 210$. Abbreviations: v.p., degenerating and collapsing channels of the venous plexus at the dorsolateral side of the internal jugular (precordial) vein, v.j., are being replaced by the lymphatic anlagen, l.a., here appearing as extra-intimal mesenchymal spaces.

in all essential details Kampmeier's observations and deductions. What is most striking is the close similarity between the marsupial animal, near one end of the mammalian scale, and man, at the other

end, in the microscopic appearance of transections of the jugular lymph sac area during its formative stages; a comparison of the photographs of respective sections, as pictured in the two publications, evinces

no telling differences between the two mammals in the morphogenetic and histogenetic events that transpire here.

The indubitable evidence for the mesenchymal origin of the jugular lymph sacs in man and mammal casts doubt on the validity of the contrary assertions by Eleanor L. Clark and others about the venous origin of the same structures in the bird. Yet more trenchant in our scepticism is not the improbability of diverse derivations of homologues, but what seems superficially to be a trivial point in method of examination. Clark made her observations mainly on whole mounts of successive stages of chick embryos which had been injected with India ink and examined under the microscope either in their living state or when fixed and cleared. Her drawing, reproduced in figure 2, of the "jugular lymph plexus" pictures the things seen in a specimen of this kind. The character, continuity and complexity of the opaquely-filled plexus are nicely exhibited, but obviously any mesenchymal spaces that might lurk, so to say, among its meshes would escape detection. To weigh this criticism, the circulatory system of a series of chick embryos were injected by the writer, and these were then serially-sectioned and stained for study; his collection comprises altogether 37 embryos, ranging in age from 75 to 180 hours (three to 7½ days) of incubation and a number (17) of which were injected, some of them with success. The data which both the injected and the uninjected specimens yielded are briefly set forth in the following, and the proof that the definitive jugular lymph plexus of the bird arises genetically like the jugular lymph sac of man and of the opossum is furnished by the photomicrographs, figures 5 to 17. As these pictures show, the most significant aspects—at any rate, those that strike the eye—come to pass in the period between 4½ and 6 days of incubation.

Figure 5, typical of a cross section through the neck of a chick embryo at the end of the 4th day, may serve the purpose of orientation. On both the right and the left side, the territory demarcated laterally by the ectoderm, medially by the aorta, dorsally by the lower margin of the myotomes, and ventrally by the precardinal

or internal jugular vein near its junction with the common cardinal vein, is the scene of the processes that are to inaugurate the jugular lymph plexus of the corresponding side. For more specific lines of reference, attention is directed in particular to the dorsal somatic intersegmental tributaries of the cardinal veins, their bifurcation into mesial and lateral branches and their anastomoses traversing the myotomes. Subsequently, these mesial and lateral divisions of the intersegmental veins tend more and more to drain into the cardinal veins by separate stems.

Needless to say, the development of the jugular lymph plexuses of the two sides is identical. Clark pictured the right plexus (fig. 2). In the present paper, preference is given to the left one, since the figures of it can be compared more conveniently with those of the mammalian, especially the human, left jugular lymph sac, which is customarily chosen for portrayal, in view of the later asymmetrical plan of the thoracic duct and its discharge via the left sac.

In the 4.5-day chick the venous plexus has made its appearance, the compound of which is to be appropriated and reconstituted by the succeeding lymph sac. As shown in figure 6, vp, it occupies the region defined above, and it is in continuity with the respective dorsal intersegmental veins, especially their lateral divisions, and directly also with the lower or terminal portion of the precardinal (internal jugular) vein, the common cardinal (duct of Cuvier) and the upper end of the postcardinal. Its association with these veins and its pattern when injected are indicated three-dimensionally in Clark's figure 2, although this is of a later stage (5 days, 20 hours). Comparing the sections, such as figure 6, with figure 1 from a 9.1-mm human embryo, verifies the identity of this venous plexus and its equivalent location in the cardino-Cuvierian territory in bird and mammal.

The first signs of the imminent formation of the jugular lymph plexus are discerned in the chick embryo of 4¾ days of incubation as faintly suggested spaces clinging here and there to some of the channels of the venous plexus just pointed

out. Only a few hours later (approx. at 5 days) the collapse of these channels and the expansion of the mesenchymal spaces engulfing them are in full sway, as illustrated in figures 8 to 10, and continue so until the 6th day. Section after section from embryos in the period between the 5th and 6th day—and only then—display such a bewildering abundance of extra-intimal cavities in the restricted territory indicated that the selection of examples for illustration can almost be a haphazard one. Coincidentally, in that period of rapid activity and change, more and more of the connections of the short-lived hemal plexus with the neighboring venous trunks and their immediate tributaries are lost, so that the shells of cut-off and shrunken vessels, and the stagnant blood trapped within, are conspicuous features in the distending lumina of the lymph plexus. The progressive segregation and discard of the venous plexus are confirmed by figures 13 to 15 from a 5.3-day embryo whose blood vascular system was injected with considerable pressure; here, the channels of the lymph plexus are seen to be empty of India ink, except for a small, enveloped and evidently evanescent vein containing particles of it. The histological evidence of the vanishing connections between the defunct and the functional veins is unmistakable. At the 6th day their traces are especially numerous, manifested as darkened cellular lines, doubtlessly endothelial, extending from the deteriorating vessels to the walls of the cardinal veins. Whether all of the original connections break away is open to question according to Clark; if the isolation of the venous plexus is not total in fact, then it is so in effect, the present writer believes, and the final valvular outlets of the jugular lymph plexus into the venous system may really be new formations (figs. 16 and 17).

We ask ourselves, what eventually is the fate of the abandoned venous channels within the cavities of the lymph plexus? In the more advanced stages (approx. 6-day) their dwindling endothelial remnants look like "ghosts," their pale-staining reaction leaving the impression that they are dissolving.

The increment and dilatation, proportionately, of the jugular lymph plexus take place before an effective valvular communication with the venous system is established, as witnessed in figure 17. The same figure suggests that the increasing distention of the plexus is responsible for the emergence of its meshes, though this process is not carried as far as that which produces the more sac-like chamber in mammals.

During the development of the chick's jugular lymph plexus—finished fundamentally in one day, that structure may harbor a great amount of blood (figs. 7, 9–11, 15–17). As already intimated, much of this is due to stasis when the precursory plexus is being eliminated from the circulation; some of the blood is unquestionably newly-formed, since hemopoiesis occurs to a limited degree in the mesenchymal interstices (fig. 10, h) embodied in the expanding lymph plexus, but to a more massive degree it occurs near the aorta and there has access to the developing thoracic duct (figs. 8 and 9). With the completion of a guarded emptying mechanism, marked by the advent of valves, the lymph plexus and its afferents, principally the thoracic duct, truly execute an hemophoric function at the onset of their activity by sweeping the accumulated blood cells along with the lymph into the venous system.

The apparently strange phenomenon of an orderly network of vessels being destroyed to make way for another one in exactly the same site, and both of them functionally related, is, nevertheless, in keeping with what happens in other systems of organs during development. Embryologists and anatomists in general habitually think of the embryo as having its eyes fastened—figuratively speaking—on the goal of creating the fully-formed individual and of reaching that goal in a straight-forward and undeviating manner. The truth is, of course, that the rapidly developing embryo is an organism of rapidly changing immediate needs which perpetually call for revisions and readjustments intrinsically; in other words, it passes through a succession of provisional stages before it attains the post-natal condition. The classic example is provided

by the development of the skeletal system, especially by its processes of radical demolition and rebuilding as it proceeds from the chondral to the osseous stage. No less impressive is the far-reaching remodeling that the human metanephros or "permanent" kidney undergoes during its formation; the terms "vestigial zone," "provisional zone" and "growth zone," given (Kampmeier, '26) to succeeding levels of the differentiating nephrogenic blastema, spell that remodeling, the first referring to the primal generation of uriniferous tubules next to the primitive renal pelvis which are doomed from the start, the second referring to the following several tiers of large, well-formed and transiently functioning uriniferous tubules which are crowded out of existence (except for occasional, abiding remnants in the form of congenital cysts) by the widening renal pelvis as this incorporates the corresponding orders of collecting tubules, and the third term referring to the peripheral tiers of developing uriniferous tubules which are destined to construct the cortex of the kidney. Likewise, every other system of organs would reveal, on close inspection, some sort of reconstitution during the embryonic period. Certainly, the embryology of the lymphatic system offers no exception to the biological principle of the urgency of provisional phases during morphogenesis.

In contradicting the prevailing and inherently static point of view that the jugular lymph sac springs as a hemal or "veno-lymphatic" plexus from the veins and becomes directly the definitive one without basic transformations, it is scarcely necessary to stress the present discussion because the accompanying photographs are convincing enough. Yet while reviewing and summarizing the data, the temptation cannot be avoided to enter the realm of speculation and interpret them physiologically; though the deductions may be logical, still they are circumstantial and tentative. The blood-vascular plexus that arises during early embryonic life as a dorsolateral outgrowth from the cardino-Cuvierian venous confluence, and that is preliminary to the coming jugular lymph sac, composes a prototype or proximate model of this structure,

analogous to the blastemal stage that foreshadows the general design of the skeletal system. If, at the same time, it functions as a drainage route for the incipiently minimal volume of lymph from the embryonic tissues, such a result is nullified very soon by the stasis of blood that impedes and perhaps blocks it, because of the back pressure from the veins. In a sense, then, this first phase in the developmental history of the jugular lymph sac may be construed as a vestigial one. The second phase is associated with the flooding of lymph in the same area, as suggested by the rapidly appearing and enlarging mesenchymal spaces around and between the stagnant blood channels. Before the pools of lymph find a "secondary" or final outlet into the veins, the dilating mesenchymal spaces fuse to form a true lymphatic plexus which engulfs the discarded and collapsing hemal one, a process that recalls the deposition of bone on the crumbling cartilaginous framework in endochondral osteogenesis. The degenerating endothelium is seen as caved-in or shrunken capsules—unless distended artificially in injected specimens—within the lumina of the lymphatic plexus, which derives its own endothelium from the bordering mesenchymal cells. In the meantime, the several connections of the hemal plexus with the parent veins are breaking away. In birds the embryonic "jugular lymph sac" retains largely a plexiform character, while in mammals it becomes truly sac-like as the mesenchymal cavities swell more and more and coalesce into a multilocular chamber in which only persisting strands and partial partitions that cross here and there are reminiscent of the earlier network. The third stage of the developing jugular lymph sac leading up to the final condition is distinguished by the establishment of a valvular opening (or openings) between it and the jugulo-subclavian venous junction. The valves, preventing reflux of blood into the sac, and constituting the controlling mechanism of expediting the prompt and unimpeded flow of lymph into the venous system, have the result that the sac now is disproportionately large as a collecting reservoir. What brings about a readjustment and relative reduction in this re-

spect can only be conjectured, for the fact is that eventually merely a part of the sac, the terminal part, will act as the main lymph passage to the veins, while the much greater remainder, the more peripheral part, participates in the events of the 4th stage, namely, the development of cervical lymph nodes—in birds, generally one node on each side, and in man and some other mammals, a packet of them. Accordingly, therefore, even the third stage of the embryonic jugular lymph sac is in part provisional.

Finally, turning to the province of homology, the writer believes that, on the basis of what has been recorded in the foregoing, including a review of the locus, anatomical relations, and mode of origin of the anterior lymph hearts in Amphibia, we can apprehend more accurately now the phylogenesis of the jugular lymph sacs of amniote vertebrates. Strictly speaking, the "vestigial" or hemal phases of these sacs, and not the succeeding provisional and final phases, are to be homologized with those hearts. To elucidate this distinction by analogy, we point to the pharyngeal arches, grooves and pouches—not their later transformations in the embryo—as the rudimentary survival and equivalence of the branchial system in fishes. No less cogent, though much less dramatic, during early embryonic life of the higher vertebrates including man is the short retention of an ancestral stage of the lymphatic system before its conversion and readaptation to altered and growing physiological demands.

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Abbreviations to plates

| | |
|----------------------------|---------------------------------|
| a, aorta | n, notochord |
| b, brachial nerve plexus | p, pronephric tubule |
| c, carotid artery | pc, precardinal or |
| cc, common cardinal vein | internal jugular vein |
| (duct of Cuvier) | pp, pleuroperitoneal cavity |
| e, esophagus | pm, prevertebral muscle mass |
| h, hemopoietic foci, or | s, spinal cord |
| developing blood cells | sl, subvertebral lymphatic |
| hu, humerus | (thoracic duct) |
| jlp, jugular lymph plexus | *, special feature indicated by |
| or sac | explanation of figures |
| la, lymphatic anlage | sy, sympathetic nerve trunk |
| lv, lateral dorsal somatic | v, valve |
| intersegmental vein | va, vagus |
| m, myotome | vp, venous plexus |
| mv, medial dorsal somatic | |
| intersegmental vein | |

PLATE 1

EXPLANATION OF FIGURES

- 5 Transection of a 4-day incubated chick embryo, uninjected, at the level of the lower end of the precardinal veins (pc) just above their confluence with the common cardinal veins (ducts of Cuvier). $\times 75$. In the area demarcated medially by the aorta, laterally by the ectoderm, ventrally by the precardinal (internal jugular) vein, and dorsally by the myotomes, the jugular lymph sac of the corresponding side will later develop. Note the anastomosis of the medial and lateral branches of the dorsal somatic intersegmental veins passing through the myotome.
- 6 Transection of a 4.5-day chick embryo, its vascular system injected with dilute India ink, at the same level as that shown in the preceding figure. $\times 75$. Note the venous plexus, precursor of the jugular lymph sac, dorsolateral to the precardinal or internal jugular vein.
- 7 Transection of a 4.7-day chick embryo, uninjected, of the pertinent area, seen in the orientation figures 5 and 6. $\times 150$. Note the channels of the venous plexus, precursor of the jugular lymph sac, dorsolateral to the precardinal vein; not to detract from the appearance of the field, these channels, some containing blood, are not labeled. A lateral stem (lv) of the dorsal somatic intersegmental veins is seen joining the precardinal.

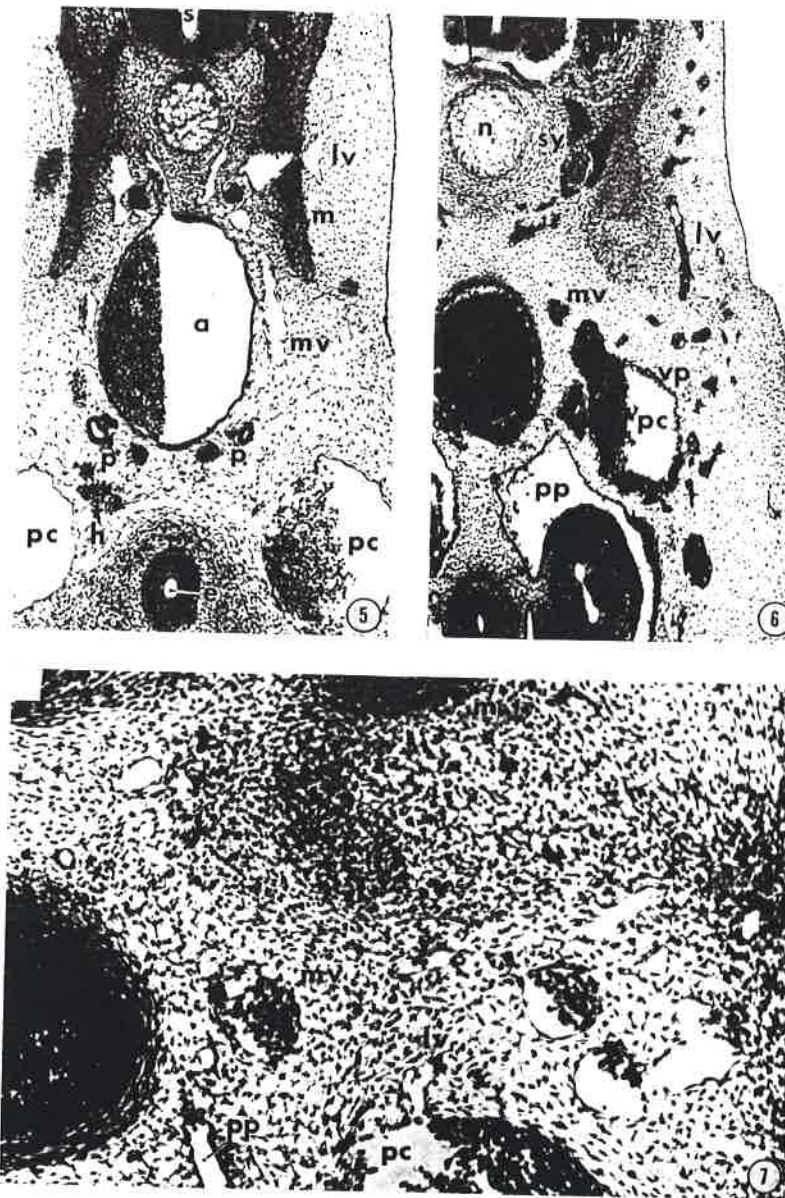


PLATE 2

EXPLANATION OF FIGURES

- 8 Transection of a 4.7-day chick embryo, uninjected, at the level of the lower end of the precardinal (internal jugular) vein just above its confluence with the common cardinal duct of Cuvier). $\times 50$. Especially to be noted are the 4 relatively large vessels lying in a horizontal row to the right (actually left in the embryo) of the aorta, a, and immediately dorsal to the precardinal vein, pc, and explained in the following figure.
- 9 The middle right portion of the inset, figure 8, enlarged, $\times 150$, to illustrate an early stage in the forming jugular lymph plexus. Of the 4 vessels dorsal to the precardinal vein, pc, the one lying next to the aorta, a, is involved in the formation of the terminal or uppermost portion of the left subvertebral lymphatic (thoracic duct); its venous precursor has begun to collapse, as evidenced by its receding endothelium and the extra-intimal space outside it; it contains many blood cells, almost all of them in development, and it lies adjacent to a hemopoietic condensation, h, in the mesenchyme. The second vessel shows the collapse of the contained vein, vp, and its replacement by an extra-intimal space, or lymphatic anlage, la, more strikingly. In the third and 4th channels such processes are barely indicated. The large black mass, *, near the aorta, represents a stagnant vessel (pronephric sinusoid?), stuffed with blood cells, that is in connection with the medial division of a dorsal intersegmental vein and can be traced back through a number of sections to its encirclement of a large, vestigial pronephric tubule.
- 10 Transection of another 4.7-day chick embryo, uninjected, at the same level as before, that is, through the area of the forming jugular lymph plexus. $\times 150$. Note the several channels showing the shrinking, degenerating veins and the origin of extra-intimal spaces (only one of these channels labeled, vp and la) around them in various stages of progress. Observe secondly the hemopoietic foci, h, one next the aorta and associated with the most medial portion (future thoracic duct approach) of the developing jugular lymph plexus, and the other with one of its middle channels (dorsal to the precardinal vein).
- 11 Transection of a 5.2-day chick embryo, uninjected, through the forming jugular lymph plexus, showing the antecedent venous plexus in various stages of regression; at * a stagnant vessel, stuffed with blood cells. $\times 150$.

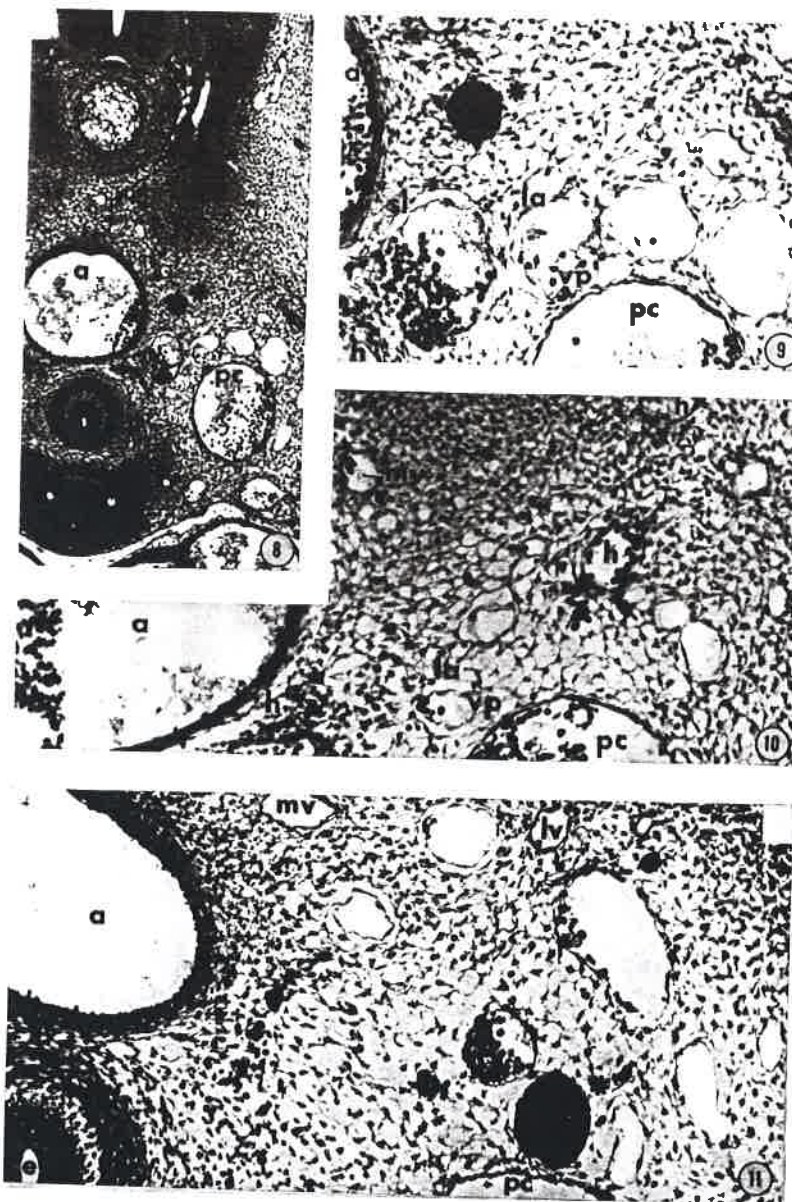


PLATE 3

EXPLANATION OF FIGURES

- 12 Transection of a 5.4-day chick embryo, its blood vascular system fully injected with India ink, at the same level as the preceding specimens. $\times 50$. The pertinent features of this orientation figure are explained in the following illustration.
- 13 The middle portion of the inset, figure 12, enlarged $\times 150$. Note that the retrogressive venous plexus and its replacement by extra-intimal mesenchymal spaces in the development of the jugular lymph plexus contains no ink as compared with the filled channels of the lateral dorsal intersegmental veins, lv.
- 14 Transection of the same embryo as that of figures 12 and 13, at the level of common cardinal vein (duct of Cuvier). $\times 150$. Note that here traces of ink, at *, have entered the retrogressive venous plexus, evidently through a still barely persisting connection with the cardinal veins.

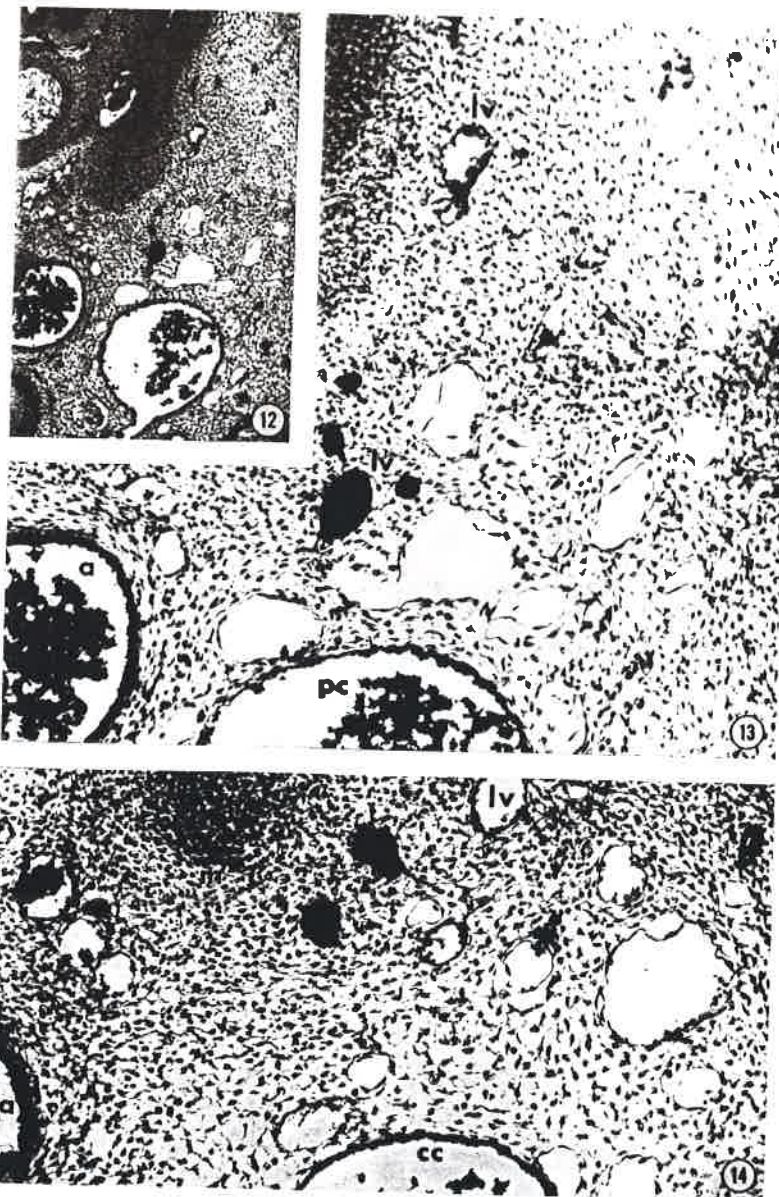


PLATE 4

EXPLANATION OF FIGURES

- 15 Transection of a 6-day chick embryo, uninjected, at the level of the precardinal (internal jugular) vein, through the developing jugular lymph plexus. $\times 150$. Observe within the lumina of the lymph plexus the shrunken shells of stagnant veins, some of them stuffed (black) with blood cells, and others appearing as remnants ("ghosts").
- 16 Transection of a 5.8-day chick embryo, uninjected, at the level of the common cardinal vein (duct of Cuvier), cc, showing a valvular communication at v, of the jugular lymph plexus with this vein. $\times 50$.
- 17 Transection of a 6.5-day chick embryo, uninjected, through the jugular lymph plexus, jlp, at the level of its developing valvular outlet, v, into the internal jugular vein, pc. $\times 50$. Note that the lateral part (black) of the lymph plexus near the brachial nerve plexus is filled with blood.

