ON THE PROVISIONAL ARRANGEMENT OF THE EMBRYONIC LYMPHATIC SYSTEM

AN ARRANGEMENT BY MEANS OF WHICH A CENTRIPETAL LYMPH
FLOW TOWARD THE VENOUS CIRCULATION IS CONTROLLED
AND REGULATED IN AN ORDERLY AND UNIFORM MANNER,
FROM THE TIME LYMPH BEGINS TO COLLECT IN THE INTER-
CELLULAR SPACES, UNTIL IT IS FORWARDED TO THE VENOUS
CIRCULATION

CHARLES F. W. McCLURE

From the Department of Comparative Anatomy, Princeton University

SIX FIGURES

One of the most interesting problems of the lymphatic system is the determination of the manner in which the continuous centripetal lymph flow is established in the embryo, in relation to the developing lymphatic vessels by which it is subsequently conveyed to the venous circulation.

It is well known that the anlagen of the lymphatic system do not normally make their appearance in the embryo until after the haemal vessels have been established. As soon, however, as the haemal vessels begin to function, lymph begins to collect in the intercellular spaces of the embryo and, as we know, is subsequently collected by a set of newly-formed vessels, the lymphatics, which convey it to the venous circulation.

Those who maintain that the lymphatics sprout centrifugally and continuously from the veins, would necessarily hold that the lymph in the intercellular spaces patiently awaits the arrival of closed and hollow outgrowths from the veins, the lymphatics, before it can be received into any portion of the lymphatic system. Such continuous outgrowths from the veins would necessarily take place in a centrifugal direction which is opposed to that of the centripetal flow of lymph they would receive.

It has always proved a difficult matter for some of us to reconcile the view that the direction of the growth of the vessels

in which the flow takes place should be opposed to that of the flow. One might expect a continuous centripetal lymph flow toward the venous circulation to be established in a gradual manner in the embryo, and to be regulated from the time lymph first made its appearance in the intercellular spaces, until it was continued on to the venous circulation. In fact, one might expect to find some provisional condition of the embryonic lymphatic system which should exactly accord with the maintenance and regulation of such a centripetal flow. *Such a provisional condition of the embryonic lymphatic system I believe I have been able to demonstrate in a positive manner in the living embryo of the trout.*

One of the most salient features noticeable in the development of the lymphatic system of the trout, as well as in that of mammals, is that the main lymphatic channels are formed through a gradual concrescence of independent and discontinuous anlagen or lymph vesicles. These independent lymph vesicles make their appearance in the embryo in a progressive manner along the lines subsequently followed by continuous lymphatic vessels, and, in certain districts of the mammalian embryo, they utilize the static line vacated by degenerating veins, so that certain lymphatic vessels of the body subsequently follow the course of abandoned veins. These independent lymph vesicles of the embryo first become concrescent to form continuous channels contiguous to the points at which the lymphatics establish typical communications with the veins. With these points of lymphatico-venous entry, the vesicles continue to become concrescent in a progressive manner, so that the out-lying or peripheral lymph vesicles are the last to establish a communication with the veins.

The view which calls for the development of the main lymphatic channels through a confluence of independent and discontinuous anlagen was advanced by Huntington and McClure¹

¹ Huntington and McClure. The development of the main lymph channels of the cat in their relations to the venous system. *Anat. Rec.*, vol. 1, 1907. Read before the American Association of Anatomists at the meeting held in New York in December, 1906.

in 1906. Since a recognition of this fact is a necessary corollary to the main issue involved in the present paper, we will first consider what constitute the main lymphatic channels in the embryo of the trout and show how, in their development, they follow this plan.

Figure 1 represents a ventral view of a reconstruction of the main lymphatics, veins, and arteries found in the regions of the head and pharynx of a rainbow trout embryo, on the twenty-second day after fertilization. This embryo was developed at a temperature of about 10.5° C. and its lymphatic system is represented, for the most part, by a continuous system of vessels which drain into the veins at typical points. The typical points of lymphatico-venous communication in the regions of the head and pharynx occur in the cardino-Cuvierian district (9); with the precardinal (jugular) vein near the caudal end of the otocyst (13); and with the precardinal, near a point where the latter leaves the cranial cavity (2). The first and last mentioned points of communication appear invariably to be retained in the adult.

The principal or main lymphatic vessels found in the regions of the head and pharynx of a twenty-two day rainbow trout embryo are as follows:

1. *The subocular lymph sacs (saccus lymphaticus subocularis, 1 in figure 1)*

The subocular lymph sacs of the trout embryo consist of two relatively huge sacs or vesicles, each of which lies ventro-medial to the eye. They are more or less triangular in form, with their apices directed forward. In the twenty-two day trout under consideration, they extend between the hyoidean artery (15) and the olfactory invagination (21), as shown in figure 2. At its postero-lateral angle, each subocular lymph sac (1) communicates directly with the lateral pharyngeal lymphatic (3 in fig. 1) and it is solely through the latter vessel that the subocular lymph sac drains into the veins.

Fig. 1 Reconstruction of the main lymphatics, arteries and veins found in the regions of the head and pharynx of a twenty-two-day rainbow trout embryo; ventral view. P. E. C. series 648. Reconstructed after the method of Born at a magnification of 200 diameters.

REFERENCE NUMBERS

1, Subocular lymph sac	11, Postcardinal vein
2, Medial pharyngeal communication	12, Duct of Cuvier
3, Lateral pharyngeal lymphatic	13, Otic communication
4, Medial pharyngeal lymphatic	14, Dorsal aorta
5, Precardinal (jugular) lymphatic	15, Hyoidian artery
6, Precardinal (jugular) vein	16, First efferent aortic arch
7, Communication between the pre-cardinal lymphatic and the lateral pharyngeal lymphatic	17, Second efferent aortic arch
8, Caudal end of otocyst	18, Third efferent aortic arch
9, Cardio-Cuvierian communication	19, Fourth efferent aortic arch
10, Lymphatic of the lateral line of the trunk	20, Caudal end of eye
	21, Olfactory invagination
	22, Carotid artery

Fig. 2 Sagittal section taken through the head and pharynx of a twenty-two-day rainbow trout embryo, showing the relations of the subocular lymph sac to the eye, the hyoidian artery and the olfactory invagination; 1, subocular lymph sac; 15, hyoidian artery; 21, olfactory invagination. P. E. C. series 816.

2. *The lateral pharyngeal lymphatic (truncus lymphaticus pharyngeus lateralis, 3 in figure 1)*

This vessel occupies a superficial position in the lateral wall of the pharynx and forms, on each side of the body, the direct anterior continuation of the lymphatic of the lateral line of the trunk (*truncus lymphaticus longitudinalis lateralis, 10 in fig. 1*). The lymphatic of the lateral line of the trunk is completely developed in the twenty-two day rainbow trout and can be followed caudad to the region of the caudal lymph heart. The lateral pharyngeal lymphatic drains the subocular lymph sac and, at a slightly later stage of development, also the dorsal region of the head and pharynx, the operculum and the lower jaw. It communicates with the veins in the cardino-Cuvierian district (9) in common with the lymphatic of the lateral line of the trunk (10).

3. *The medial pharyngeal lymphatic (truncus lymphaticus pharyngeus medialis, 4 in figure 1)*

This vessel occupies a more central position and is more deeply situated than the lateral pharyngeal lymphatic. It follows an oblique course, in a postero-anterior direction, from about the middle of the lateral pharyngeal lymphatic with which it subsequently communicates, to open into the precardinal vein just caudal to the point where this vein emerges from the cranial cavity (2 in fig. 1).

4. *The precardinal or jugular lymphatics (truncus lymphaticus precardinalis vel jugularis, 5 in figure 1)*

These vessels develop along the line of the precardinal veins. They are not completely established on the twenty-second day in the form of continuous channels and are represented by such vessels only near the caudal end of the pharynx, where they communicate with the lateral pharyngeal lymphatic (7 in fig. 1).

All of the continuous lymphatic channels described above, as occurring on the twenty-second day in the embryo of the rain-

bow trout, can be readily injected from the veins, through any one of the typical points of communication which are established between the lymphatics and the veins. By injecting into the subocular lymph sacs it is also possible to fill the continuous lymphatic vessels with injecta, as well as the veins. At this particular stage of development, in the absence of lymphatico-venous valves, blood may flow freely into the lymphatics from the veins, at the typical points at which the lymphatics communicate with the veins. In rainbow trout embryos slightly older than twenty-two days, and in which lymphatico-venous valves have been formed, the application of chloretone apparently vitiates the normal function of these valves. Blood may then also flow freely from the veins into the lymphatics and fill up completely all of the continuous lymphatic channels, including the subocular lymph sacs. If such embryos are removed to water in which no chloretone is present, the blood will flow back from the lymphatics into the veins.

The age of the rainbow, steelhead or brook trout embryo in which a continuous system of lymphatic channels, as shown in figure 1, is met with for the first time, naturally varies with the temperature of the water in which development takes place. Much variation is also met with in the rate at which the lymphatic system of the trout develops in different embryos of the same age, as well as upon opposite sides of the same embryo. When developed at a temperature of about 10.5° C., a continuous lymphatic system, as shown in figure 1, is usually found in rainbow and steelhead trout embryos on the twenty-second day after fertilization, which is six or seven days after its earliest anlagen can first be observed in sections.

In all stages of development, prior to the establishment of such a continuous system of lymphatics as shown in figure 1, a condition is invariably met with in the embryo in which the lymphatic system is represented by a progressively appearing series of discontinuous anlagen, that present varying degrees of concrescence with one another to form continuous lymphatic vessels which communicate with the veins, only at the typical points at which the lymphatics establish communications with the

Fig. 3 Reconstruction of the lymphatics, arteries and veins found in the regions of the head and pharynx of a twenty-day rainbow trout embryo; ventral view. P. E. C. series 668. Reconstructed after the method of Born at a magnification of 200 diameters; reconstruction of an injected embryo. For reference to numbers see under figure 1.

veins. Two such stages of development are herewith presented in illustration of this fact.

Figures 3 and 4 represent, respectively, reconstructions of the lymphatics, veins, and arteries found in the regions of the head and pharynx of a rainbow trout embryo on the twentieth and

Fig. 4 Reconstruction of the lymphatics, arteries and veins found in the regions of the head and pharynx of a twenty-one-day rainbow trout embryo; ventral view. P. E. C. series 646. Reconstructed after the method of Born at a magnification of 200 diameters; reconstruction of an injected embryo. For reference to numbers see under figure 1.

twenty-first days after fertilization. These embryos were developed at a temperature of about 10.5°C . When compared with the conditions found in the embryo on the twenty-second day (fig. 1), it is seen that the subocular lymph sacs (1 in figs. 3 and 4) are entirely independent of the veins and of other independent lymph vesicles, and that a series of discontinuous

and independent lymph vesicles lie exactly in the line subsequently followed by continuous lymphatic channels on the twenty-second day (fig. 1). It is also seen that these lymph vesicles present varying degrees of concrescence beginning at the points at which the lymphatics establish typical communications with the veins. It may also be observed that none of these independent lymph vesicles communicate with the veins except those which lie contiguous to the points at which the lymphatics establish typical communications with the veins.

For the purpose of the present paper reconstructions of the twenty and twenty-one day trout are sufficient to illustrate the general principle of development followed by the main lymphatic channels.

The main question involved in the present issue is, however, what functional significance does the presence in the embryo of such an independent and discontinuous series of lymph vesicles imply? *A study of one of these lymph vesicles, the subocular lymph sacs, as observed and experimented upon in the living trout embryo gives, I believe, a positive answer to this question.*

When rainbow and steelhead trout embryos are developed at a temperature of about 10.5°C., the development of the anlagen of the subocular lymph sacs is initiated on about the sixteenth day after fertilization. These anlagen first appear as small clear vesicles which lie in the mesenchyme in an area between the hyoidean artery and the eye and just dorsal to the maxillary ridge. These small vesicles finally become confluent to form a larger vesicle which gradually increases in size. Injection experiments upon the living trout embryo, controlled by sections, have shown that the anlagen of the subocular lymph sacs never establish a communication with the veins in the neighborhood of the sacs. They have also shown that the subocular lymph sacs cannot be injected from the veins at any stage of their development, until after the independent and discontinuous anlagen of the lateral pharyngeal have become concrescent with one another to form a continuous vessel and until a communication has been established between this vessel and the subocular

lymph sac (compare figures 3 and 4 with figure 1). In other words, at no stage of development has it been possible to inject the subocular lymph sac of the trout embryo except by way of the lateral pharyngeal lymphatic, which signifies that the subocular lymph sac of the trout embryo is entirely independent of the veins and of other lymphatics, until this communication has been made.

Fig. 5 Photograph of the ventral aspect of the head of a twenty-day rainbow trout embryo on which an attempt was made to inject the lymphatics and the veins by injecting into the subocular lymph sacs. Spalteholz preparation. 1, subocular lymph sacs.

Injection experiments have also proved that the subocular lymph sacs of the trout embryo do not grow caudad and that they are invariably bounded posteriorly by the hyoidean artery; (compare 15, hyoidean artery, with 1, subocular lymph sac, in figures 2, 3 and 4). Figure 5 is a photograph of a Spalteholz preparation of a twenty-day rainbow trout embryo on which an attempt was made to inject the lymphatics and the veins by injecting into the subocular lymph sacs (1 in fig. 5). The figure shows the position occupied by the subocular lymph sacs

in the living embryo and, since the sacs alone filled with the injecta, is illustrative of an experiment which proves that the subocular lymph sacs have not grown caudad, nor do they communicate with the lateral pharyngeal lymphatic, nor with the veins at the stage of development presented by this twenty-day trout.

Fig. 6 Transverse section taken through the subocular lymph sacs of a twenty-one-day rainbow trout embryo in which, as independent structures, the sacs have reached the maximum stage of their development. P. E. C. series 646. Injected embryo. 1, subocular lymph sac; 6, precardinal (jugular) vein; 20, eye; 22, carotid artery.

The subocular lymph sacs of the trout embryo are non-pulsatile in character and are lined by an endothelium around which no muscular coat is formed. During the stage of their independence they become gradually distended with lymph which must necessarily enter them in a centripetal direction from the

intercellular spaces of the head. As this lymph gradually increases in amount the subocular sacs of the trout can be easily observed in the living embryo, and are especially prominent in the rainbow trout between the nineteenth and twenty-first days. By the time the subocular lymph sacs have attained their maximum size as independent structures, on the twenty-first day (figs. 4 and 6), a considerable pressure must be exerted by their lymph upon their walls, which would account for the distended appearance presented by the sacs (1) at this stage (fig. 6). As soon, however, as the subocular lymph sacs establish their communication with the lateral pharyngeal lymphatic, so that their lymph can flow continuously and centripetally to the veins, this pressure against their walls is immediately released, and the sacs then appear less prominently in the living embryo, due to a partial collapse of their walls.

It is thus seen that, during the period of its independence, the subocular lymph sac of the trout embryo serves as a local and independent reservoir for the reception of lymph which enters it in a centripetal direction from the intercellular spaces; that it retains this lymph only temporarily, until the sac establishes a communication with the lateral pharyngeal lymphatic, through which a continuous centripetal lymph flow may then pass from the intercellular spaces to the venous circulation.

The subocular lymph sacs of the trout embryo therefore furnish us with a striking example of the fact that, not only do independent and discontinuous anlagen of the lymphatic system actually exist, but, that they can also be observed and be experimented upon in the living embryo.

The functional rôle played by the subocular lymph sacs of the trout embryo, during the stage of their independence, as well as after they have established a communication with the venous circulation, undoubtedly gives us the clue to the function assumed by the independent lymph vesicles of the embryo in general. It also explains the manner in which a continuous centripetal lymph flow is established in the embryo, between the intercellular spaces and the venous circulation, in relation to the developing lymphatic vessels.

On the basis of the functional rôle played by the subocular lymph sacs—and this can be actually demonstrated in the living trout embryo—it is highly probable that the independent lymph vesicles, of the embryo in general, also serve as local reservoirs for the temporary retention of lymph which enters them in a centripetal direction from the intercellular spaces; that these lymph vesicles become progressively concrescent with one another to form continuous channels, through which the lymph collected and temporarily retained by them is then forwarded to the venous circulation. In this manner the centripetal flow of lymph which continuously enters these independent lymph vesicles from the outlying intercellular spaces, is continued on to the venous circulation.

It may be mentioned here, incidentally, that the functional rôle played by the subocular lymph sacs of the trout embryo during the stage of their independence, is also evidence of the fact that the lymphatics of fishes function solely in the capacity of lymphatics at the time of their inception, and that they are therefore not transformed veins.

In case the independent lymph vesicles of the embryo should fail to become concrescent with one another and with the veins, at the typical points of lymphatico-venous entry, an oedematous condition of the body would undoubtedly arise, and the ontogenetic condition, in which only independent and discontinuous anlagen are present, might then be retained in the adult. That such might actually be the case seems to be borne out by the conditions observed in an oedematous human foetus already described by Smith and Birmingham.² These investigators have described a case in which that peculiar and rare condition known as oedematous foetus was found to depend upon the complete absence of the thoracic duct, lymphatic glands and lymphatic trunks in general, and in which the lymph was stored in what they described as “greatly distended tissue spaces” which neither communicated with one another, nor with the veins.

² Smith and Birmingham. Absent thoracic duct causing oedema of a foetus. Jour. Anat. and Physiol., vol. 23.

Only two possible conclusions can be drawn regarding the significance of the complete absence of continuous lymphatic trunks and of the presence only of independent and discontinuous lymph-containing tissue spaces in this human foetus: Either there has been a complete failure on the part of the lymphatic system even to initiate its development, so that these 'tissue spaces' are in no sense related to the true lymphatic system, or, the presence of these spaces signifies a condition in which the normal development of the lymphatic system has been arrested at an early ontogenetic stage.

Huntington and the writer³ have repeatedly described and figured the presence of independent lymph vesicles or lymph spaces in the mammalian embryo, and Huntington⁴ has recently made a more extensive and detailed study of these structures, as they occur in the subelavian and primitive ulnar regions of the cat. Although one is not given the opportunity of studying these independent lymph vesicles in the living embryo of the mammal, as is the case in the trout, it would now seem quite a waste of time to parley further over the question of their presence in the mammalian embryo, or, that it is through the concrescence of such independent vesicles that the main lymphatic channels of mammals are formed.

The oedematous human foetus described by Smith and Birmingham appears to present us with the most striking evidence, not only of the fact that the presence of independent lymph vesicles may actually be demonstrated, in certain circumstances, as functional structures in mammals, but, also, that the functional rôle played by them is similar to that played by the subocular lymph sacs of the trout during their independent stage. It is

³ Huntington and McClure. The anatomy and development of the jugular lymph sacs in the domestic cat (*Felis domestica*). Amer. Jour. Anat., vol. 10, 1910, fig. 66.

⁴ Huntington. The development of the mammalian jugular lymph sac, of the tributary primitive ulnar lymphatic and the thoracic ducts from the viewpoint of recent investigations of lymphatic ontogeny, together with a consideration of the genetic relations of lymphatic and haemal vascular channels in the embryos of amniotes. Amer. Jour. Anat., vol. 16, 1914. Also, The development of the lymphatic drainage of the anterior limb in embryos of the cat. Proc. Amer. Ass. Anat., Anat. Rec., vol. 9, 1915

highly probable, therefore, that the conditions found in this human foetus indicate that the development of the lymphatic system had been arrested at a normal ontogenetic stage; that its oedematous condition was due to the circumstance that the independent and discontinuous anlagen of the lymphatic system had failed to become conrescent with one another and with the veins, in order to establish a continuous system of channels, through which a continuous centripetal lymph flow could pass from the outlying tissue spaces to the venous circulation.