Resumen por el autor, Otto F. Kampmeier

El desarrollo de los linfáticos anteriores y corazones linfáticos
de los embriones de los anuros

El orígen y desarrollo del seno linfático maxilar primario,
lifáticos yugulares y corazones linfáticos anteriores de embriones
de sapo es objeto de descripción en el presente trabajo. El
seno se origina a expensas de pequeños esbozos discontínuos que
aparecen como engrosamientos del endotelio de las yugulares
externas en vías de desarrollo o como islotes en el mesenquima
que rodea a las anteriores. Al principio macizos, estos esbozos
adquieren una cavidad y mediante proliferación se alargan, se
funden, ramifican y forman de este modo una red complicada.
Esta red se transforman en un reservorio espacioso mediante
expansión de los canales interanastomóticos y reducción de los
cordones mesenquimóticos que los separan. El linfático yugu-
lar se desarrolla a expensas de un plexo venolinfático que de-
riva de las tres primeras venas intersegmentarias (tributarios
dorsales del seno venoso pronéfrico), cuando estas se separan
del sistema venoso. Después el linfatico yugular establece
continuidad con el séno linfático maxilar primario y con el cora-
zón linfático anterior.

El corazón linfatico anterior se origina en una porción circun-
scrita del plexo veno-linfático, mencionado anteriormente, al
nivel de la tercera vena intersegmentaria original. El esbozo
plexiforme se desarrolla en la cámara cardiaca por distensión y
fusión de sus canales reunidos. Temporalmente se aisla del
plexo veno-linfático circunyacente, pero persiste unido con
las venas en la boca de la tercera vena intersegmental, de la
cual deriva la vena vertebral anterior. Las comunicaciones
entre el corazón linfátido y los linfáticos aferentes se reestablecen
ulteriomente. La formación de las válvulas y la histogénesis de
las paredes del corazón son también objeto de descripción.

THE DEVELOPMENT OF THE ANTERIOR LYMPHATICS AND LYMPH HEARTS IN ANURAN EMBRYOS[1]

OTTO F. KAMPMEIER

Department of Anatomy, College of Medicine, University of Illinois, Chicago

THIRTY-FIVE FIGURES INCLUDING EIGHT COLORED PLATES[2]

THE LYMPHATIC GROUND-PLAN OF THE TADPOLE

As is well known, the vessels which collect the lymphatic fluid and convey it to the lymph hearts and thus to the veins in the fully developed anuran Amphibia are in the form of extensive subcutaneous sacs and deep sinuses. This condition, however, is a relatively late acquisition in development, appearing during the metamorphosis of the individual. Before this period, the lymphatic conduit system consists of narrower ducts and capillary networks, similar to those found in the higher vertebrates. In fact, we can recongnize three periods in the development of the lymph channels in Anura: firstly, the initial formative period, secondly, a phase of specific ducts and plexuses, and, thirdly, the final condition, characterized by broad lymph sacs and sinuses—periods which in a general way coincide with the three into which we arbitrarily divide the embryogeny of frog and toad, namely, early embryonic, larval or tadpole, and metamorphic phases. To follow intelligently the nature of events which occur during the formation of the anuran lymphatic system from its inception to its final configuration, as well as to emphasize particular components and to propose a terminology which will facilitate comparison with other vertebrates, it seems expedient to

[1] The present communication represents a portion of a monograph intended for publication in 1918. Other papers which will follow complete the subject-matter of this monograph. The reason why it was broken into a number of separate parts is explained in a footnote of the first installment which appeared in The Anatomical Record, vol. 19, July, 1920.

[2] The cost of illustrations in part borne by the Anatomical Laboratories.

delineate the topography of the lymphatics functional in an intermediate stage.

In Bufo tadpoles,[3] 12 to 15 mm. long, the chief lymph vessels are already laid down, so that we may say the second phase of lymphatic organization, suggested above, begins at this time.

[3] I spent the spring and summer of 1913 with great profit and pleasure at the Anatomical Institute of the University of Munich, where, through the kindness of Professor Rückert, I was able to enjoy all the facilities of those delightful laboratories. I wish also to mention Dr. H. Marcus, who placed his series of larval Gymnophiona at my disposal. Further, I express my gratitude to Mr. Otto Balbach, of the University of Pittsburgh, who prepared the later series of my numerous sectioned anuran embryos.

Toad embryos constitute by far the bulk of the material used in the investigation. These specimens are of two species, the American common toad, Bufo lentiginosus, and the European, Bufo vulgaris (?), and are respectively from New Jersey and Wisconsin, and from the marshes along the Isar River near Munich. The writer neglected to determine with absolute certainty the specific name of the latter form before leaving Munich. There are but two species which can be considered, Bufo vulgaris and B. viridis, but after comparing them as to distribution, breeding habits, etc., as described in the standard works of Zoology, he is confident that it is Bufo vulgaris. The descriptions and figures are based mainly on the embryos of this European form which were gathered later in the course of the investigation when the writer had attained greater success in the preparation of tissues so profusely filled with yolk as are amphibian embryos. The ova of these Anura were collected shortly after laying and developed in the laboratory aquaria. To procure a closely graded ontogenetic series, active individuals were fixed and preserved at intervals of three to four hours.

The ordinary methods of technique were employed in the preparation of the serial sections. Before the embryos exhibited movement, they were fixed directly in Zenker's fluid, but later embryos were first anesthetized in a weak chloretone solution to prevent the distortion or tearing of the delicate tissues which might result from the writhing or twitching of the body when placed in the irritating fixative. The difficulties at first encountered in making satisfactory serial sections, apparently due to the brittleness of the yolk-laden tissues when xylol was used as the clearing reagent, were overcome by using cedar oil instead and by diminishing the time of paraffin infiltration to a minimum. Both the graphic method and the modified Born's wax-plate process were enlisted in the execution of the reconstructions. In every case, the outline drawings of the sections were made with the Edinger projection apparatus. The writer also attempted to inject the vascular channels of a number of embryos, but on the whole he met with little success. The continuous layer of brown pigment in the skin of the toad larvae hides the underlying structures, and the cannula needle can therefore not be directed with the same degree of certainty as when transparent fish embryos or the much larger pig or chick embryos are injected under the binocular microscope.
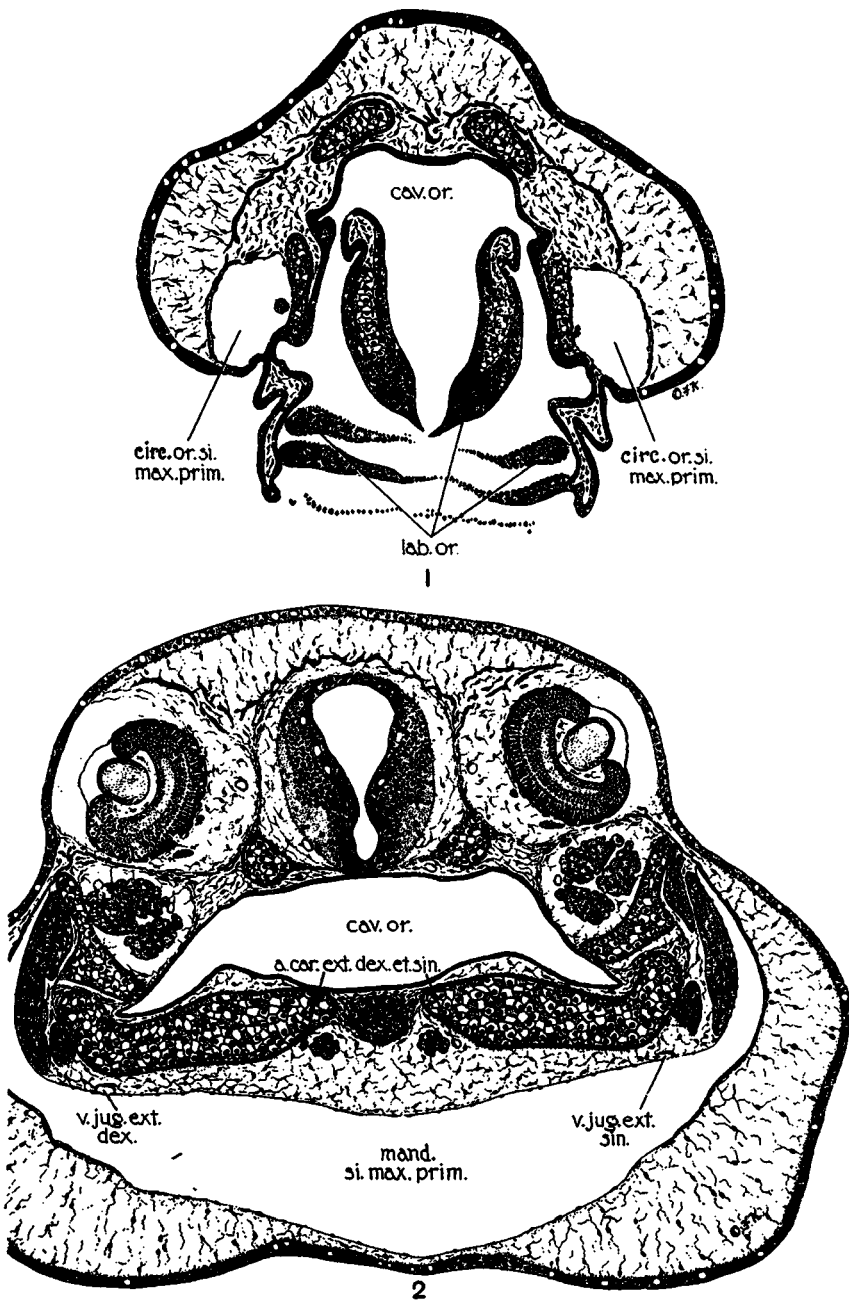
Fig. 1 Transverse section of a 13-mm. embryo of Bufo vulgaris at the level of the mouth. × 50. *circor. si. max. prim.*, circumoral division of the primary maxillary sinus; *cav. or.*, cavum oris; *lab. or.*, labial structures of the larval sucking mouth.

Fig. 2 Same, at the level of the eyes. × 50. *mand. si. max. prim.*, mandibular division of sinus lymphaticus maxillaris primigenius; *v. jug. ext. dex.* and *sin.*, vena jugularis externa dextra and sinistra; *a. car. ext. dex. et sin.*, arteria carotis externa dextra and sinistra; *cav. or.*, cavum oris.

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The most marked feature of the disposition of the lymphatics in the head is the relatively enormous expanse of a lymph sinus situated in the ventral and lateral cephalic territory. Its limits in a 26-mm. frog larva are shown in Hoyer's sketches, illustrated in Wiederscheim's 'Vergleichende Anatomie der Wirbeltiere' (7th et al. editions), and in the wax reconstruction of the vascular channels in the head of a toad embryo in figure 28. This lymph reservoir, which the writer designates the primary maxillary sinus (sinus lymphaticus primigenius maxillaris)[4] because eventually it is resolved into the secondary lymph sinuses in the region of the jaws, is developed very early and, in general form, extent and proportions, is virtually complete in 9- or 10-mm. toad embryos, hence, at a period when most of the other lymphatics are still in the formative state. Figure 28 shows that this sinus does not possess a simple contour, but is composed of several interconnecting chambers of diverse shape and size. For convenience and clearness, we may refer to the several subdivisions by different names. The broad, roughly rectangular division (figs. 2, and 28, *mand. si. max. prim.*) on the ventral side of the head may be regarded as the mandibular one; it is the largest, the first to develop, and the other portions of the sinus arise from it by outgrowth and extension. In continuity with it anteriorly is the circumoral division (figs. 1 and 28, *circ. or si. max. prim.*), which encircles the mouth opening. The third division, a pair of temporal chambers (figs. 3 and 28, *temp. si. max. prim.*), appears in the wax model as two lateral wing-like expansions of the mandibular sac; these extend as far as the pronephroi, where each contracts into a narrow duct which leads to the anterior lymph heart of the respective side. The fourth division of the primary maxillary sinus may be termed the pericardial (figs. 3 and 28, *pericard. si. max. prim.*); it constitutes a second path of communication between the mandibular and temporal sacs, but at a deeper level. It is paired and branches, as a more slender and somewhat plexiform channel, from the mandibular sac near

[4] Hoyer calls this the 'Kehlsack' and Jourdain 'sac gulaire,' and in my paper on the origin of the lymphatics in Bufo ('15) it is spoken of as the ventral cephalic sinus, but these terms are too general.

its posterior margin, but in its course it curves dorsally, that is, centrally or inwardly, and, closely associated in position with the external jugular vein, passes back along the heart towards
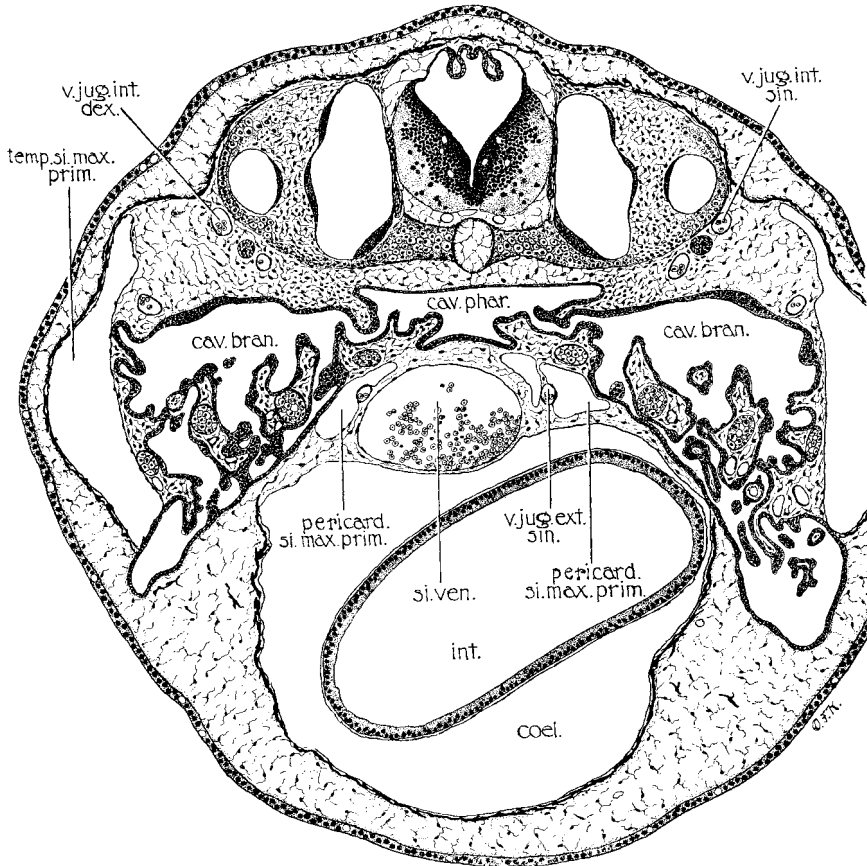

Fig. 3 Same, at the level of the auditory capsule. × 50. *temp.* and *pericard. si. max. prim.*, temporal and pericardial divisions of the primary maxillary sinus; *v. jug. int.* and *ext. dex.* and *sin.*, venae jugulares internae and externae dextrae and sinistrae; *si. ven.*, sinus venosus; *cav. phar.*, cavum pharyngeus; *cav. bran.*, cavum branchialis; *int.*, intestinum; *coel.*, coelom.

the sinus venosus and thence makes a broad sweep outward along the duct of Cuvier to join the hinder end of the temporal sac.
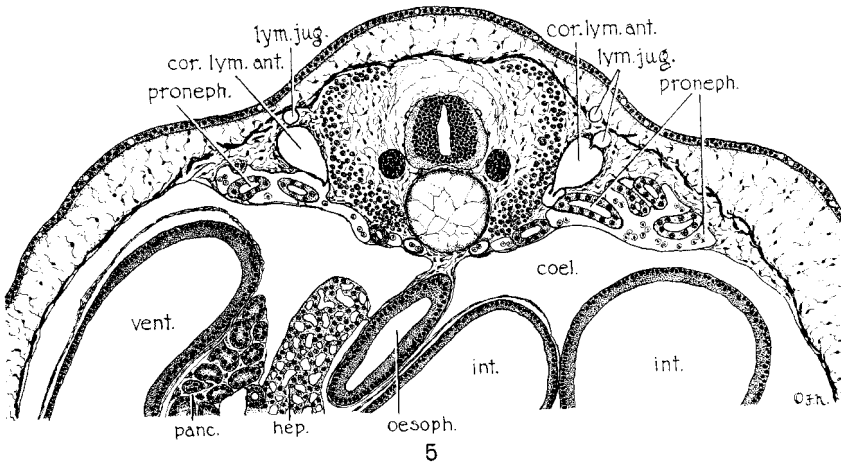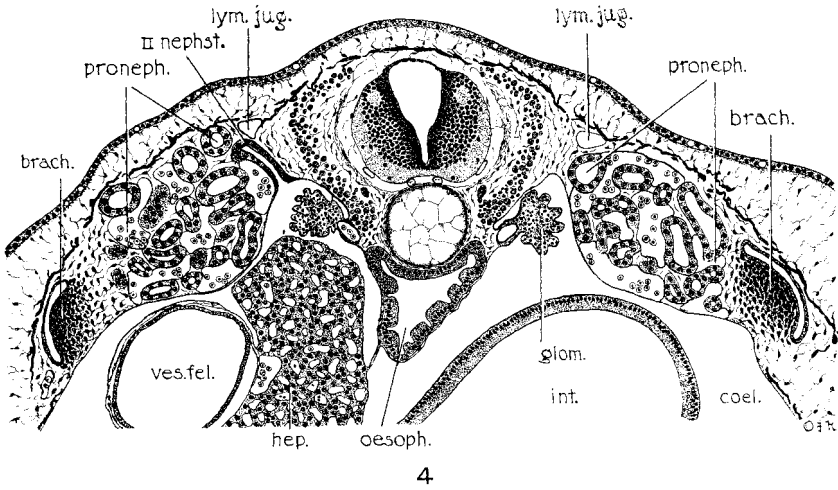
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Fig. 4 Same, at the level of the anterior limb bud (*brach.*). × 50. *lym. jug.*,
lymphatica jugularis; *proneph.*, pronephric tubules and sinusoids; *II nephst.*,
2nd nephrostome; *glom.*, pronephric glomerulus; *int.*, intestinum; *oesoph.*, oeso-
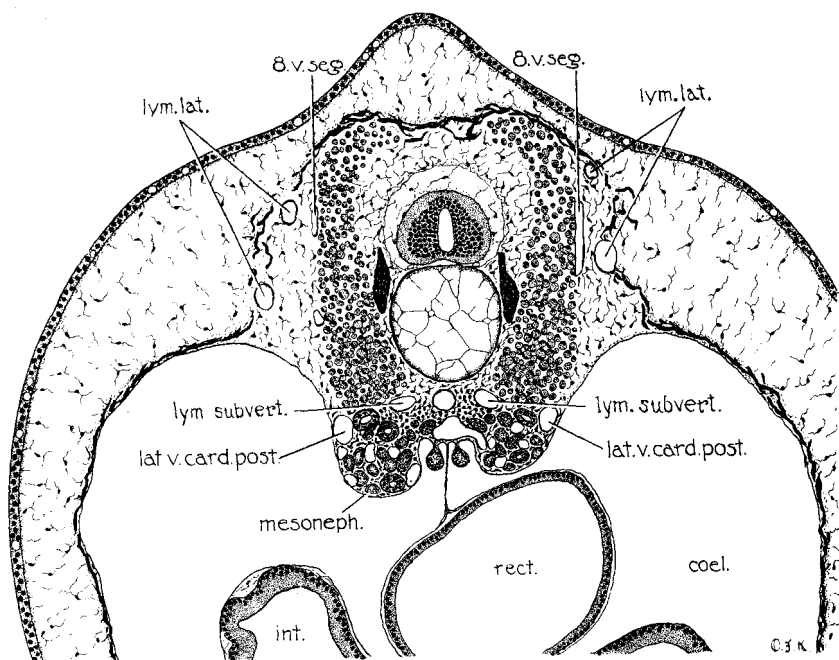phagus; *ves. fel.*, vesica fellea; *hep.*, hepar; *coel.*, coelom.

Fig. 5 Same, at the level of the 3rd spinal ganglia. × 50. *cor. lym. ant.*,
cor lymphaticum anterius; *vent.*, venter. Other references as in the preceding
figure. Lying medial to the pronephric tubules and sinusoids (*proneph.*) are, in
the order named, the primary excretory duct, the subcardinal vein and the aorta.

The primary maxillary sinus receives the lymphatic drainage of the head, as indicated in Hoyer's sketches. The lymph then flows posteriorly towards the anterior lymph heart of the same side through the channel (fig. 28, *lym. jug.*), which has been mentioned as a caudal prolongation of the temporal portion of the sinus, though genetically it has an independent origin. Hoyer has called this vessel the 'cephalic duct' or 'Kopfgefäss,' but the term jugular lymphatic (lymphatica jugularis)[5] seems more appropriate on account of its probable homology with a similar vessel in all other vertebrates. It lies immediately dorsal to the pronephros (fig. 4) and only a short distance below the skin. Near the lymph heart a tributary is given off which extends to the anlage of the forelimb, at this time a knob-like condensation of mesenchyme beneath the operculum, and, as it is the parent of the future lymph vessels of the arm, this tributary may be called the brachial lymphatic (lymphatica brachialis).
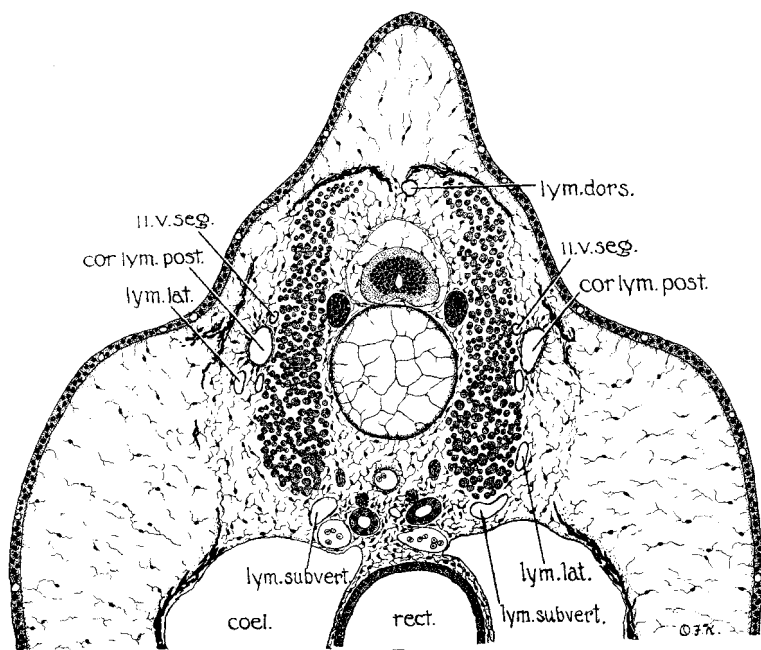
The relative size, shape, position, and connections of the pair of anterior lymph hearts during the second embryonic phase are exhibited in the wax reconstruction (fig. 28) and in the section of a 13-mm. embryo (fig. 5). Each heart is globular in form, placed superiorly at the posterior limit of the pronephros and is in continuity with both vein and lymphatic duct. It is located in the triangular area, bounded by skin, myotome, and the roof of the coelom at the level of the third spinal ganglion. On its ventral side it opens at the junction of the pronephric sinus and a short dorsal venous extension, the rudiment of the anterior vertebral vein of the adult. In the opposite wall of the heart the afferent lymphatic vessel has its entrance.

Not only the lymphatic drainage of the head is poured into the anterior lymph hearts, but also the greater quantity of the lymph from the trunk is conveyed to them by two pairs of important ducts, the subvertebral lymphatics (lymphaticae subvertebrales), lying deep, and the lateral lymphatics (lymphaticae laterales) of the trunk situated superficially, one on each side. The latter

[5] The usage of the word 'lymphatic' and its Latin form, 'lymphatica,' has been made clear in the author's paper in The Anatomical Record, vol. 16, no. 6, August, 1919.

Fig. 6

8.v.seg.   8.v.seg.
lym.lat.   lym.lat.
lym subvert.   lym. subvert.
lat v.card.post.   lat.v.card.post.
mesoneph.
rect.   coel.
int.

6

lym.dors.
11.v.seg.   11.v.seg.
cor lym. post.   cor lym. post.
lym.lat.
lym.subvert.   lym.lat.
coel.   rect.   lym.subvert.

7

are a direct continuation backwards of the jugular lymphatics, as illustrated in figure 28, and together they possess a common opening into the lymph hearts. In its course caudalward, each lateral lymph duct runs between the myotomes and the epidermis (fig. 6, *lym. lat.*), in the lateral-line region. About halfway towards the posterior lymph heart, it sends off a branch which passes over the upper edge of the myotome to fuse with its fellow of the opposite side and assuming a median position (fig. 7, *lym. dors.*), proceeds distally into the tail as the dorsal lymphatic (lymphatica dorsalis).

The paired subvertebral lymphatic, corresponding to the thoracic ducts of the higher vertebrates, has a position along the aorta and dorsal to the postcardinal veins. Anteriorly, it curves outward under the lower margins of the myotomes to join the anterior end of the lateral lymph ducts in the immediate neighborhood of the anterior lymph hearts. It retains the axial location (fig. 6, *lym. subvert.*) throughout almost its entire extent and later becomes connected with its companion by occasional anastomoses. In the vicinity of the posterior lymph heart, the duct again bends outward to reunite with the lateral lymph vessel of the same side (fig. 7). The common duct so formed later combines with the opposite one in the ventral midline and is prolonged caudally into the tail as the ventral caudal lymphatic (lymphatica ventralis) in the base of the ventral tail fin. In this region, too, the iliac lymphatic (lymphatica iliaca) is given off to the hind limb bud.

Fig. 6 Transverse section of a 15-mm. embryo of Bufo vulgaris through the trunk at the level of the 9th spinal ganglia. *lym. lat.*, lymphatica lateralis; this duct is plexiform in character; *lym. subvert.*, lymphatica subvertebralis (thoracic duct); *8 v. seg.*, 8th intersegmental vein; *lat. v. card. post.*, lateral division of the postcardinal vein; the medial divisions (subcardinals) have fused to form the postcava which lies ventral to the aorta; *mesoneph.*, mesonephric tubules and sinusoids; *rect.*, rectum; *int.*, intestinum; *coel.*, coelom.

Fig. 7 Same, at the level of the 11th spinal ganglia. × 50. *cor lym. post.*, cor lymphaticum posterius; *lym. dors.*, lymphatic dorsalis; *11 v. seg.*, 11th intersegmental vein. Other references as in the preceding figures. Medial to the subvertebral lymphatics are the aorta, primary excretory ducts and the postcardinal veins.

The paired posterior lymph heart is similar in shape to the anterior, though somewhat smaller in size at this period (15-mm. embryo), and lies lateral to the myotomes in the intersegment of the 11th and 12th (fig. 7, *cor. lym. post.*). It joins the 11th intersegmental vein (*11 v. seg.*) which becomes, as shown previously,[6] the proximal portion of the posterior vertebral vein. The heart receives the lymph stream from the hinder regions of the trunk and the tail through the lateral lymph duct.

All of the main lymphatic conduits described possess subsidiaries and capillary plexuses, the ramifications of which in frog larvae are admirably shown in injected specimens, as illustrated by Hoyer.

In the present paper, the origin and development of the primary lymph sinus, the jugular lymphatics, and the anterior lymph hearts will be considered. The formation of the lymphatics of the trunk and tail, including the posterior lymph hearts, will be taken up in a succeeding article.

THE DEVELOPMENT OF THE PRIMARY MAXILLARY LYMPH SINUS[7]

In 5-mm. embryos (Bufo vulgaris) a crude vascular plexus exists ventral to the oropharyngeal cavity and has its greatest concentration in the vicinity of the thyroid diverticulum. From this plexus the external jugulars[8] and external carotids and their tributaries subsequently differentiate. But at this time veins and arteries are still broadly confluent; all channels are alike in histological appearance, and merely the definite and constant position of certain ones enables us to pick out the future arterial and venous components. Farther back towards the heart, however, a division has already occurred between them, and the external jugulars and carotids are independent, the former curving laterally around the ventricle to join the common cardinal veins

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[6] Anatomical Record, vol. 9, July, 1920.

[7] A short description of the genesis of the primary lymph sinus in the head of Bufo embryos was published by the writer in The American Journal of Anatomy, vol. 17, 1915.

[8] In using the term 'external jugular vein' the author is following Gruby and Ecker; Goette and many other authors refer to this vein as the 'inferior jugular.'

and the latter being in continuity with the aortic arches. In the reconstruction reproduced in figure 29 their topographical relations are clearly indicated, though this deals with a later stage, a 6-mm. embryo, in which the demarcation between jugular (*v. jug. ext.*) and carotid (*a. car. ext.*) is complete except anteriorly, where they are still in broad plexiform connection.

The inception of the primary maxillary sinus takes place in 5-mm. embryos during the period of the indifferent jugulocarotid plexus, just described. Its initial anlagen arise along those channels which are to become the external jugular veins, and at first many of them are in the form of short knot-like cellular thickenings adhering to their lining. Such a lymphatic anlage is shown in the photomicrograph, figure 8, as a compact protuberance (*lym.*) of the intima of the blood vessl (*v. jug. ext. dex.*). A transverse section of another sinus anlage of the same specimen, but from the opposite side, is pictured in figure 9, *B*. In longitudinal extent, it passes through seven sections (each 6 μ thick). It is a solid cell cord or column attached to the wall of the vein (*v. jug. ext. sin.*) by its anterior end, while throughout the remainder of its course it lies free in the mesenchyme ventral and parallel to this vessel.

Besides the adherent lymphatic anlagen, there are at this stage other anlagen, which, though they be similar to them in size, shape, and location, are not in immediate contact with the lining of the blood channel, and the question naturally arises: Were such anlagen formerly connected with the haemal endothelium, or did they arise independently?   Observations on the succeeding genetic stage, as well as the investigation of the developing lymphatics of the trunk region, furnish evidence that points to the independent origin of such anlagen and besides reduces the significance which we would attach to the adhesion of some of the earliest lymphatic anlagen to the primitive blood channels. The theoretical aspects of this problem will be discussed after the steps in the development of the primary maxillary lymph sinus have been described.

The lymphatic anlagen, like the endothelium of all blood channels, especially in the head region during early development,

are stuffed with large yolk globules from tip to tip—a fact that clearly distinguishes them from the surrounding mesenchymal cells which have for the most part lost their yolk content. The nuclei of incipient endothelium, regardless of whether haemal or lymphatic, show no difference, except possibly in chromatic density when compared with those of mesenchyme; indeed, the endothelium presents a very unspecialized appearance. The fact of the longer retention of yolk spherules by the cells of vascular anlagen and channels was reported by the author ('15) and emphasized as a diagnostic trait of considerable value in discriminating between these tissues during the earlier embryonic period. Their distinctions were accurately expressed in the colored figures of that paper, to which the reader is referred.

The next older stage, a 6-mm. embryo, is characterized by the numerical increase of sinus anlagen along the external jugular veins, by their growth in length and budding of branches, their detachment from the venous intima at the original point of contact, and by their acquisition of lumina. The reconstruction in figure 29 displays the number, size, form, affinities, and distribution of these lymphatic anlagen. It furnishes a convincing picture to show that the sinus does not originate by centrifugal sprouting from any specific foci, but that it has a multiple origin in proximate relation with vessels of the primitive vascular network, and that the initial anlagen are discontinuous. Further, it shows the bilateral origin of the sinus and also that its principal or mandibular division is the first component to be formed, the other divisions, such as the circumoral, temporal and pericardial appearing somewhat later.

The time at which the individual lymphatic anlagen that are adherent to the venous wall retract from it varies greatly, neither the time of their beginning nor their length entirely conditioning it. In the reconstruction (fig. 29) some of them still cling to the blood channel, while others, even smaller ones, lie independently in the surrounding tissue. Nor is the size of the anlage a criterion of the possession of a lumen; one may acquire such very early, even in its incipient stage, while another may remain solid for a longer period of time. When a lumen does appear, it is at
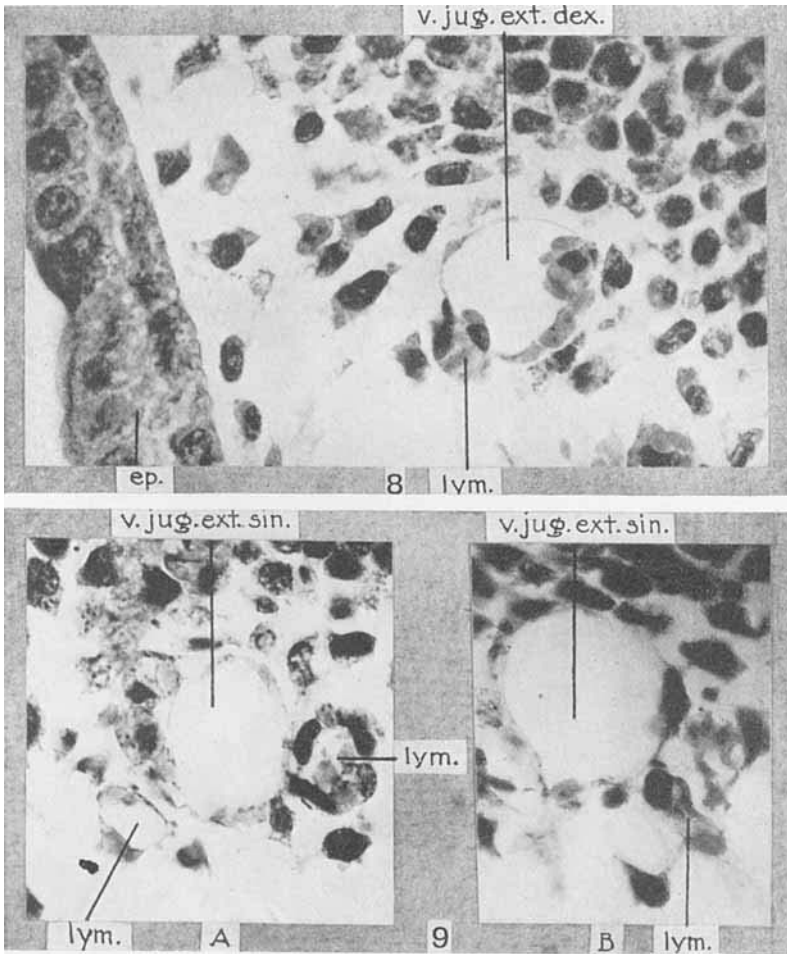
Fig. 8   Photomicrograph of a transverse section through the right ventral cephalic region in a 5-mm. embryo of Bufo vulgaris (Kampmeier Embryological Collection, series B 25, slide 1, section 71).   × 690.   (Zeiss Apochromat. Obj. 4 and Compensat. Project. Oc. 4,).   *ep.* epidermis; *v. jug. ext. dex.*, vena jugularis externa dextra; *lym.*, and initial lymphatic anlage of the primary maxillary sinus; the structure lying within the upper right-hand portion of the lumen of the vein is a yolk-filled blood cell.   In this and the following photographs, the yolk globules can be easily distinguished from the dense cell nuclei by their smaller oval shape and their uniform gray color.

Fig. 9   Photomicrograph of transverse sections through the left ventral cephalic region in a 6-mm. embryo of Bufo vulgaris (K. E. C., series B 54, slide 1, sections 75 (*A*) and 80 (*B*).   × 690.   *v. jug. ext. sin.*, vena jugularis externa sinistra; *lym.*, initial anlagen of the primary maxillary sinus.
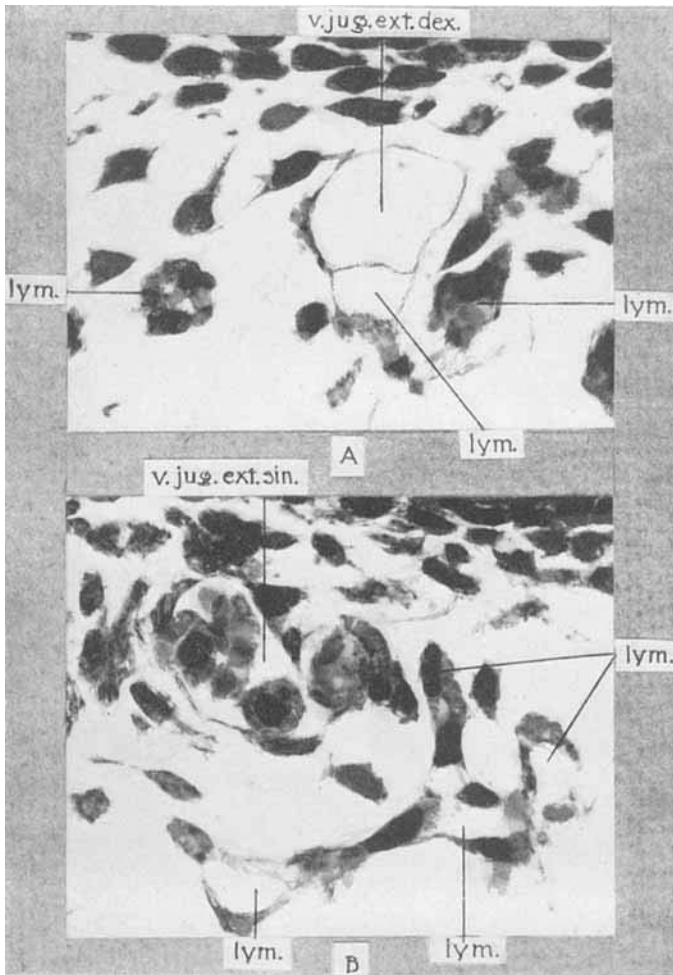
Fig. 10 (A) Photomicrograph of a transverse section through the right ventral cephalic region of a 6-mm. embryo of Bufo vulgaris (K. E. C., series B 53, slide 1, section 90). × 690. (B) Section through the left ventral cephalic region of a 7-mm. embryo (series B 52, slide 1, section 84). × 690. v. jug. ext. dex. and sin., vena jugularis externa dextra and sinistra; lym., anlagen of the primary maxillary sinus.

first a cleft or vacuole in the cytoplasm[9] between the large prominent yolk globules (fig. 10, A), or if the anlage be larger, it may consist of a number of crevices which soon coalesce to produce a more conspicuous cavity, (figs. 9. A, and 10). The lumen naturally expands with the growth of the anlage, but during several successive stages the confines of these lymphatics, like those of the haemal vessels, remain irregular and of varying thickness and appear gnarled, particularly in section (fig. 10), owing to the groups of large ovoid yolk globules which they contain and which do not entirely vanish until a relatively late period of sinus formation.

From now on the development of the sinus makes rapid progress. The discrete lymphatic anlagen of the same side establish continuity with one another by end-to-end fusion and begin to send out endothelial extensions in a ventromedial direction. These sprouts actively proliferate, branch and rebranch, and freely anastomose with one another in such a way as to produce a plexus, the meshes of which all lie in the same plane. As the identical condition prevails on the opposite side of the head, the two plexuses approach each other, meet and combine in the midline and so create a broad intricate network (fig. 11, from *lym.* to *lym.*) extending in a curved plane from the vicinity of one external jugular to that of the other through the loose mesenchyme between the thyroid and the epidermis on the ventral surface of the head. This network is the anlage of the principal or mandibular division of the primary maxillary sinus and is shown in the reconstruction in figure 30 (*si. mand.*). In the drawing, the vascular channels are pictured in a flat plane, though in reality the most distal structures bend dorsolaterally. It may

[9] The formation of the lumen, as indicated, raises the question, whether it is of intracellular or intercellular origin. The answer rests partly on our definitions of 'cell' and 'syncytium.' Are the spaces of mesenchyme to be considered as 'intercellular' or 'intracellular'? The originally solid lymphatic anlagen, described above, are probably, like other mesenchymal tissue, syncytial in nature, and accordingly I would look upon the vacuole-like beginnings of their lumen as being intracellular in situation. Subsequently, with the expansion of the lymphatic anlage into a definite vessel and the appearance of distinct cell boundaries in its endothelium, the lumen acquires its intercellular character.
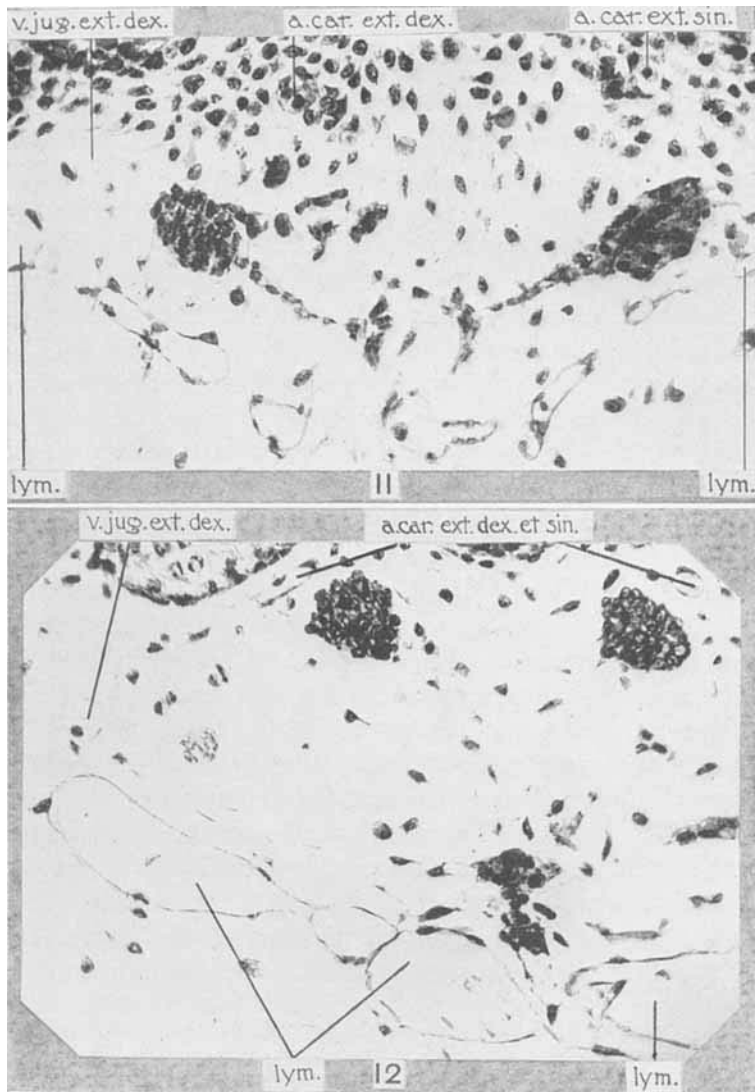
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Fig. 11 Photomicrograph of a transverse section through the ventral cephalic
region in a 7-mm. embryo of Bufo vulgaris (K. E. C., series B 27, slide 1, section
100). × 290 (Leitz 4-mm. Obj. and Zeiss Compensat. Project. Oc. 4). *a. car.
ext. dex.* and *sin.*, arteria carotis externa dextra and sinistra; *v. jug. ext. dex.*,

be noted that the outer limit of the principal or mandibular plexus is sharply defined by a pair of broader, longitudinal vessels which genetically represent the oldest portion. In this stage (a 7-mm. embryo) also the anlagen of the other divisions have made their appearance as outgrowths from the principal one. Anteriorly, the circumoral division (*si. circor.*) is an extension, on either side, growing forward along a ring-like vessel which is a branch of the external jugulars and encircles the mouth opening. Eventually the two halves of this division, by further elongation, meet and unite in front of it. Posteriorly, the pericardial division (*si. pericard.*) of the sinus consists of a pair of caudally directed extensions, closely accompanying the external jugular veins towards the sinus venosus, where in subsequent stages they become prolonged outward to join the terminal portion of the temporal division of the same side. In the reconstruction under consideration each temporal division (*si. temp.*) is shown as a derivative of a slender lymph vessel which extends laterally around the oropharyngeal cavity in association with a tributary of the external jugular vein. At this time the temporal division has already made considerable advance in plexus formation, but further tips continue to proliferate and to anastomose. One of these offshoots, passing back in the broad expanse of loose tissue lateral to the aortic arches, is highly distended locally (fig. 30), a condition manifestly produced by the pressure of the lymph collected within its lumen. A similar saccular enlargement of the temporal plexus exists also on the opposite side. In several sections on the right side, the writer was unable to follow with certainty its connection with the remaining part of the plexus, and on the reconstruction this point has been indicated by an

vena juglaris externa dextra; the heavy masses in the center of the figure are muscle anlagen; in a curved line from *lym.* to *lym.*, sections of the interanastomosing channels of the primary maxillary lymph sinus during its plexiform stage (cf. fig. 30).

Fig. 12 Photomicrograph of a transverse section through the ventral cephalic region in an 8-mm. embryo of Bufo vulgaris (K. E. C., series B 49, slide 1, section 88). × 290. *lym.*, the channels of the plexiform primary maxillary sinus are beginning to coalesce with one another by their expansion. Other references as in figure 11.

interrogation mark. It is probable that during the fixation of the embryo, the very slender connecting channel had collapsed or contracted into such a delicate strand that it became impossible to distinguish it from the surrounding mesenchymal reticulum.

The reconstruction (fig. 30) shows that the primary outgrowths of the circumoral, temporal, and pericardial divisions from the principal plexus keep close to haemal vessels, potential veins. In fact, frequently, and particularly in the case of the pericardial division, the lymphatic extension adheres to the wall of the blood vessel. The writer has been unable to decide whether or not, in the elongation of such lymph channels, the endothelium of the blood vessel contributes cells to the growing tip. It is conceivable that the latter might simply advance along a path of least resistance or in accordance with certain stresses or currents that may closely parallel the blood vessel. As yet we are entirely ignorant of the presence or absence of any such pronounced currents in the tissue interstices before the advent of the haemal and lymphatic capillary systems, and the suggestion that the paths invariably taken by these primary lymphatic extensions may be predestined by the existence of definite antecedent streams, acting as a stimulus or directive force to the proliferating endothelium, is pure conjecture. A cross-section of the pericardial division illustrating the adhesion of the lymph vessel to the haemal one is shown in figure 14 (*lym.* and *v. jug. ext. sin.*).

During the formation of the plexus phase of the primary maxillary sinus, the sprouts and the most recently established anastomoses are usually solid, the acquisition of lumina, however, occurring very soon. During this period, too, the number of yolk spherules in the lining cells are still very abundant.

The next phase in the development of the lymph sinus is the transformation of the plexus into a spacious and uninterrupted chamber. This process is a rapid one, being practically finished in the embryonic period between 8- and 10-mm. stages (B. vulgaris). The genetic changes consist in the progressive expansion of all the anastomosing channels, so that the gaps in the network are reduced and the mesenchyme filling them is compressed

into trabeculae, which become more and more attenuated, and finally break and disappear as the sinus becomes more greatly distended in its vertical, that is, dorsoventral, diameter. These successive steps are clearly exhibited in the inserted photomicrographs, figures 11, 12, and 13, the last two illustrating how the mesenchymal strands are drawn out and tear and how their remnants persist for a time as longer or shorter spurs which project into the sinus cavity.

During the further growth and enlargement of the sinus, I was unable to find the addition of separate mesenchymal spaces by concrescence, such as I described in the development of the thoracic duct in the pig ('12) or those of McClure ('15) in the formation of the subocular lymph sac in the trout, or those of Huntington ('11) on the growth of the periaortic lymphatics in Chelydra.

I can but believe that the coalescence of the originally discontinuous lymphatic anlagen, the formation of the intricate lymphatic plexus and its conversion into the relatively enormous sinus is largely, perhaps wholly, due to the accumulation within their lumen of lymph, which, as it increases in quantity, increases the internal pressure on its walls and achieves the extension and distention of the developing sinus, for *during this important genetic period the sinus possesses no outlet; it is not confluent with the veins.* The saccular and expanded posterior prolongations of the temporal plexus shown in the reconstruction (fig. 30) certainly point to such an interpretation. A similar view was expressed by McClure ('15) in his preliminary paper on the development of the anterior lymphatics in teleost embryos.

Coincident with the expansion of the lymph sinus, its lining cells assume all of the attributes of typical endothelia. The cells become much flattened, and their nuclei, which in earlier stages resembled those of mesenchyme in their spherical shape and their coarse chromatic texture, become more and more compact and dense like the intimal nuclei of older vascular channels. The yolk corpuscles in the cytoplasm of the endothelium also gradually disappear, although in 9- and 10-mm. embryos a few are still to be found.

In embryos, approximately 10 or 11 mm. long, the primary maxillary sinus acquires an outlet. The posterior extremity of the temporal division by further backward prolongation (figs. 34 and 35) becomes confluent with the jugular lymphatic, which in turn gains access to the anterior lymph heart and conveys thither the lymph collected by the sinus.

During the later larval and metamorphic periods, the primary maxillary sinus, as well as the other lymph channels laid down in the embryo, are converted into the superficial and deep lymph sacs found in the adult.[10] Such changes will be reserved for a later paper.

From the foregoing account of the appearance and relations of the early adherent anlagen of the primary maxillary lymph sinus, reinforced by the evidence of the photomicrographs illustrating it, the conclusion is forced upon one that they are probably derivatives by proliferation from the walls of the external jugular components of the early unspecialized jugulocarotid vascular plexus. Formerly ('15) I believed that these observations afforded fairly decisive evidence in favor of the origin of lymphatics from venous epithelium, and I suggested tentatively that certain discontinuous mesenchymal spaces of Amniotes, which had been described previously as incipient lymphatics, might have been derived early from neighboring blood channels in a manner hardly perceptible on account of the absence of any special differential characteristic in either the vascular intima or the mesenchyme. But after investigating more thoroughly other lymphatic channels in anuran embryos, as well as considering the evidence contained in the mass of literature which has accumulated in recent years on the problem of vasculogenesis, that opin-

[10] In The Anatomical Record, vol. 16, 1919, the writer stated that topographical relations and genetic data show the primary maxillary lymph sinus of anuran tadpoles to correspond to the subocular lymph sinus of fishes. Since then I have carried on a comparative study of the lymphatic system in the different classes of vertebrates, and the available data force me to modify that statement. Possibly only the dorsal lateral extensions of the principal portion of the primary maxillary sinus are concerned in the homology. Further observations bearing on this question will be considered in the comparative anatomy of the lymphatic system which is in process of preparation.
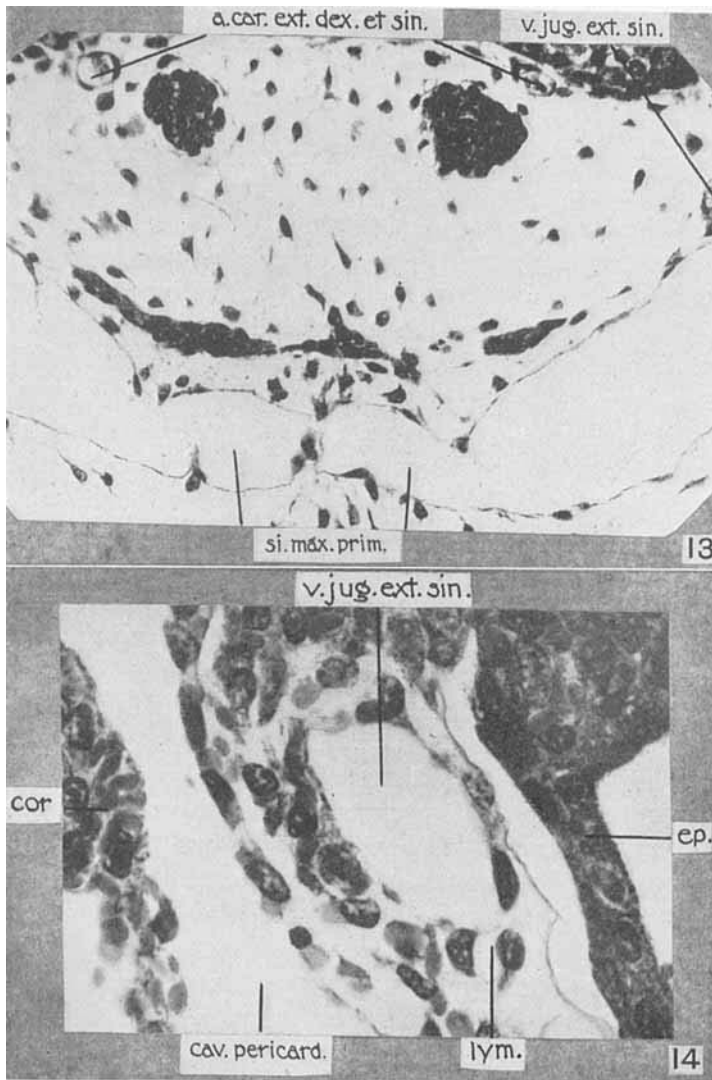
Fig. 13 Photomicrograph of a transverse setion through the ventral cephalic region in a 9-mm. embryo of Bufo vulgaris (K. E. C., series B 2, slide 1, section 115). × 290. *si. max. prim.*, primary maxillary lymph sinus; the spurs of tissue projecting into its lumen are the vestiges of the former bands of mesenchyme between the lymph channels during the plexus stage of the sinus. Other references as in previous figures.

Fig. 14 Photomicrograph of a transverse section through the left ventral region of the body at the level of the heart in a 7-mm. embryo of Bufo vulgaris (K. E. C., series B 27, slide 1, section 178). × 690. *cor*, wall of the heart; *cav. pericard.*, cavum pericardium; *ep.*, epidermis; *v. jug. ext. sin.*, vena jugularis externa sinistra; *lym.*, anlage of the pericardial division of the primary maxillary sinus.

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ion loses weight—a source of gratification to the writer in so far
as he is not compelled to regard his earliest work ('12) as funda-
mentally wrong in its deductions. The recent investigations
have brushed away many difficulties, and the interpretation of
the observations rests on firmer ground; views which were thought
conflicting only a few years ago can now be reconciled.[11] The
origin of the earliest vascular anlagen in the embryo is the basic
problem of vasculogenesis, not that of vessels, regardless of their
vascular function, which develop later. If it be true that the
earliest anlagen arise from the mesenchyme, as most of the
modern research on vasculogenesis would indicate, then the two
views of lymphatic development, the venous origin and the
mesenchymal origin of lymph vessels, are not in diametrical
opposition as was formerly vehemently asserted. Indeed, there
may be a number of variations in the genesis of such channels,
but the differences can now be judged superficial, presupposing,
of course, that the haemal and lymphatic systems are not pri-
mordially and phyletically distinct, as some investigators tacitly
hold. Lymphatic anlagen may proliferate from components of
the early indifferent embryonic vascular plexus, or certain channels
may separate from it (just as arteries and veins are differentiated
from it) and assume a lymphatic function; and, again, they may
be formed directly from mesenchyme independently of vascular
channels already existing. What determines the several varia-
tions of lymphatic development is still obscure, although the time
and the site at which they first appear, as conditioned by physio-
logical needs, may be the causative factors. The first method
is illustrated by the origin of the primary maxillary sinus which, with
the exception of the anterior lymph hearts, is the first lymphatic to
appear in Bufo embryos, and perhaps most of the anlagen of
which originate, as has just been described, in connection with
the endothelium of potential haemal vessels before that endo-

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[11] Anyone wishing to follow the controversy regarding the origin of lymphatics
is referred to the numerous papers which have appeared during the last decade
in America on the problem of lymphatic development. The larger papers of
Sabin, Huntington, and McClure on the development of the mammalian lym-
phatic system contain a comprehensive list of the literature.

thelium has become specialized, that is, has acquired the attributes of the typical flattened lining cells. The second method is seen in the formation of the jugular lymph sac of mammals, and in that of the anterior lymph hearts and the jugular lymph ducts of the toad. The development of the latter structures will be described in following sections of this paper, but it may be stated here that they arise from vessels which, at first, are freely confluent with the embryonic blood vessels and function as such, but later separate and become an integral part of the lymphatic channel system. The third method, the formation of a lymph vessel by the fusion of mesenchymal spaces, somewhat like the origin of the earliest vascular channels, is illustrated by the development of a considerable portion, at least, of the thoracic duct and other large lymph vessels in mammals, birds, reptiles, and fishes, as portrayed in numerous papers that have appeared within the last decade. It was therefore not surprising to discover this method active also, perhaps solely, in the formation of the large lymph ducts in Anura which arise later than the anterior lymph hearts and primary maxillary sinus, at a time when the blood circulatory system of the embryo had become better organized and its components more specialized.

THE DEVELOPMENT OF THE JUGULAR LYMPHATIC

Hoyer describes the development of the jugular lymphatic (cephalic duct) in frog embryos as a centrifugal outgrowth of the anterior lymph heart, but the writer's observations show that in toad embryos, at least, its origin is not so simple.

The jugular lymphatics (fig. 28, *lym. jug.*), one on each side, develop at the same time as the anterior lymph hearts and in the same general region so that they might be discussed together, though for systematic reasons they will be treated separately. Figures 31 to 35, inclusive, which illustrate reconstructions of the important structures in the territory of the left pronephros in several consecutive stages, furnish a clear idea of the salient and progressive events that occur. Besides the vascular channels which are directly and indirectly concerned in the formation of the lymphatics, other organs, such as the pronephros, spinal

ganglia[12] and a segment of the neural tube, were introduced in the reconstructions for the purpose of orientation.

I ('20) described the series of intersegmental vessels that appear in the development of the venous system as dorsal tributaries of the pre- and postcardinal trunks and found them situated at the intersegments of successive myotomes. The first two reconstructions (figs. 31 to 32), besides illustrating the beginning of the anterior lymph heart as a circumscribed plexus of the proximal portion of the third intersegmental, shows the development of a more open-meshed network of vessels formed by anastomoses between the first, second, and third intersegmentals. The jugular lymph duct is derived from the latter plexus. Passing from the 6- to the 7-mm. stage, the genetic changes consist in the separation of this intersegmental plexus (which in view of its former and its future functions may temporarily be called a venolymphatic) from the precardinal vein and the pronephric venous sinus. The channels of connection contract in caliber, like any other small redundant vessel, and finally are cut off entirely. This is indicated in the reconstruction in figure 33, where the points marked by a star still show minute and slender connections, the last traces of the originally freely confluent condition of the intersegmental veins and their parent trunk. Farther forward in the figure are two other vestiges in the form of venous spurs extending towards the lymphatic duct. As the veno-lymphatic plexus (potentially lymphatic) is severed from the veins, its channels distend, evidently due to the accumulation of the lymph within their lumen.

While the foregoing is taking place, a notable event, the transient isolation of the lymph heart anlage from the surrounding lymphatic plexus, occurs, a process which will be considered more fully in the following section. Such a phase is illustrated in the reconstruction in figure 34. The secondary junction between heart and afferent lymphatic is brought about a little later. The

[12] The first pair of spinal ganglia are evanescent structures in the anourous Amphibia, being present in toad embryos (B. vulgaris) only during the 6-mm. stage and vanishing completely very soon after. The second pair become the first of the adult.

reconstruction exhibits a number of other features. The jugular lymphatic (*lym. jug.*) by growth cephalad and the temporal division of the primary maxillary lymph sinus (*temp. si. max. prim.*) by growth caudad (figs. 30, 34, and 35) have met and become continuous. Further, as shown in figure 34, the jugular and the lateral-line (*lym. lat.*) lymphatics, united from the beginning, develop prominent ventral branches lateral to the pronephros. The other tributaries, extending dorsally and showing a metameric tendency, unquestionably represent the distal portions of the intersegmental vessels from which the jugular lymphatic was derived. Finally, the minute connection (starred) between this plexiform duct and the pronephric sinusoids may be noted, which has managed to persist until this time. In a later stage (10-mm. embryo) the jugular, in common with the lateral-line lymphatic, has reunited with the lymph heart, and farther forward the junction with the temporal division of the primary maxillary sinus has expanded (fig. 35).

A few words respecting the venous circulation of the region under consideration will explain certain difficulties. Since the anterior intersegmental veins function as haemal conduits before their transformation into the plexus of the jugular lymphatic, as soon as their complete separation from the cardinal venous trunk is accomplished, the region of the myotomes which they drained would be left without a blood vascular return, but for the development of secondary channels from the cardinal veins. Accordingly, such tributaries are laid down at this time, but they are situated chiefly on the inner suface of the myotomes and accompany the spinal nerves and ganglia; here, besides receiving branches from the myotomes, they communicate broadly with similar channels from the aorta. In order not to complicate the reconstruction more than was necessary, the entire medial blood vascular plexus, except the main cardinal tributaries, was omitted. Besides these medial segmental tributaries, two or three lateral ones develop (figs. 34 and 35) in proximity to the lymph heart and are closely pressed against the outer side of the myotomes. At a later period, these venules anastomose, become larger, and combine to form the definitive anterior vertebral vein and its branches (fig. 35).

THE DEVELOPMENT OF THE ANTERIOR LYMPH HEARTS[13]

Jourdain ('83) probably was the first to make a statement respecting the development of the lymph hearts in Anura. But his paper is chiefly concerned with the formation of several lymph sinuses in the frog, and his allusion to the hearts is very cursory, these being dismissed in a few sentences. He pointed out that a small pulsating vesicle, the posterior lymph heart, is visible, one on each side, at the base of the tail in tadpoles on which the hind limbs are budding, and that it conducts the lymph into a branch of the postcardinal vein. On the other hand, the lymph from the anterior regions of the larva flows directly, according to him, into the precardinal vein, as in fishes. The anterior pair of lymph hearts are considered independent (?) structures, which do not appear until the pectoral girdle has been formed.

[13] It would seem superfluous again to draw the distinction between 'lymph heart' and 'lymph sac' or 'sinus', were it not for the confusion of terms and ideas that is evident in several recent papers on the lymphatic system. In Mrs. Eleanor L. Clark's paper ('15) on the early lymphatics of the chick, the difference between lymph sacs and lymph hearts is disregarded, as may be instanced by the following quotation: "According to Baranski and Fedorowicz, the lymph hearts are formed from two or three lymphatics instead of from a luxuriant plexus, as in birds and mammals. However, Knower and Kampmeier state that in frog and toad embryos, the anterior lymph heart is formed from numerous lymphatic capillaries." In criticism, I wish to state that the researches of Baranski and Fedorowicz, here mentioned, deal only with the genesis of the posterior lymph hearts in Anura, and genetic peculiarities distinguish them from their fellows in the anterior region of the body. Further, Knower is mistated, and my paper, to which reference was made, published in 1915, is absolutely not concerned with the development of the anterior pair of lymph hearts, but describes the origin of a few lymphatic ducts and especially that of the large lymph sinus of the head which is not the same thing as the lymph heart. My studies of the heart were briefly reported for the first time before the American Anatomists during the Christmas holidays of 1916, a year after Mrs. Clark's article appeared. In the Amphibia, lymph heart and lymph sac or sinus are distinct structures, one possesses muscular walls and pulsates, the other is a modified lymph duct or a transformed lymph capillary plexus.

Miss Sabin also uses lymph heart and lymph sac indiscriminately. Thus ('13, p. 56), she has the following sentence: "Weliky, Jossifov, and Favaro thought that the posterior lymph heart arose from the dilatation of the caudal lymph trunks which grow from the anterior lymph hearts, and Jourdain describes them as being formed by a rapid destruction of connective tissue." Jourdain's account, here mentioned (Comptes Rendues, 96, 1883), does not pertain to the formation of lymph hearts, but refers to that of the lymph sacs.

In 1891 Field published his excellent work on the development of the pronephros in the frog, and in a footnote ('91, p. 240) described briefly, though correctly, a 'peculiar sac' which he found in 8-mm. embryos lateral to the myotomes at the niveau of the third nephrostome and joined to the postcardinal vein. "Respecting the fate and the significance of this singular structure," he says, "I have no suggestions to offer." Reference to this observation is made by Gaupp ('99, pt. 2, p. 380) who calls it a 'Blutbläschen' of unknown function. We now know that this enigmatical organ is the anterior lymph heart.

Hoyer ('05), in his work on the formation of the lymphatic system in frog larvae, states that the anterior lymph heart makes its appearance during the stage when the external gills begin to vanish, as a small spindle-shaped evagination from the short anterior vertebral vein anlage at the point where this vein branches dorsally from the pronephric venous plexus. At that time the walls of the fusiform heart are composed of an inner endothelium and an outer layer of stellate cells. In 6-mm. embryos the heart has become larger, but it is still in broad, open communication with the vein and contains numerous blood corpuscles in its cavity. Its walls become thicker and a few cross-striated muscle fibers are visible in the outer coat. During this stage in the living specimen the heart occasionally quivers, but distinct rhythmic contractions do not become evident until later, when the embryo has reached the length of 12 mm. and the muscle elements have increased in number and in configuration. In the meantime valves have appeared, one at the junction of heart and vein and another at the opening of the lymph vessel into the heart. After the formation of these structures, blood cells are only exceptionally found in the heart chamber. The fundamental changes in the development of the heart have now occurred, and in subsequent stages it merely grows larger and acquires its definitive character. But it retains its original position lateral to the second myotome throughout the entire period of genesis and growth up to metamorphosis.

Knower, in a short paper ('08), remarks that the anterior lymph heart is the first lymphatic to be formed in the frog and

agrees with Hoyer that it makes its appearance in approximately
6-mm. embryos (Rana palustris, R. virescens, R. sylvatica).[14]
He observed that during this period the heart is situated dorsal
to the posterior end of the pronephros and arises from the 4th
intersegmental vein, thus differing from Hoyer. Knower does
not state explicitly how it originates, but I assume that he regards
it as a local expansion of the vein. According to him, the heart
opens directly into the plexiform venous sinus of the pronephros
just back of the last nephrostome. Striated muscle fibers appear
early in its walls, and in 8-mm. embryos already are arranged in
bundles which branch freely; he believes that these fibers are
derived from the adjacent myotomes, the fourth and the fifth,
since the heart is developed in the intersegment in proximity to
the ventrolateral portions of these muscle segments. Finally, he
notes the development of valves at both the afferent and efferent
portal of the heart.

A very brief preliminary account of the development of the
anterior lymph hearts in Bufo was presented by the writer before
the American Anatomists in 1916.

Morphogenesis

Except that they state definitely the time of appearance and
the location of the anterior lymph heart in frog embryos, neither
Hoyer nor Knower offers a detailed description of its formation;
their accounts are brief and rather general. After more extensive
study, in which numerous graphic reconstructions and some wax
models were made, the writer is able to demonstrate with greater
preciseness, perhaps, its origin and progressive changes. Such
an exposition will show that, in Bufo embryos at least, its genetic
history and the nature of its changes are not so simple as Hoyer's
and Knower's descriptions would lead us to suppose. The first
indefinite rudiments are already suggested in approximately
4-mm. embryos (Bufo vulgaris), thus appreciably earlier than

[14] The early origin of the anterior lymph heart in the frog was indicated by
Knower five years earlier (before the American Society of Zoologists, 1903), two
years before Hoyer's first paper on the development of the lymphatics in the frog
appeared.

was specified for the frog; yet, I am inclined to believe that even in frog larvae the first hint of a lymph heart may be demonstrated earlier by means of reconstructions, which, if accurately executed,
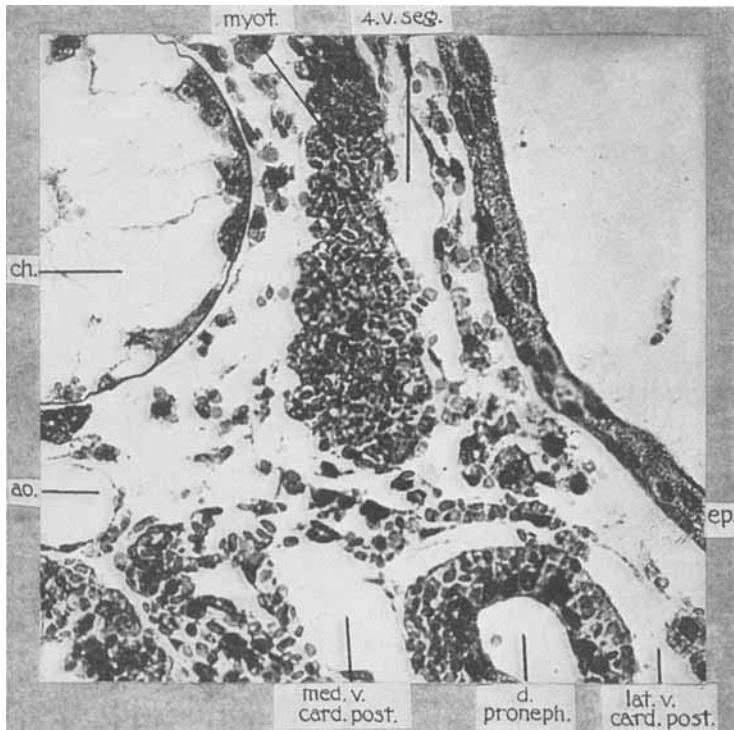

Fig. 15   Photomicrograph of a transverse section through the left lateral and anterior trunk region in a 5-mm. embryo of Bufo vulgaris (K. E. C., series B 44, slide 2, section 142).   × 340.   (Zeiss Apochromat. Obj. 8 and Compensat. Project. Oc. 4).   *4 v. seg.*, 4th intersegmental vein; *med.* and *lat. v. card. post.*, medial (subcardinal) and lateral divisions of the postcardinal vein; *d. proneph.*, pronephric or primary excretory duct; *ao.*, aorta; *ch.*, chorda dorsalis; *myot.*, myotome; *ep.*, epidermis.

bring to view significant twists and turns and other topographical details that frequently escape the strictest scrutiny of serial sections.   However, the relative time at which the lymph heart arises is a matter of little importance; we are interested more in

the manner in which it originates; but here, too, my observations
are not in agreement with the views expressed by the above-
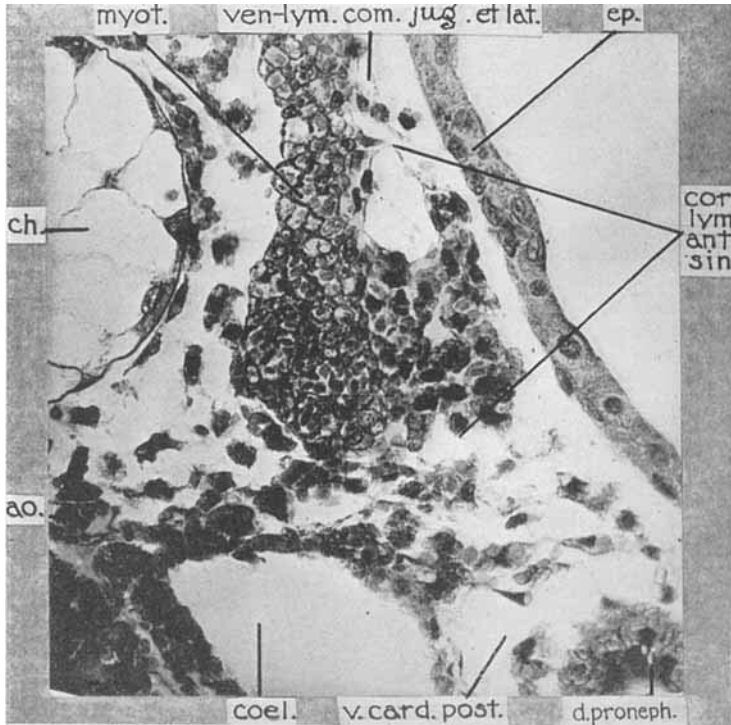named investigators.　　In Bufo embryos at least, it arises neither

Fig. 16　Photomicrograph of a transverse section through the left anterior
lymph heart region in a 5-mm. embryo of Bufo vulgaris (K. E. C., series B 44,
slide 2, section 126).　× 340.　The line of demarcation and the difference of
appearance between the cells of the myotome (*myot.*) and those of the lymph
heart anlage (*cor. lym. ant. sin.*) is clearly expressed; *ven-lym. com. jug. et lat.*,
common segment of the jugular and the lateral line venolymphatics.　Other
references as in figure 15.

as an evagination of the anterior vertebral vein nor as a direct
expansion of a particular intersegmental vein, although it is
conceivable how its appearance in certain developmental stages
might lead to such suppositions.　　The readiest way of obtaining

a lucid idea of the morphogenesis of the anterior lymph heart is to examine a consecutive series of reconstructions representing different genetic stages. Such a series is pictured in figures 31 to 35, inclusive, attention to which has already been directed in the preceding section on the development of the jugular lymph duct.

The reconstruction in figure 31, reproducing the conditions in a 4-mm. embryo, shows the vague beginnings of the anterior lymph heart as an incipient vascular plexus between the second and the fourth intersegmental vessels and in connection with the proximal portion of the third. It is so inconspicuous and ill-defined that the observer would overlook it but for the striking changes that occur in the same locality soon after.

In 5-mm. embryos, the above venous, or better, venolymphatic plexus, the anlage of the anterior lymph heart, has become more sharply outlined. In comparison with the preceding stage, the plexus not only has joined the second and fourth intersegmental vessels by longitudinal anastomosis, but also has increased the number of its connections with the pronephric sinus from one, the original mouth of the third intersegmental, to several.

By the distention of the interjoined channels of the lymph-heart plexus, these coalesce, resulting in a single cavity. In figure 32 the loop-hole in the anterior part of the anlage is still indicative of its previous plexiform state. Viewed from the side, as pictured the contour of the anlage already suggests its future globular form. In reality, however, its shape at this time is lenticular, for its lateromedial diameter is not much greater than the third intersegmental vessel from which it sprang, and accordingly in transverse section through its center (fig. 17, cor. lym. ant. sin.) it would appear as a spindle-shaped expansion of this vessel. The connection of the heart with the pronephric venous sinus and with the surrounding intersegmental network, which, as already shown, is involved in the formation of lymphatic ducts, vary little in position and in number, as a comparison of several specimens of the same age has shown. At the lower margin of the lymph-heart anlage (fig. 32) the delta-like confluence with the pronephric sinusoids is to be regarded as a complication of the

mouth of the former third intersegmental vein, and the extensions
at the upper margin as the distal portion of this vessel. The
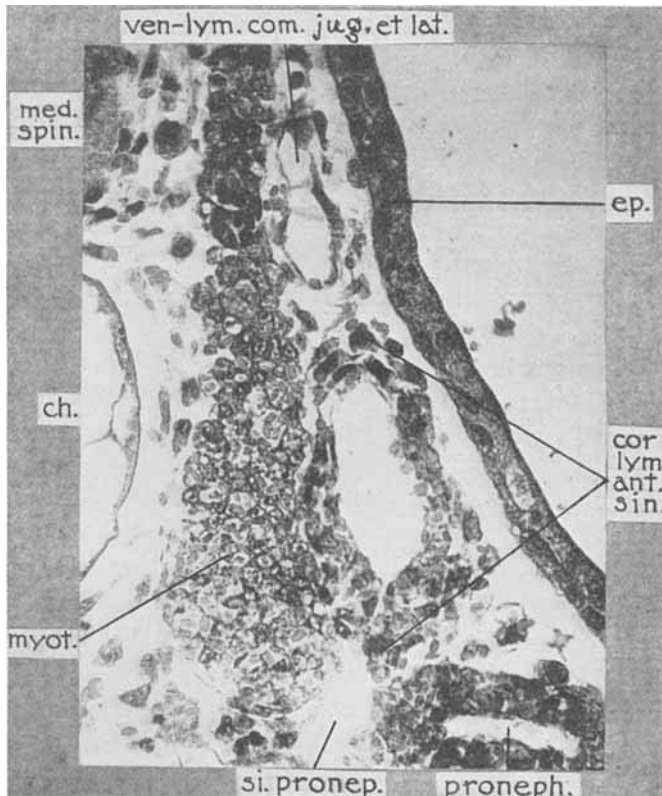anterior and posterior junctions of the lymph heart with the

Fig. 17 Photomicrograph of a transverse section through the left anterior
lymph heart region in a 6-mm. embryo of Bufo vulgaris (K. E. C., series B 54,
slide 2, section 130).   × 340.   *si. proneph.*, the mouth of the original 3rd inter-
segmental vein, branch of the pronephric sinus; *proneph.*, pronephric tubule;
med. spin., medulla spinalis. Other references as in figure 15.

second and fourth intersegmentals, respectively, may also be
resolved into plexiform channels. Thus, four fairly constant
groups of connections may be recognized, a ventral, a dorsal,
an anterior, and a posterior group.

In 7-mm. embryos, the connections between the lymph heart and the circumjacent venolymphatic plexus begin to break away. An early stage in this process is shown in figure 33.   The anterior connection is still broad; on the dorsal surface of the heart one has already severed relations and another is very much constricted; the one on the posterior surface, too, shows signs of contraction when compared with its homologue in figure 32.   On the ventral aspect of the heart one channel of confluence is just being pinched off, but the more anterior connections are fusing into one and so constitute the anlage of the anterior vertebral vein and the lymphaticovenous tap.   During these progressive events, the heart becomes more spheroidal as the area between the myotomes and the epidermis widens, associated with the rounding out of the back and sides of the embryo (fig. 18).

In 8-mm. embryos, mere vestiges, in the form of small projections, remain of the former union between the lymph heart and the neighboring lymph vessels, as delineated in the reconstruction in figure 35, but in every case their coincidence with the points of union of earlier stages can be made out readily.   At this period, then, there seems to be no open passage whatsoever between the cavity of the lymph heart and the remainder of the lymphatic conduit system.   It is a blind globular chamber attached to the anlage of the vertebral vein at its anterior and ventromedial surface and is confluent with it.

During the period between 8- and 10-mm. stages the secondary or permanent communication is established between the lymph heart and the afferent lymph duct.   A comparison of figures 34 and 35 and the photomicrographs, figures 19 to 23, shows plainly how this is accomplished.   By uniform growth and dilatation of the lymph heart as well as of the circumjacent lymph vessels, the common segment of the jugular and lateral ducts (*lym. com. jug. et. lat.*) and the dorsal aspect of the heart are gradually brought together, this approximation continuing until the duct comes to lie in a shallow groove-like indentation or depression of the heart wall.   Along this line of contact the first afferent ostium appears.   In later stages, as more tributaries of the aforesaid lymph channels are formed, some of them, situated nearest the heart, cross over its surface and come to lie against it, and

eventually break through at certain points, so increasing the num-
ber of portals of entry for the lymph stream, as shown in the
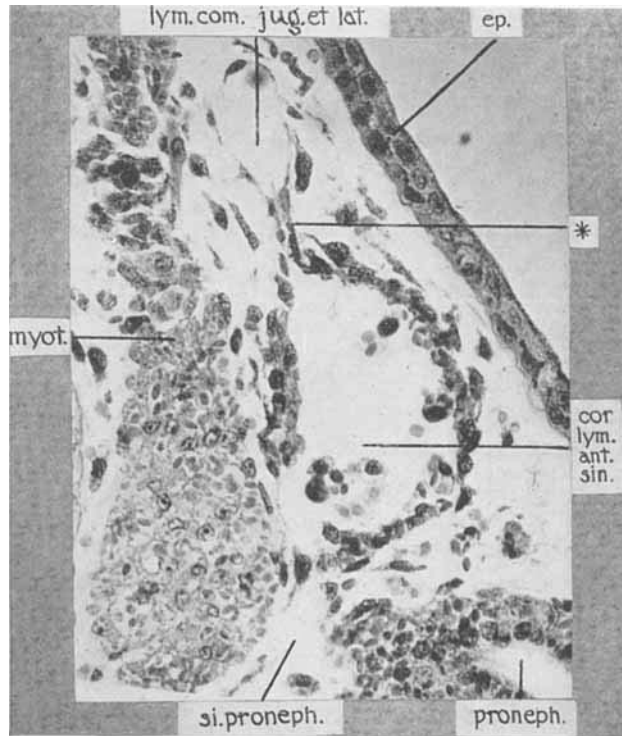drawing (fig. 24) of the lymph heart in a young toad.   In figure 35

Fig. 18   Photomicrograph of a transverse section through the left anterior
lymph heart region in a 7-mm. embryo of Bufo vulgaris (K. E. C., series B 27,
slide. 2, section 83).   × 340.   *lym. com. jug. et lat.*, common segment of the
lymphatica jugularis and lymphatica lateralis; *, temporary breaking away of
the lymphatics just mentioned from the lymph heart (*cor. lym. ant. sin.*).   Other
references as in the preceding figure.

such a condition is already intimated by the lymph vessel which
branches off from the jugular duct and passes diagonally over the
outer surface of the lymph heart.[15]

[15] During the period of metamorphosis, the plexus of lymphatic vessels in the
vicinity of the anterior lymph heart develop into the sub-scapular lymph sinus.
The description of the transformation of the lymphatic system in the tadpole

One detail still remains to be considered in the morphogenesis of the anterior lymph heart, namely, the shifting of the efferent portal, or ostium venosum.   In the earlier stages, this connection is with
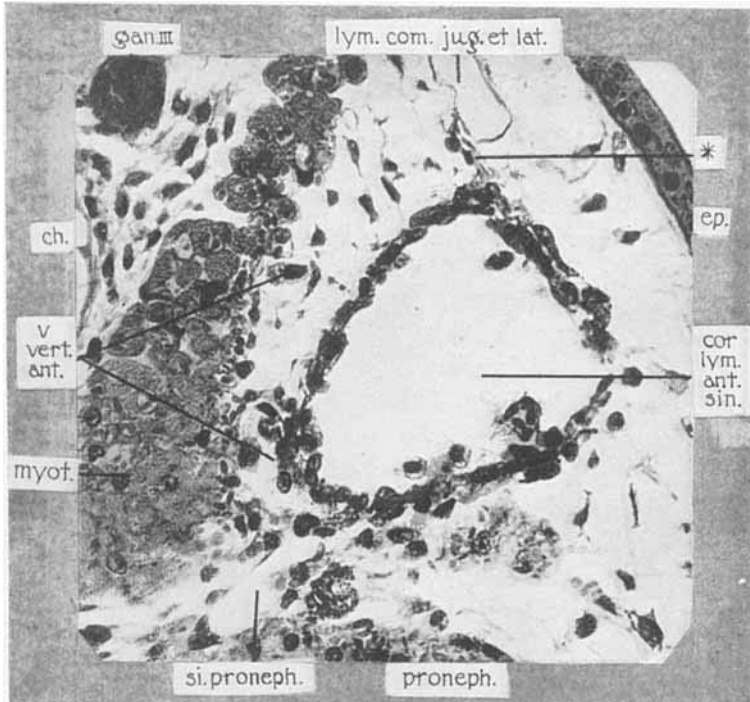

Fig. 19   Photomicrograph of a transverse section through the left anterior lymph heart region in an 8-mm. embryo of Bufo vulgaris (K. E. C., series B 49, slide 2, section 112).   × 340.   *v. vert. ant.*, vena vertebralis anterior, branch of the pronephric sinus (*si. proneph.*), its mouth being that of the original third intersegmental vein; *, point of former connection, in the form of a small spur, between the common segment of the jugular and lateral lymphatics and the lymph heart; *gan. III*, third spinal ganglion.   Other references as before.

the pronephric venous sinus and is found squarely on the ventral side of the heart (fig. 32).   This condition is changed by three factors: first, the breaking away of the posterior channel of the

into that of the fully formed animal represents another section of the original monograph and will appear later as a separate paper.

multiple junction and the amalgamation of the other channels into a larger one; secondly, the outgrowth of the anterior vertebral vein (*v. vert. ant.*) just internal to the latter, and, thirdly, the distention of the lymph-heart cavity, the bulging of which is more pronounced laterally than medially where the myotomes resist its expansion (cf. photomicrographs figs. 18 to 22). As a result of the interaction of these factors, the lymphaticovenous tap is shifted forward and medially. The ultimate condition has not yet been attained, however, for in the young toad the junction is at the anterior, more conical, end of the heart (fig. 24). Between the stage figured in figure 35 and the final one, it is evident, therefore, that considerable displacement still occurs, but to specify all of the underlying causes is impossible and is of little importance. Unquestionably, it is correlated with the stresses and strains due to other bodily changes that take place in the neighborhood of the lymph heart during development, such as the atrophy of the pronephros and the proximal segment of the postcardinal,[16] the absorption of the pronephric sinus by the internal jugular, the consequent shifting of the mouth of the anterior vertebral vein, the differentiation of the myotomes, and the rearrangement of the resulting muscles, to mention only a few of the most evident modifications.

Histogenesis

a. The lymph heart wall. Having described the conformation of the lymph heart and its venous relations, the development of its walls and valves remains to be discussed.

In early stages (4- and 5-mm. embryos) when the anterior intersegmental vessels have just been established, the area between the epidermis and the myotomes is very narrow, not much wider than is sufficient to accommodate these vessels (fig. 15). The mesenchyme, too, is very scanty here except in the region of the 3rd intersegmental vein, where its yolk-laden cells soon become more numerous and are locally massed against

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[16] The medial division of the postcardinal vein has been shown by the writer (Anat. Rec., vol. 19, 1920) to correspond to the sub-cardinal vein of higher vertebrates.

the confines of that channel. This is especially true during the stage when the lymph-heart anlage is plexiform, a section of which is shown in the photomicrograph, figure 16 (*cor. lym. ant. sin.*). As suggested in the figure, the masses of mesenchymal cells are not uniformly arranged around the outlines of the lymph-heart plexus, but are irregularly distributed, at one level being crowded against its lateral side, at another, against its medial, and more frequently between the meshes of its interanastomosing channels. Incidentally, it is evident that the reconstruction, which simply represents an enlarged cast of the lumen of the channels, does not exhibit all of the essential features of the developing structure, and to acquire a correct conception of the genetic processes, the sections as portrayed in the photomicrographs must be examined together with the reconstructions. The mesenchymal cells are so closely packed together and so filled with yolk globules that it is impossible to determine their individual boundaries. At several points, too, such aggregations seem to bound the cavity of the lymph heart anlage directly, at least no distinct intima lining it can be recognized. Otherwise the lining is quite sharply defined, though only part of the endothelial cells tend to the flattened shape, while others still retain the unspecialized form in which the nuclei are in general either oval or spherical and resemble those of ordinary mesenchymal cells. The nuclei of these mesenchymal masses stain deeply and are coarsely chromatic, and many of them have an indented circumference which conforms to the large yolk bodies in the cytoplasm.

At another level of the lymph-heart plexus, just back of the section shown in figure 16, primitive spherical blood cells, also stuffed with yolk globules, are crowded together and block the lumen of a connecting channel. Generally speaking, there is already a marked difference between the nuclei of circulating blood cells and those of mesenchymal cells; the former are dense and opaque and take almost a black color when stained with haemotoxylin, while the latter possess lighter staining areas between the large chromatic granules. A few exceptions, however, were observed; several of the nuclei of the mesenchymal

cell aggregations approach the haemal nuclei in density, though the writer is unable to demonstrate decisively the transition and conversion of one into the other. This observation immediately calls to mind the researches of Miller ('13) and Allen ('13) on the development of the thoracic duct in the chick and the caudal lymph heart in Polistotrema stouti, respectively, where it was discovered that some mesenchymal cells were converted into blood cells during the early genetic stages of these lymphatics. Consequently the question arises: Does the incipient anterior lymph heart in Anura also function transiently as a haemopoietic organ? Do some of the cells of the mesenchymal masses contiguous to and between the channels of the plexiform anlage become differentiated into blood corpuscles? All my efforts to demonstrate this proved futile in the face of that prime obstacle, the abundance of yolk, which obscures and erases the more delicate tissue distinctions.

In 6-mm. embryos not only has the periphery of the lymph-heart lumen become more definite than in the previous stage, but the surrounding masses of mesenchymal cells are becoming more evenly spread out over its outer surface (fig. 17).

In the next older stage (7 mm.) the rearrangement of the cells composing the walls of the lymph heart is such that in general two layers may be distinguished (fig. 18), a lining or internal layer and a covering layer, but which, as yet, are not sharply delimited. This indistinctness is further emphasized by the fact that the intimal cells are not all flattened, as we should expect in this relatively advanced stage, but still retain their generalized character. Indeed, one is not able to discern any striking difference between them and the other mesenchymal cells; as regards size, form, and appearance, the nuclei seem identical. The yolk globules have decreased in number in both layers, and for the first time one can get a glimpse of the shape of the cell body. Some of the cells of the outer or covering layer are becoming definitely fusiform, with their long axis directed parallel to the circumference of the heart cavity. A considerable number of blood cells are present in the latter (figs. 18 to 22), a fact of no special significance, however, for the heart is in broad

open communication with the pronephric venous sinus and no valve has yet been established at this lymphaticovenous junction.

In the subsequent period of development the wall of the lymph heart changes very slowly in character. Instead of increasing in thickness, it becomes relatively thinner during the time between 9- and 13- or 14-mm. embryos, due, in the first place, to the progressive flattening of the cells of the intima layer; secondly, to the attenuation of the covering cells, and, thirdly, to the loss of the large yolk corpuscles. In fact, during these stages, the second or covering layer does not form a complete investment of the endothelium, for there are bare spots (figs. 20 and 21) where endothelium constitutes the only line of demarcation between the cavity of the lymph heart and the surrounding mesenchymal reticulum. The scantiness of the covering layer at this time is probably explained by the slow specialization of its cells and the more rapid expansion of the heart lumen with the resultant stretching of its lining cells. In 16-mm. embryos it again forms a continuous single cell-sheet (fig. 23), though it is still quite as thin as the endothelial layer, and is composed of slender spindle-shaped cells which show delicate striations. These cells, differentiated, as we have seen, from the mesenchymal cell aggregations so conspicuous during the initial stages of the lymph heart, compose the anlage cf its muscle coat.

Knower claims there is evidence that the cells of the muscle coat are derived from the adjacent myotomes, but the writer is unable to furnish proof for the contention. In 5-mm. embryos a radical difference already obtains between the cells of the myotomes and those which surround the lymph-heart anlage—a distinction strikingly revealed in figure 16. Nevertheless, this fact does not discount the possibility that in much earlier stages the potential muscle cells of the lymph heart may proliferate from the myotomal elements; but, if this is found to be true, then it will be equally true that other mesenchymal cells of the same region have a similar source, provided the absence of any visible difference whatsoever between the cells of the mesenchyme and those of the lymph-heart anlage is any criterion of the similarity of origin.

The further development and thickening of the muscle coat is not consummated until some time after metamorphosis, for even in the young toad it is not conspicuous and is composed of only three or four cell layers.
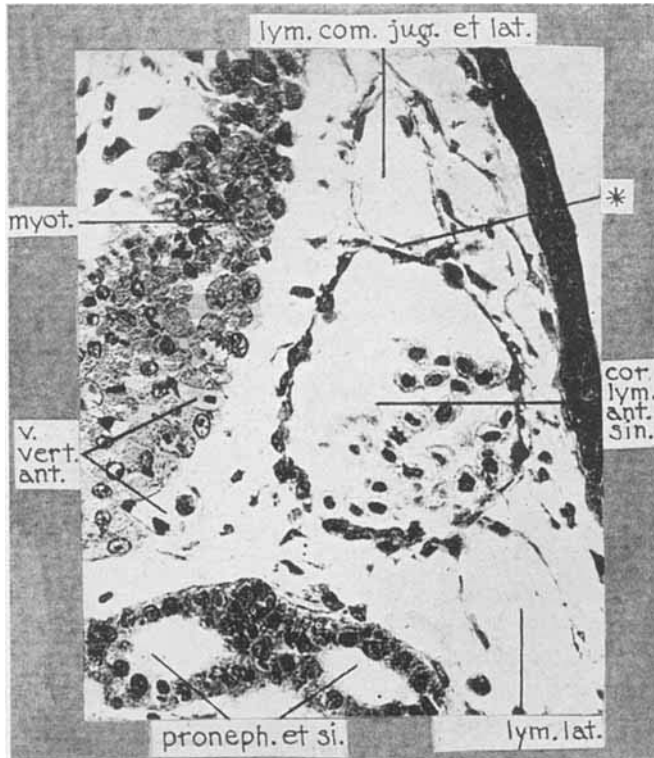

Fig. 20 Same, in a 9-mm. embryo of Bufo vulgaris (K. E. C., series B 13, slide 3, section 14). × 340. *, the common segment of the jugular and lateral lymphatics by expansion have again come in contact with the lymph heart; *lym. lat.*, a ventral branch of the lateral lymphatic (lymphatica lateralis) and continuous with the dorsal one at a further level. Other references as before.

The intima, too, of the lymph heart is slow in acquiring its definitive character, which is not attained until approximately in 15- or 16-mm. stages. Even in 10- or 11-mm. embryos the endothelial cells show little advance over those present in the heart of somewhat younger individuals. Some do have the finished
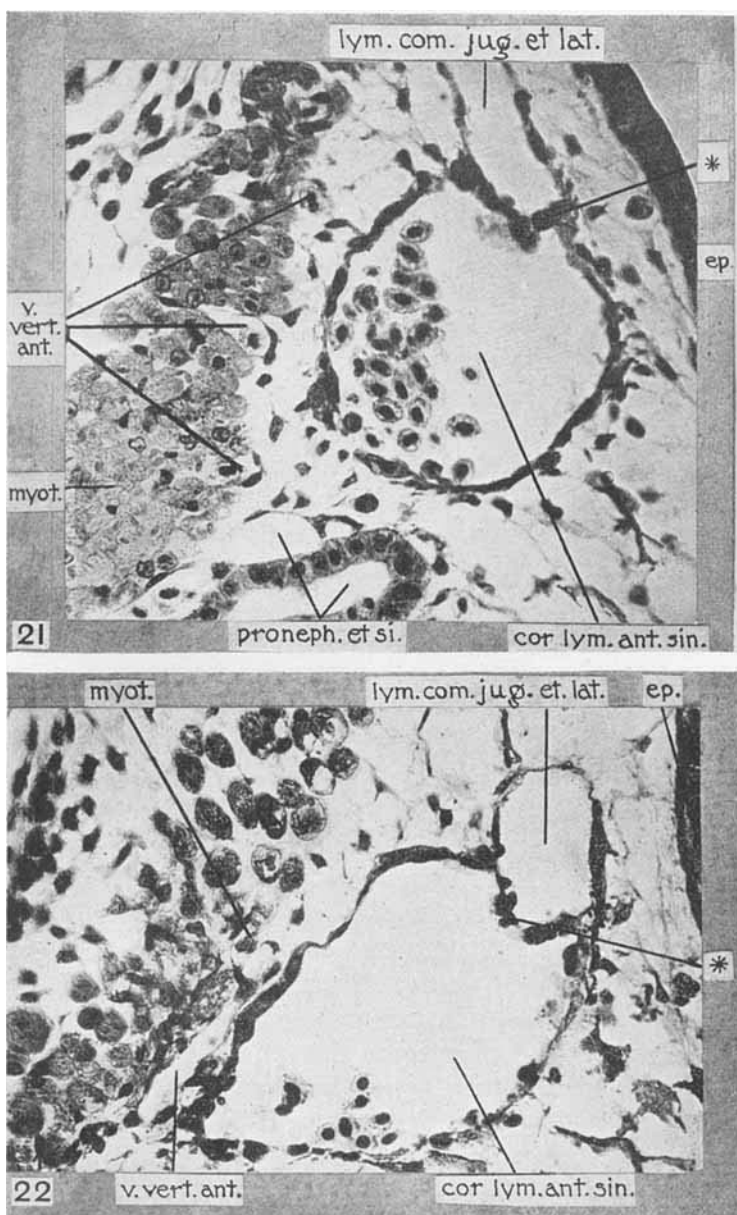
lym. com. jug. et lat.

*

ep.

v.
vert.
ant.

myot.

21 proneph. et si. cor lym. ant. sin.

myot. lym. com. jug. et. lat. ep.

*

22 v. vert. ant. cor lym. ant. sin.

Fig. 21   Photomicrograph of a transverse section through the left anterior lymph heart region in a 10-mm. embryo of Bufo vulgaris (K. E. C., series B 34, slide 2, section 83).   × 340.   *, the common segment of the jugular and lateral lymphatics has indented the dorsal wall of the lymph heart, which has become thickened at this point.   Other references as before.

Fig. 22   Same, in a 12-mm. embryo of Bufo vulgaris (K. E. C., series B 11, slide 3, section 34).   × 340.   *, the wall between the lymph duct and the lymph heart has broken through in the middle and the two flaps so formed represent the valves of the afferent portal.   Other references as before.

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form, in so far as their nuclei appear compressed and uniformly dense, but others again contain large, spherical, and coarsely chromatic nuclei and protrude into the heart cavity like little humps or hillocks, which call to mind the observations of certain investigators on haemopoiesis where endothelium germinated blood cells, but the author was unable to discover an undoubted case where one became constricted off.

The further differentiation and thickening of the heart wall occurs during the period of growth after metamorphosis, and histological examination of a section through the lymph heart of the adult anuran reveals three well-defined coats or layers: a tunica interna or intima, a tunica media, and a tunica externa or adventitia. The first is composed of the layer of highly flattened lining cells and a very thin stratum of connective tissue, probably elastic in nature, immediately external to them. As we should expect from the great energy displayed by the lymph hearts during life, the muscular tunica media, the second coat, is the broadest layer of the heart. Its muscle cells or fibers are of varying length and thickness and group themselves into small bundles which branch and interlace in a complex manner. Hoyer ('04) claims that the individual muscle fibers themselves branch and anastomose and possess numerous cross bands, which call to mind the intercalated discs of human cardiac muscle. A large number of elastic strands are also contained in the media. No sharp boundaries exist between media and adventitia. The latter is made up of fibrillar connective tissue in which are scattered pigment cells. The nerve fibers to the anterior pair of lymph hearts are apparently supplied by the III spinal nerve. According to Waldeyer ('64), both medullated and non-medullated nerve fibers are found in the walls of the fully developed lymph hearts.

Before discussing the formation of the valves, a variable feature may be mentioned in connection with the development of the walls. In about half of the lymph hearts examined between 8- and 16-mm. stages, a strand or trabecula, sometimes delicate and sometimes fairly thick, bridged the cavity. Occasionally they were imperfect, simply projecting as slender filaments

(fig. 26).   It is possible that these trabeculae correspond to the incomplete partition which, according to Radwanska ('06), is of constant occurrence in the anterior lymph hearts of adult frogs.

b. The afferent portals.   As was indicated earlier, during the first part of its functional life the lymph heart of the anuran embryo possesses but two valvular openings, a lymphaticovenous or efferent one and the entrance of the afferent lymph vessel. It is only in later embryonic and postmetamorphic periods that the number of afferent gateways is increased from one to about twelve.   The development of this type will be considered first.

In the discussion of the morphogenesis of the lymph heart the writer has described how the developing lymphatic plexus surrounding it temporarily detaches and recedes from it and how the longitudinal channel of the plexus, the common segment of the jugular and lateral-line ducts lying dorsal to the heart, again comes into juxtaposition with it by the dilatation of both structures, whereupon the permanent communication is established. Figure 20 is a section of the lymph-heart region during the phase of simple apposition.   Here the heart wall, having the same appearance and thickness as elsewhere along its periphery, separates the cavity of the heart (*cor. lym. ant. sin.*) from that of the lymph duct (*lym. com. jug. et lat.*), and there is as yet no indication of the future opening between the two.   In the next older stage (10-mm.), the heart and vessel are more intimately applied to each other by the partial invagination of the latter into the heart, as shown in figure 21 (*); in the reconstruction (fig. 35) the vessel lies in a shallow furrow of its heart wall at *af*.   It is along this surface of contact that the partition dividing the two cavities thickens considerably (fig. 21) by the proliferation of its cells. Somewhat later, a cleft develops in the center of the thickened area (fig. 22, *) by the separation of its cells, evidently the effect of the increasing pressure within the afferent lymph duct.   The margins of the simple rupture now serve as the valve.   These, by further proliferation, may become longer, and as they converge and project into the lumen of the lymph heart they produce the typical teat-like form in section (fig. 23, *).   The other valvular afferent portals which arise later (fig. 24, *af.*) are developed in

a similar way. In a young toad (Bufo lentiginosus), shortly after the period of metamorphosis, the author observed five such points of entry in the anterior lymph heart, but doubtlessly their
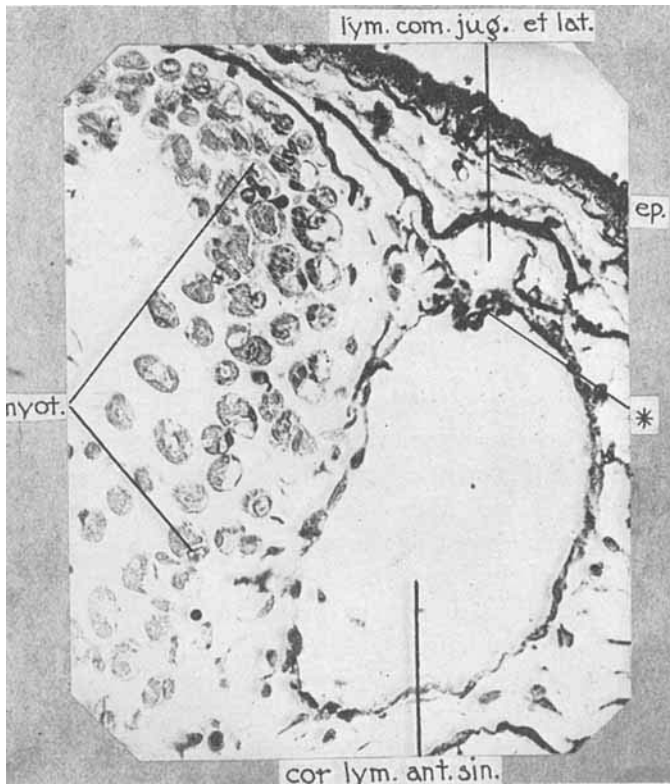

Fig. 23 Photomicrograph of a transverse section through the left anterior lymph heart region in a 16-mm. embryo of Bufo vulgaris (K. E. C., series B 39, slide 5, section 51). × 340. *, valve at the afferent portal. Other references as before.

number is increased with the growth of the toad towards maturity, for Radwanska ('06) counted more than a dozen on the same organs in adult frogs.

c. The efferent portal, or ostium venosum. The formation of the valve at the lymphaticovenous junction is perhaps not so

diagrammatic. It develops, however, at the same time as the other valve. In 7-mm. embryos the lymph heart is still in broad open communication with the anlage of the vertebral vein (fig. 18). In the next few succeeding stages (8-, 9-, and 10-mm. embryos) the junction becomes progressively constricted by the local thickening of its surrounding wall. In fact, in some cases it was observed that the cell proliferation was so considerable as to block almost entirely the channel of connection (figs. 19 and 22).
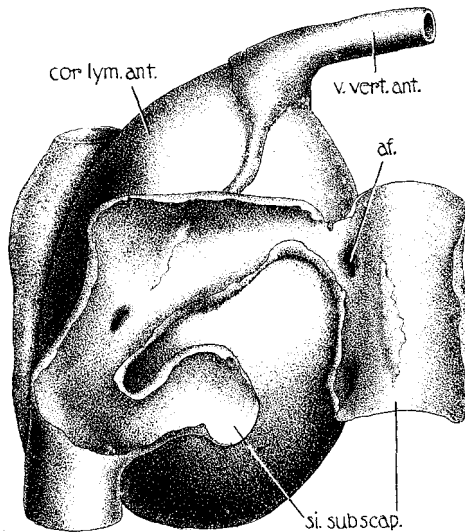

Fig. 24 Quasischematic reconstruction of the left anterior lymph heart of the young toad immediately after metamorphosis. × 150. Ventromedial view. *cor lym. ant.*, cor lymphaticum anterius; *v. vert. ant.*, vena vertebralis anterior; *si. subscap.*, subscapular sinus; *af.*, one of the afferent portals.

Then, by the elongation of its thickened sides (fig. 25,*) associated with the expansion of the venous lumen up and around it towards the lymph-heart wall, the connection becomes telescoped, as it were, into the cavity of the vein, so that the thickened cell masses project as the lips of the valve (fig. 26,*). This process is completed in 10- to 12-mm. toad embryos (B. vulgaris). In the outline sketches in figure 27, the formation of both the afferent and the efferent portal is expressed graphically.
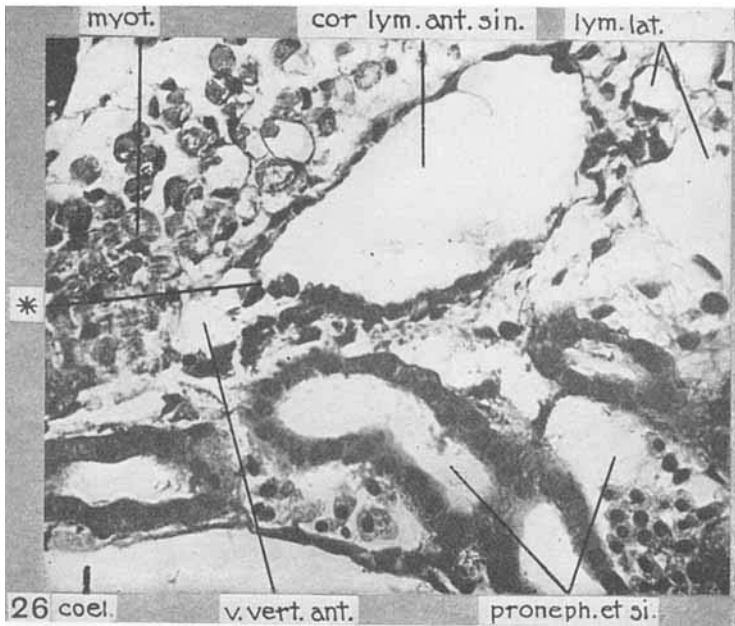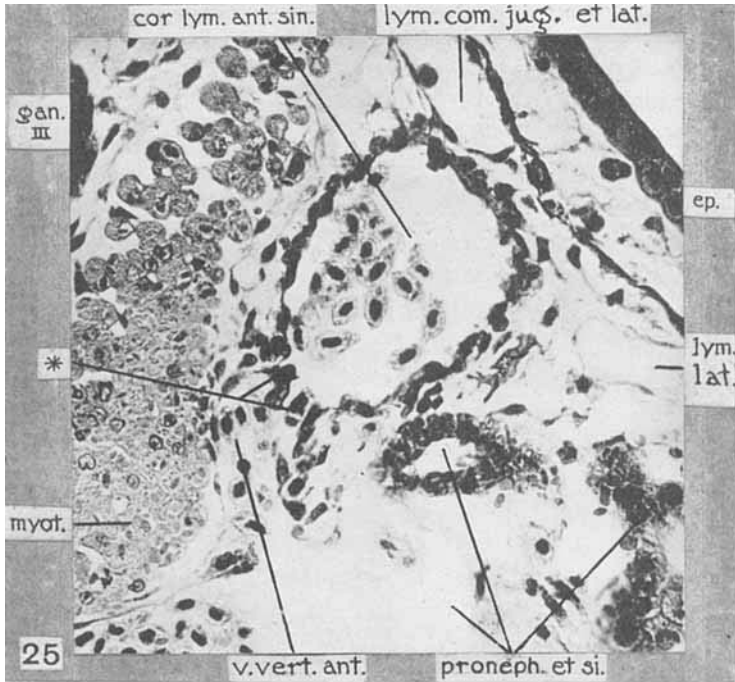
Fig. 25 Photomicrograph of a transverse section through the left anterior lymph heart region in a 10-mm. embryo of Bufo vulgaris (K. E. C., series B 34, slide 2, section 68). × 340. *, formation of the valve at the efferent portal. Other references as before.

Fig. 26 Same, in a 12-mm. embryo of Bufo vulgaris (K. E. C., series B 11, slide 3, section 41). × 340. *, valve of the efferent portal. Other references as before.
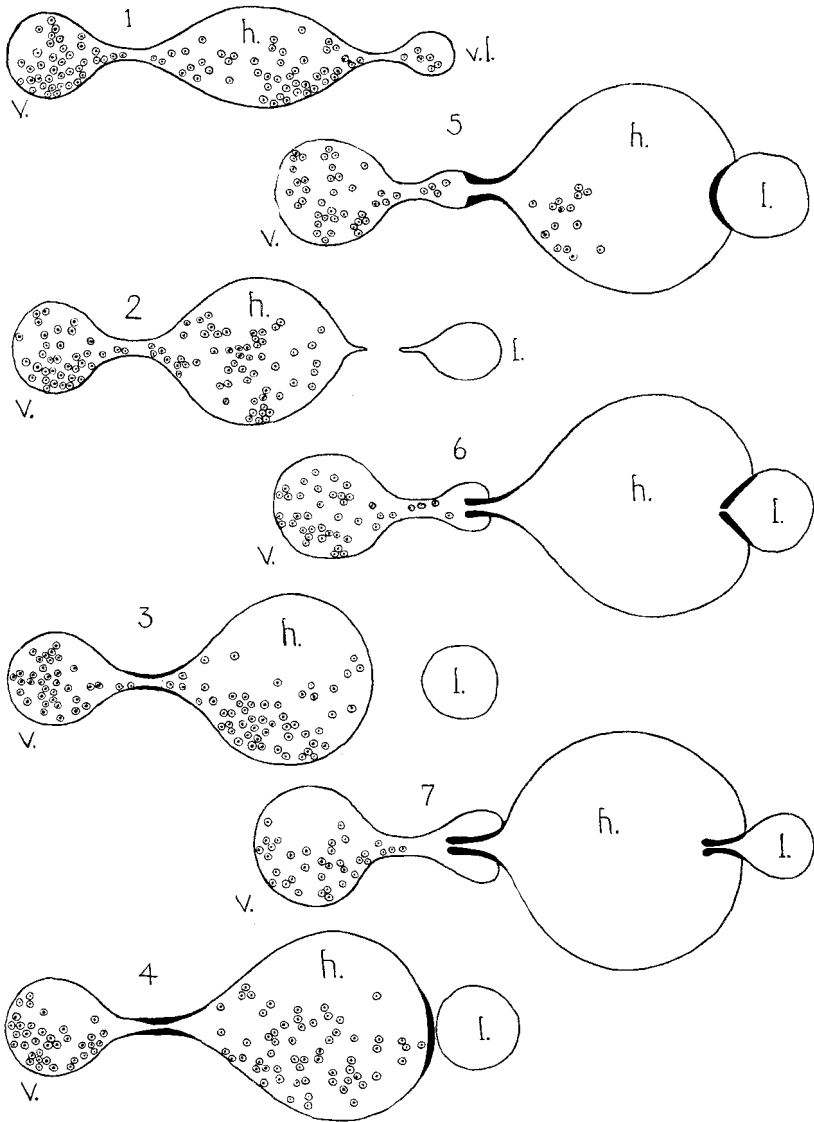
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Fig. 27   Diagrams (1 to 7) illustrating the formation of the afferent and efferent ostia of the lymph heart (based on transverse sections).   *v.*, vein; *h.*, lymph heart; *v. l.*, venolymphatic, a channel of the intersegmental vein plexus, and converted into the afferent lymph vessel, *l.*

Since blood cells have free access to the cavity of the lymph heart before the appearance of the valve at the lymphatico-venous junction, in stages up to and including 10-mm. embryos, they are abundant in it (figs. 18 to 22 and 25). In 12-mm. embryos and later (figs. 23 and 26), they are rarely present, and we may conclude from this and the fact that the valves are now functionally complete and efficient that the pulsations of the lymph heart commence at this time, for the first few contractions would certainly cause the evacuation of all haemal elements.

On account of the abundance of pigment in the integument of Bufo embryos, it was impossible to determine accurately by direct observation on the living specimen at which time the pulsations of the anterior lymph heart commenced, but, according to Hoyer ('05), they first become evident as irregular quiverings in the more transparent frog embryos (R. temporaria) when they are 12 to 13 mm. long. Later the pulsations of the lymphatic heart become more rhythmic, but the beats coincide neither with those of the haemal heart nor with those of its companion on the opposite side. In the mature animal it throbs as often as sixty to seventy times every minute, and since its capacity is about 0.5 cu. mm. (Radwanska, '06), the quantity of lymph pumped into the anterior vertebral vein during this period is 30 cu. mm., and in one hour reaches the relatively considerable amount of 180 cu. mm. During systole of the lymph heart, the efferent valve, projecting into the vein, opens for the discharge of the lymph, but closes and prevents the backflow of blood into the heart chamber during diastole. Similarly, the afferent gateways permit the entrance of the lymphatic current from the circumjacent lymph sinuses, yet avert its reflux during systole.

SUMMARY

1. On the development of the primary maxillary lymph sinus

The sinus begins in approximately 5-mm. embryos of Bufo vulgaris in the form of small discontinuous anlagen, which appear either as cellular thickenings of the endothelium of the developing jugular veins or as islands lying in the mesenchyma in the immediate vicinity of these vessels.

During development all vascular anlagen of the head region, both haemal and lymphatic, can be distinguished from the surrounding mesenchyma by the greater number of yolk globules present in their endothelium.

The originally solid lymphatic anlagen acquire lumina, which have their inception as small crevice-like spaces in the cytoplasm between the large yolk globules.

By continued proliferation and growth, the individual anlagen increase in length, bud collateral branches, coalesce with one another, and in time form a complex tubular network extending in a curved plane from the region of one external jugular vein to that of the opposite side; this network represents the principal or mandibular division of the primary maxillary lymph sinus.

The other divisions, the circumoral, temporal, and pericardial, arise from the mandibular division by outgrowth and extension.

The lymphatic network becomes transformed into a spacious and uninterrupted sinus by the progressive expansion of all the anastomosing channels and by the reduction and tearing of the intervening mesenchymal strands and trabeculae.

During the preceding genetic stages, the sinus possesses no outlet; it is not confluent with the veins. The sinus receives an outlet in approximately 10-mm. embryos as the posterior prolongations of its temporal divisions join the jugular lymphatics and thereby are placed in communication with the anterior lymph hearts and through them with the venous system.

The extension and distention of the developing sinus are probably achieved by the increasing internal pressure on its walls of the accumulating lymph before an exit is established. During the expansion of the sinus, the lining cells become progressively flattened and assume typical endothelial qualities.

2. On the development of the jugular lymphatic

In 5- to 6-mm. embryos, the first three intersegmental veins, which are dorsal vertical tributaries of the pronephric venous sinus (common segment of pre- and postcardinal veins), become joined longitudinally by interanastomoses and consequently take on a plexiform character.

The aforesaid intersegmental vein plexus, which in view of its original relations and its future function may be called a venolymphatic one, gives rise to the jugular lymphatic, the important change consisting in its gradual separation from the veins (pronephric venous sinus).

By the expansion, approximation, and fusion of the longitudinal components of the plexus, the main channel of the jugular lymphatic is definitely established, and it eventually makes connection anteriorly with the temporal division of the primary maxillary lymph sinus and at its posterior end, in common with the lateral line lymphatic, joins the anterior lymph heart.

3. On the development of the anterior lymph heart

The anterior lymph heart, on either side, arises from a circumscribed portion of the venolymphatic plexus, mentioned in the preceding section, at the level and in the axis of the original 3rd intersegmental vein.

The plexiform anlage of the lymph heart becomes transformed into the uninterrupted heart chamber by the expansion and fusion of its interjoined channels.

The developing lymph heart in approximately 7- or 8-mm. embryos severs connection with the circumjacent venolymphatic plexus, but remains in continuity with the venous system via the mouth of the former 3rd intersegmental vein, now the mouth of the anterior vertebral vein.

A communication is reestablished between lymph heart and afferent lymphatic, the common segment of the jugular and lateral-line lymphatics, in approximately 10-mm. embryos; this is accomplished by the gradual approximation of the two structures, due to their growth and dilatation, and by the perforation

of the intervening wall at the line of contact to form a teat-like valve. Other permanent afferent portals are formed later in a similar manner, there being five of these in Bufo lentiginosus immediately after the period of metamorphosis.

The valve at the efferent or lymphaticovenous tap is developed from a circular endothelial cushion projecting into the lumen of the anterior vertebral vein, followed by the telescoping of this valvular portion of the heart deeper into the lumen of the vein.

During the later development of the heart the efferent tap is shifted forward from a ventral position on the heart to an anterior one.

While the heart is expanding, the lining cells become progressively flattened; the mesenchymal cells external to these become spindle shaped and ultimately develop into muscle cells.

Before the efferent valve has become differentiated, numerous blood corpuscles are found in the heart cavity. The evacuation of these elements doubtlessly occurs at the first vigorous contractions of the heart.

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APPENDIX

At the time when the above paper had already appeared in proof, I found a reference in the literature to an article by Bles on "The life-history of Xenopus laevis" (Trans. Roy. Soc. Edinb., vol. xli, 1905) in which he described and pictured the anterior lymph hearts in the larvae of this anuran. Reference to this paper will be made in my work on the comparative morphology of the systemic lymphatics which is in preparation.

PLATES

28 Reconstruction of the larger haemal and lymphatic vessels in the head and anterior trunk region of a 7.5-mm. embryo of Bufo lentiginosus (K. E. C., series B 31), dorsal view. $\times$ 50.

Structures not colored: lymphatics, anterior end of spinal cord and brain, olfactory, optic, and auditory vesicles, and pronephros and its duct; the latter structures omitted on the right side.

Lymphatics: *si. circ. or.*, circumoral division of the sinus maxillaris primigenius; *si. mand.*, mandibular division; *si. temp.*, temporal division; *si. pericard.*, pericardial division; *lym. jug.*, lymphatica jugularis; *cor. lym. ant. dex.* and *sin.*, cor lymphaticum anterius dextrum and sinistrum; *lym. lat.*, lymphatica lateralis.

Veins (blue): A portion of the sinus venosus is shown ventral to the myelencephalon joined by the hepatic sinusoids, the external jugular veins and cuvierian ducts.   The external jugular accompanies the pericardial lymphatic and anteriorly receives two branches, a medial (hidden by the mesencephalon), probably the anlage of the vena lingualis, and a lateral, lying closely against the inner side of the principal and circumoral divisions of the primary maxillary sinus and representing the future vena mandibularis and branches.   In the region of the pronephric sinusoids (a large section omitted on the right side) the cuvierian duct is joined by the precardinal or internal jugular, which passes laterally around the auditory vesicle and possesses three large tributaries, the vena orbitonasalis, the vena ophthalmica, and a large intracranial vein.   The lateral and medial (subcardinal) divisions of the postcardinal, situated along the pronephric duct, and anteriorly, near the pronephric sinus, receive the anterior vertebral vein into which the anterior lymph heart opens.

Arteries (red): The heart, external carotids and ventral roots of the aortic arches are not shown in the drawing.   The radices aortae are broadly divergent in the region of the auditory vesicles, where they connect with the dorsal roots of the aortic arches which, as they curve ventrad, lie closely against the inner side of the temporal lymphatics.   Anteriorly the radices aortae are continued forward as the internal carotids which give off in the order named the following important branches: arteria palatina, a. ophthalmica, and a. carotis cerebralis.   The pronephric glomeruli branch from the radices aortae immediately anterior to their convergence and fusion to form one trunk (ventral to the spinal cord).
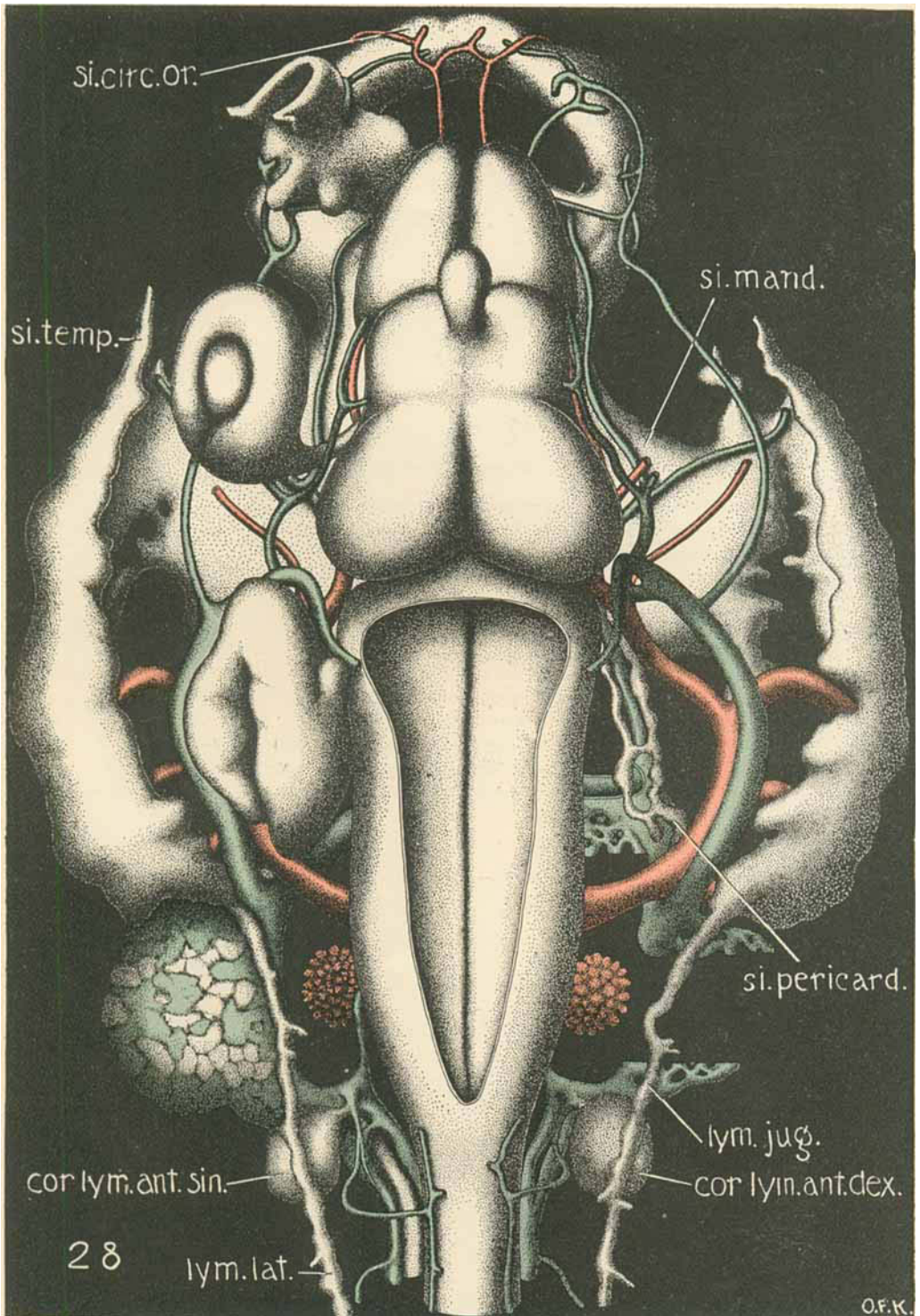
si.circ.or.

si.mand.

si.temp.

si.pericard.

lym.jug.

cor lym.ant.sin.

cor lym.ant.dex.

28

lym.lat.

O.F.K.

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29   Reconstruction of the vascular channels of the ventral cephalic region in a 6-mm. embryo of Bufo vulgaris (K. E. C., series B 53), ventral veiw.   × 125.

v. jug. ext., vena jugularis externa.

a. car. ext., arteria carotis externa.

g. thyr., glandula thyroidea.

ao., aortic arches.

vent., ventriculus of the heart.

d. Cuv., ductus Cuvieri.

sin. ven., sinus venosus; its cut edge shows its attachment to the liver, for at this period the hepatic sinusoids open directly into it.

The lymphatics, the anlagen of the sinus maxillaris primigenius arise independently of each other along the venous components of the jugulocarotid plexus; some of them have severed contact with the blood vessel wall, while others still adhere to it.
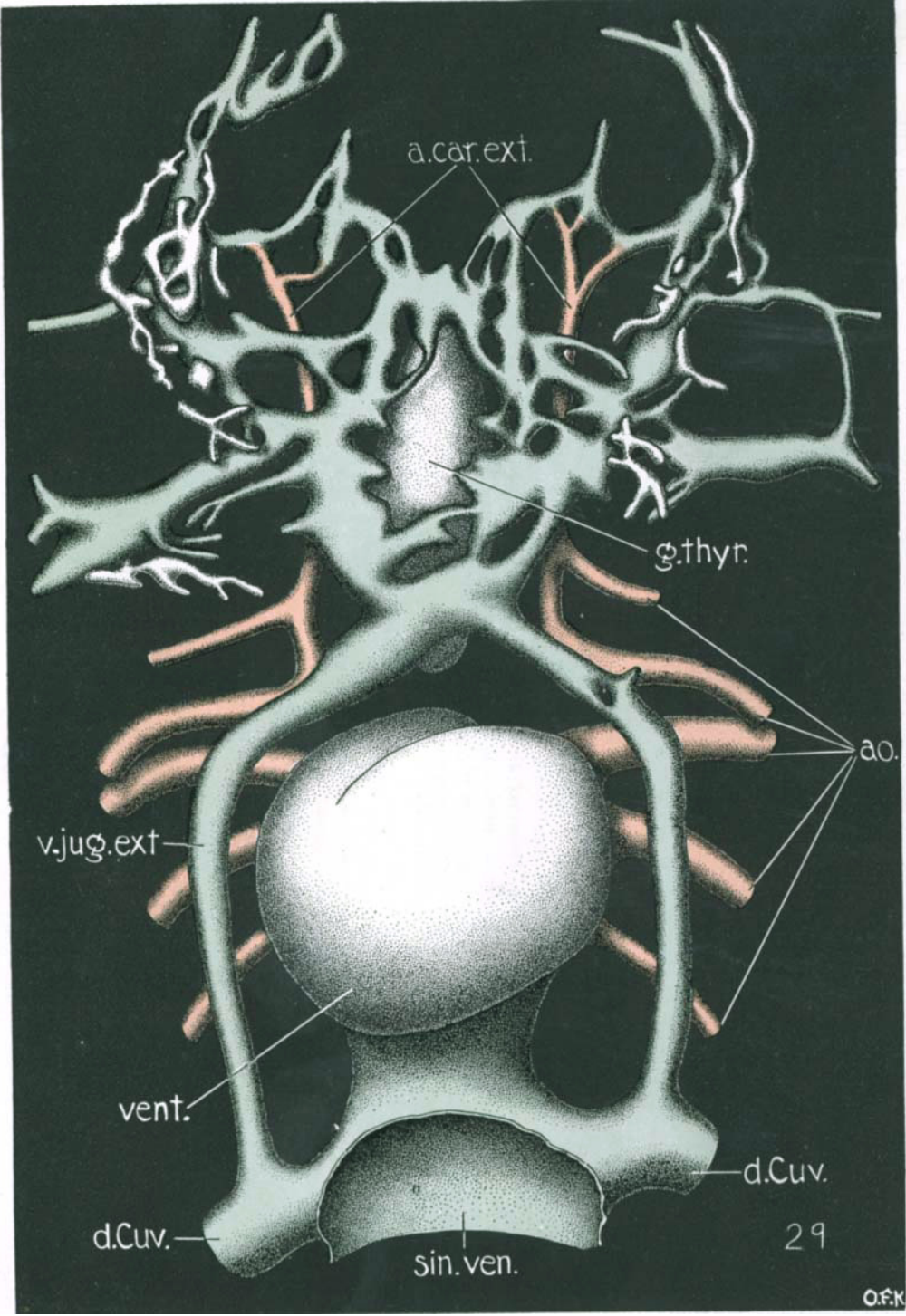
PLATE 3

30   Reconstruction of the vascular channels of the ventral cephalic region in a 7-mm. embryo of Bufo vulgaris (K. E. C., series B 27), ventral view. × 125.

si. circ. or., circumoral division of the sinus maxillaris primigenius.

si. mand., mandibular division of the sinus maxillaris primigenius.

si. temp., temporal division of the sinus maxillaris primigenius.

si. pericard., pericardial division of the sinus maxillaris primigenius.

g. thyr., glandula thyroidea.

a. car. ext., arteria carotis externa.

ao., aortic arches.

v. jug. ext., vena jugularis externa.

d. Cuv., ductus Cuvieri.

v. card. post., vena cardinalis posterior.

sin. ven., sinus venosus.

v. jug. int., vena jugularis interna.

vent., ventriculus of the heart.

At the point marked ?, the author is unable to decide whether a direct connection exists or not.
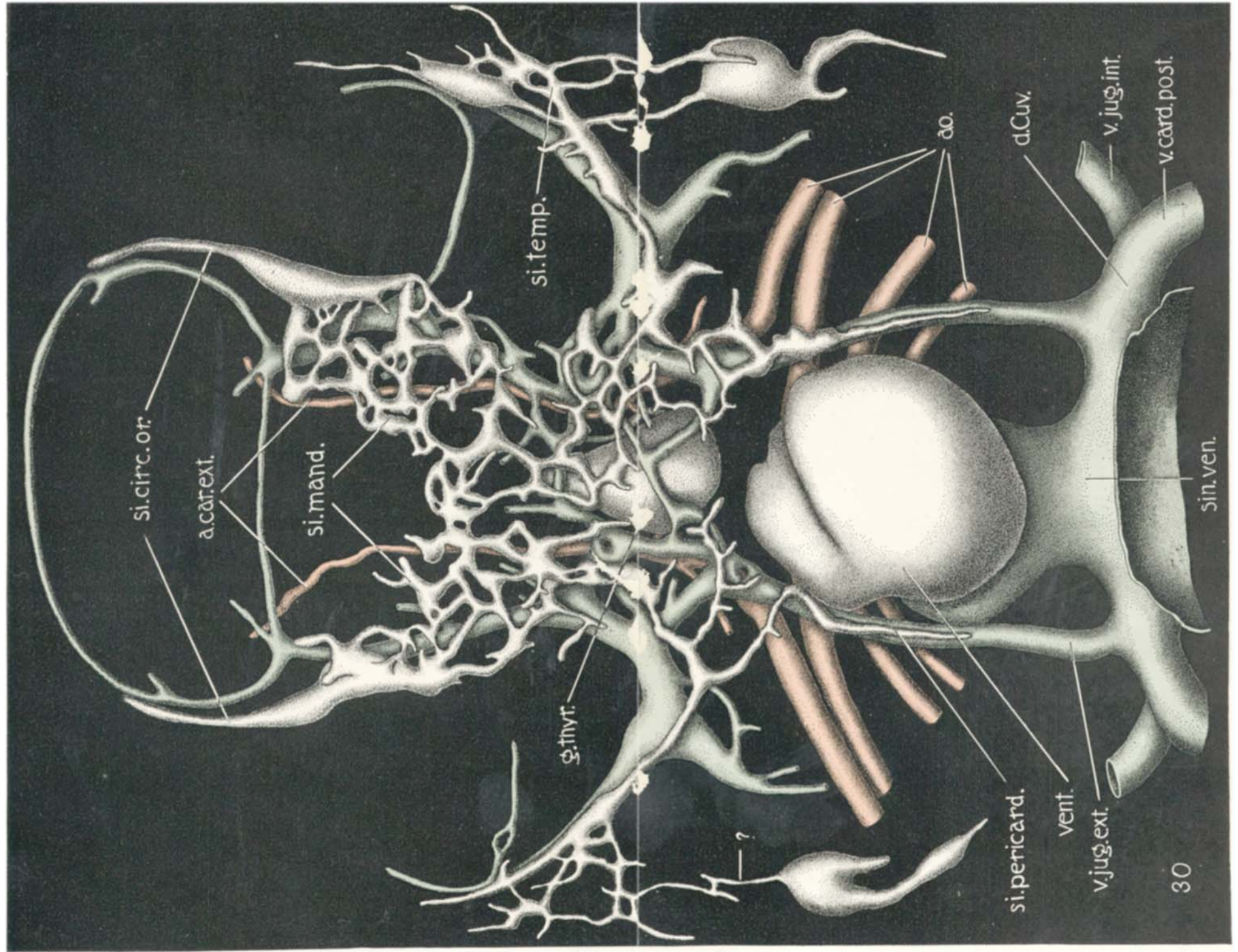
118

PLATE 3

30

31 Reconstruction of the vascular channels and other structures in the region of the left pronephros in a 4-mm. embryo of Bufo vulgaris (K. E. C., series B 45), lateral view. × 166.

1–4 v. seg., 1st to the 4th intersegmental veins; the beginning of the formation of the venous plexus in the development of the anterior lymph heart is already indicated by the short and irregular branches of the 3rd intersegmental vein.

v. jug. int., vena jugularis interna (precardinal)

v. jug. ext. vena jugularis externa

v. card. post., vena cardinalis externa

d. Cuv., ductus Cuvieri

nephst., I, II, et III, nephrostomes of the pronephros

d. proneph., pronephric or primary excretory duct

med. spin., medulla spinalis

II, III, IV, 2nd, 3rd and 4th spinal ganglia

vag., a ganglionic prolongation from the vagus group back along the medulla oblongata; it may be a vestige associated with the lateral-line organs and later disappears.

A reconstruction of the above structures in a 5-mm. embryo, representing an intermediate stage between that pictured on the opposite plate and that on the following one, was omitted with several other illustrations to reduce the cost of publication, although it revealed very strikingly the lymph heart plexus before its coalescence into a uniform cavity, a process almost completed in plate 5.
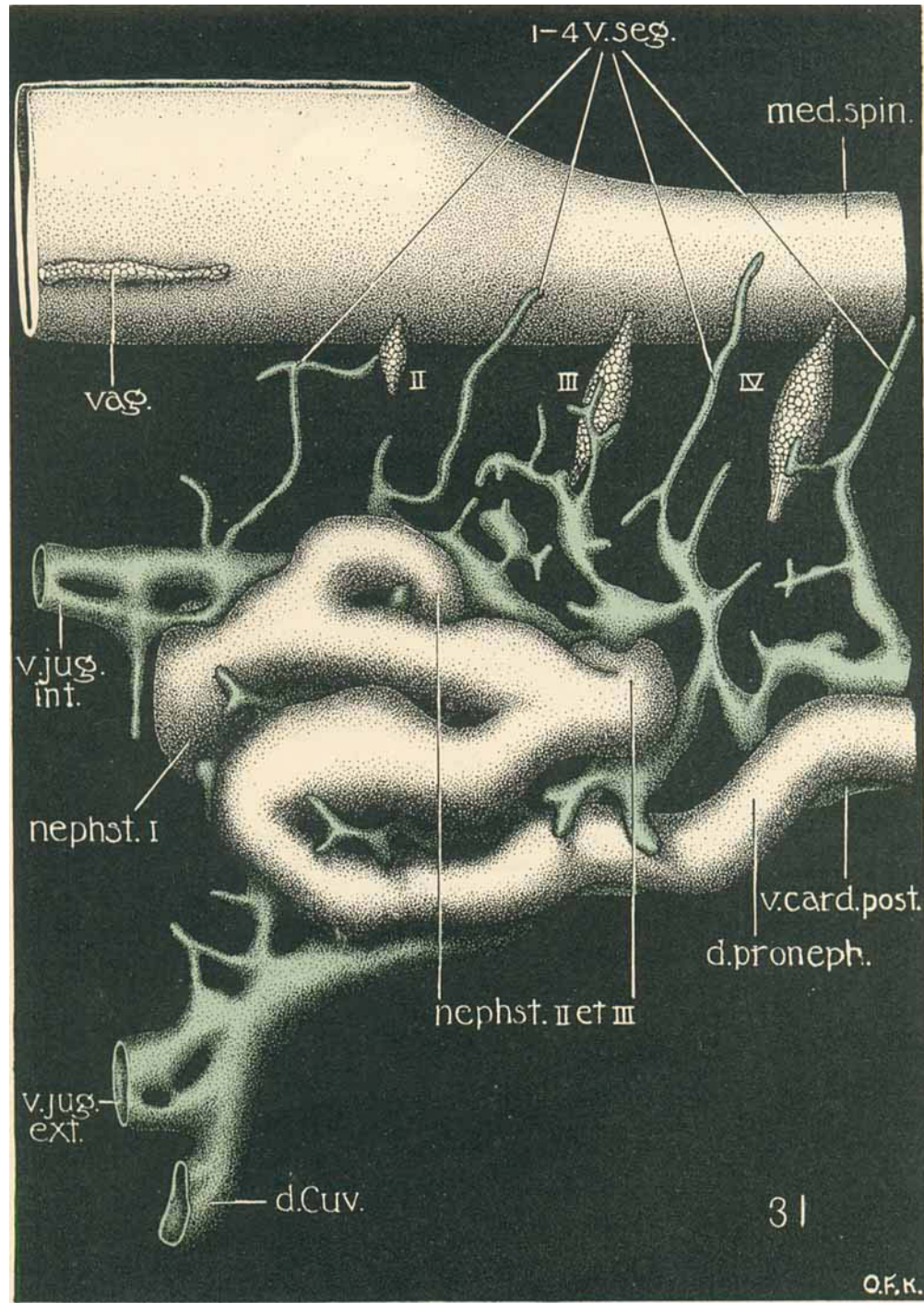
PLATE 5

32 Reconstruction of the vascular channels and other structures in the region of the left pronephros in a 6-mm. embryo of Bufo vulgaris (K. E. C., series B 54), lateral view. × 166.

1-4 v. seg., 1st to the 4th intersegmental veins; by the formation of interanastomosis between them a plexus results, which may be called a venolymphatic one, in view of the fact that it subsequently gives rise to the lymph vessels in this region.

cor. lym. ant., anlage of the anterior lymph heart.

v. jug. int., vena jugularis interna.

v. jug. ext., vena jugularis externa.

v. card. post., vena cardinalis posterior.

d. Cuv., ductus Cuvieri.

d. proneph., pronephric duct.

nephst. I, II, et III, pronephric nephrostomes.

med. spin., medulla spinalis.

I. II, III and *IV,* spinal ganglia; the 1st spinal ganglion is a very transitory and vestigial structure appearing for the first time in 6-mm. embryos and disappearing very soon after; the 2nd spinal ganglion becomes the 1st of the adult.

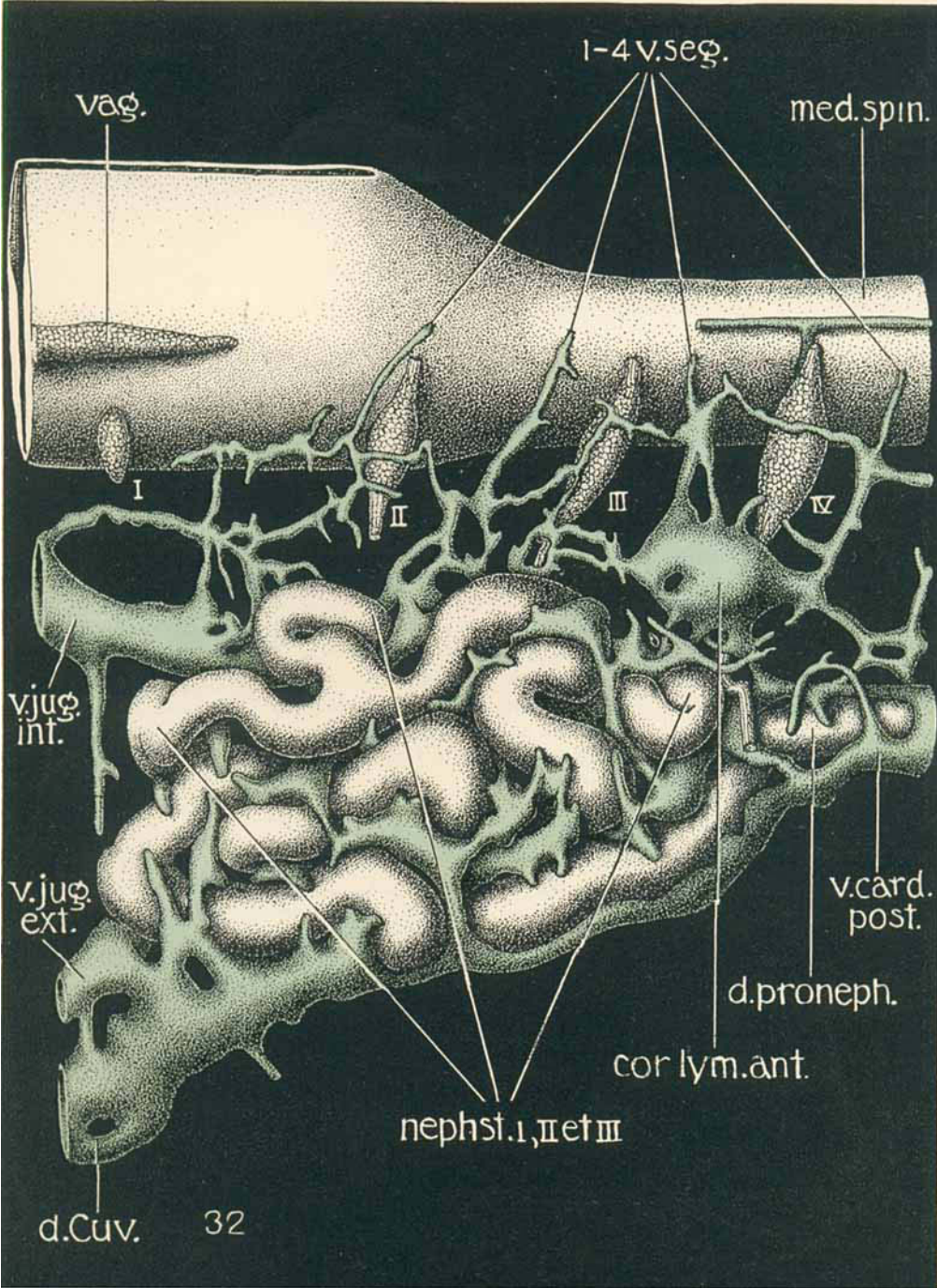*vag.,* ganglionic prolongation of the vagus group (cf. fig. 31).

PLATE 6

33 Reconstruction of the vascular channels and other structures in the region of the left pronephros in a 7-mm. embryo of Bufo vulgaris (K. E. C., series B 27), lateral view. × 166.

lym. jug., lymphatica jugularis; at the points marked * there is still a minute connection between the pronephric sinus and the lymphatic.

temp. s. max. prim., posterior tip of the temporal division of the sinus maxillaris primigenius.

lym. lat., lymphatica lateralis; this lymphatic in early stages is broadly plexiform and has a dorsal and a ventral division.

cor. lym. ant., cor lymphaticum anterius; the surrounding lymphatics are severing connection with the heart; the lymphaticovenous junction is on the ventral surface of the heart, and anterior to this are two small venous branches which later become consolidated with it to form the anterior vertebral vein.

v. jug. ext., vena jugularis externa.

d. Cuv., ductus Cuvieri.

gan. jug. (vag.), ganglion jugulare of the vagus group.

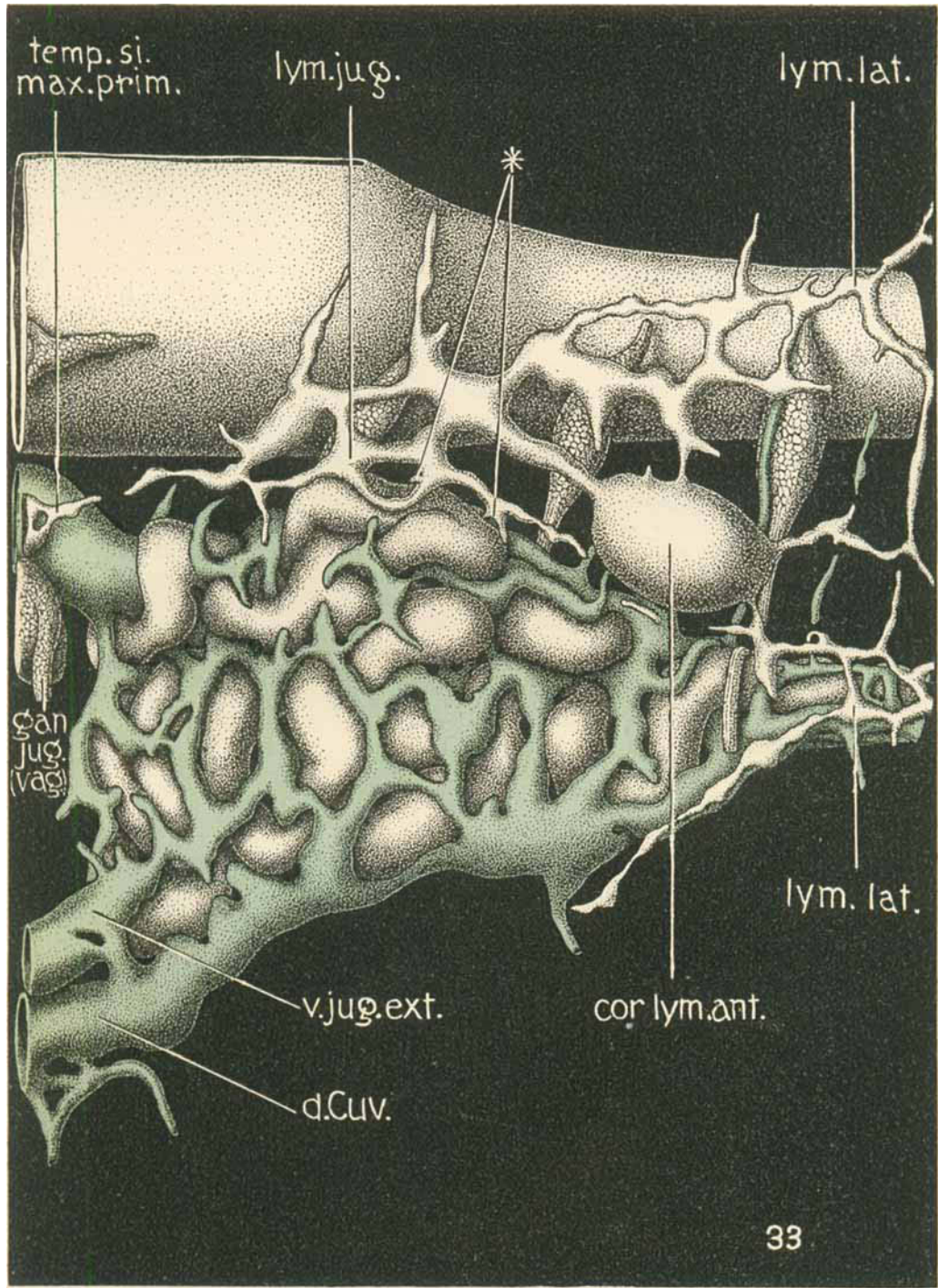Other structures as in the preceding plates.

temp. si.
max. prim.

lym. jug.

lym. lat.

*

gan
jug.
(vag)

lym. lat.

v. jug. ext.

cor lym. ant.

d. Cuv.

33

34 Reconstruction of the vascular channels and other structures in the region of the left pronephros in an 8-mm. embryo of Bufo vulgaris (K. E. C., series B 49), lateral view; $\times$ 166.

lym. jug., lymphatica jugularis; the point marked * still shows a minute vestigial connection with the pronephric sinus.

temp. s. max. prim., temporal division of the primary maxillary sinus.

lym. lat., lymphatica lateralis (both dorsal and ventral divisions); the common channel of the jugular and the lateral lymphatic dorsal to the lymph heart is referred to in the text and photomicrographs as the common segment of these vessels.

cor. lym. ant., cor lymphaticum anterius; the surrounding lymphatic plexus has temporarily severed ts connection with the heart.

v. vert. ant., vena vertebralis anterior.

v. jug. int., vena jugularis interna.

v. jug. ext., vena jugularis externa.

v. card. post., vena cardinalis posterior.

d. Cuv., ductus Cuvieri.

med. spin., medulla spinalis.

gan. jug. (vag.), ganglion jugulare of the vagus group; *vag.* (cf. figs. 31–33). Other structures as before.
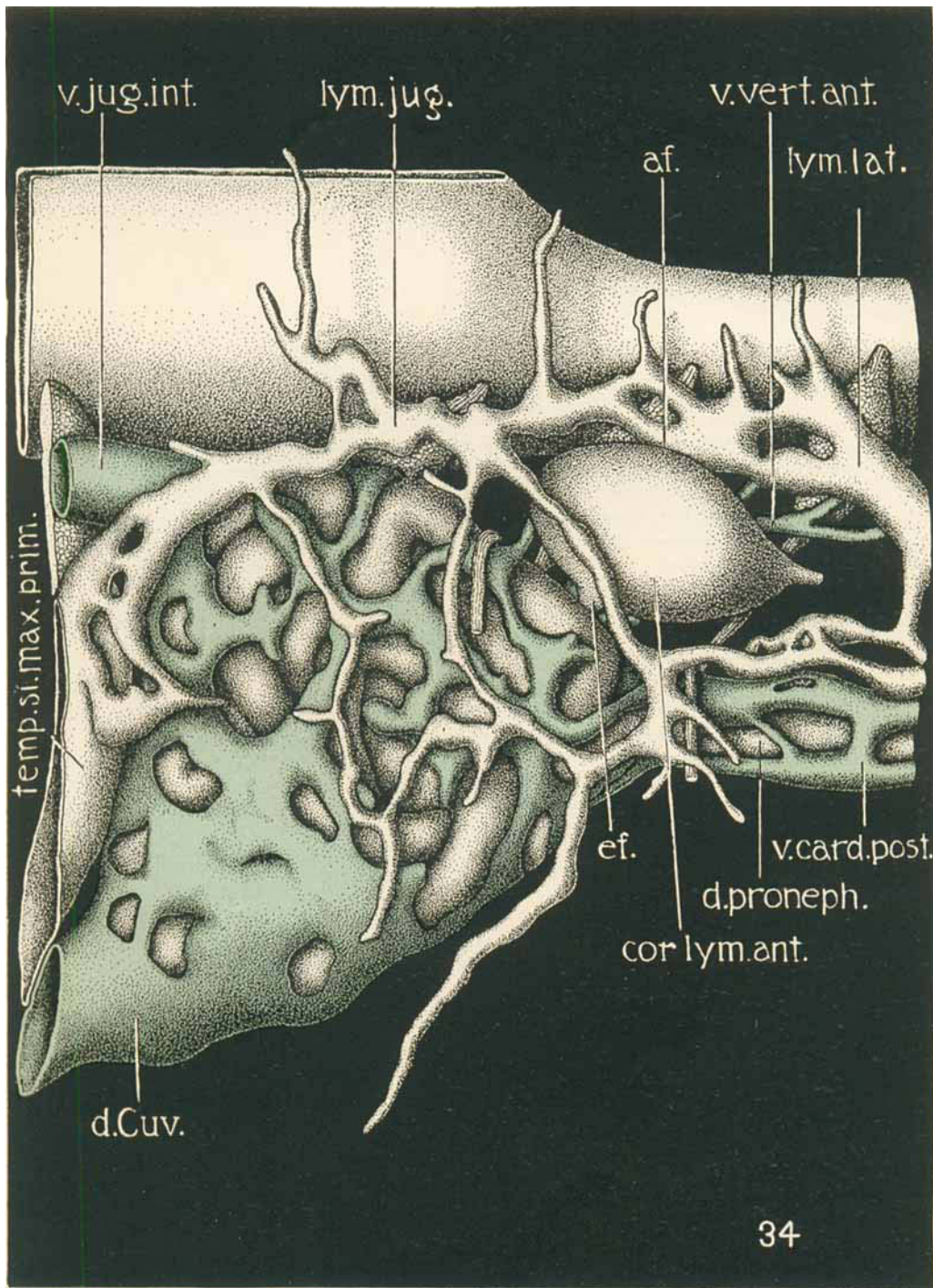
35 Reconstruction of the vascular channels and other structures in the region of the left pronephros in a 10-mm. embryo of Bufo vulgaris (K. E. C., series B 34), lateral view. $\times$ 166.

cor. lym. ant., cor. lymphaticum anterius; the lymphaticovenous junction or efferent portal is shown at *ef.; af.*, indicates the point at which the afferent portal is being established.

lym. jug., lymphatica jugularis.

lym. lat., lymphatica lateralis.

temp. s. max. prim., posterior portion of the temporal division of the primary maxillary sinus.

v. vert. ant., vena vertebralis anterior.

v. jug. int., vena jugularis interna.

v. card. post., vena cardinalis posterior showing both the medial (subcardinal) and lateral divisions.

d. Cuv., ductus Cuvieri.

d. proneph., pronephric duct.

Other structures as before.

35