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THE TOPOGRAPHY OF THE LYMPH HEART IN THE DOMESTIC CHICKEN *(Gallus Domesticus)**

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ABSTRACT

Although at the time of hatching, the lymph heart of the domestic chicken (Gallus domesticus) *is usually considered to have atrophied, our findings suggest otherwise. Apart from the lymphatic-venous (L-V) anastomoses between the thoracoabdominal lymph trunks and the superior vena cava in the chest, there are two other L-V anastomoses in the sacral region. These L-V communications connect the lymph heart with three inter-segmental veins and with the L-V sinus of the dura mater. The topography of the lymph heart was demonstrated using direct and indirect blue dye injections depending on the age of the chicken. Lymphatic continuity was noted up to the 15th week of age from lymphatics of the hindlimb into the lymph heart via the thoracoabdominal lymph trunk, including communications of the lymph heart with the venous system and dural sinus. At 16 weeks of age, lymph flow apparently ceases caudally, but continues superiorly into the venous system. In the egg, the lymph heart may help propel interstitial fluid in the motionless fetus. After hatching, the lymph heart may facilitate fluid propulsion through L-V communications in the spinal canal.*

**Dedicated to Prof. Dr. Schaller (Vienna) on the occasion of his 70th birthday.*

Contradictory reports exist as to the postnatal persistence (or regression) of the lymph heart and pelvic lymph-venous (L-V) anastomoses of the domestic chicken (*G. domesticus*). Several authors maintain (1-3), that the lymph heart atrophies soon after hatching as the allantoic circulation becomes obliterated, its lymph circulation ceases (1), and in its place there is a slightly enlarged lymphatic trunk (4). The L-V connection in the pelvic area also presumably atrophies (5), so that lymph only enters the blood circulatory system at the jugular-subclavian venous juncture. On the other hand, others claim that rudiments of the lymph heart are detectable up to the 30-35th day after hatching (6,7), or may even persist in the adult chicken (8).

The reason for these differences probably are traceable to the difficulty of color-marking and accurate localization of the lymph heart with its pale color and minute size of only 2mm. Indirect marking by dye injection into a metatarsal footpad has previously been unsuccessful in demonstrating lymph flow to the lymph heart. Direct injection into major lymphatics of the domestic chicken has also been technically unfeasible (9), whereas a method previously used in ratite birds and waterfowl (10) has proved impractical. Although these latter species have a well developed lymphatic copulatory organ in which the lymph heart is functionally integrated and can be readily marked by dye injection into the lymphatic corpus spongio-

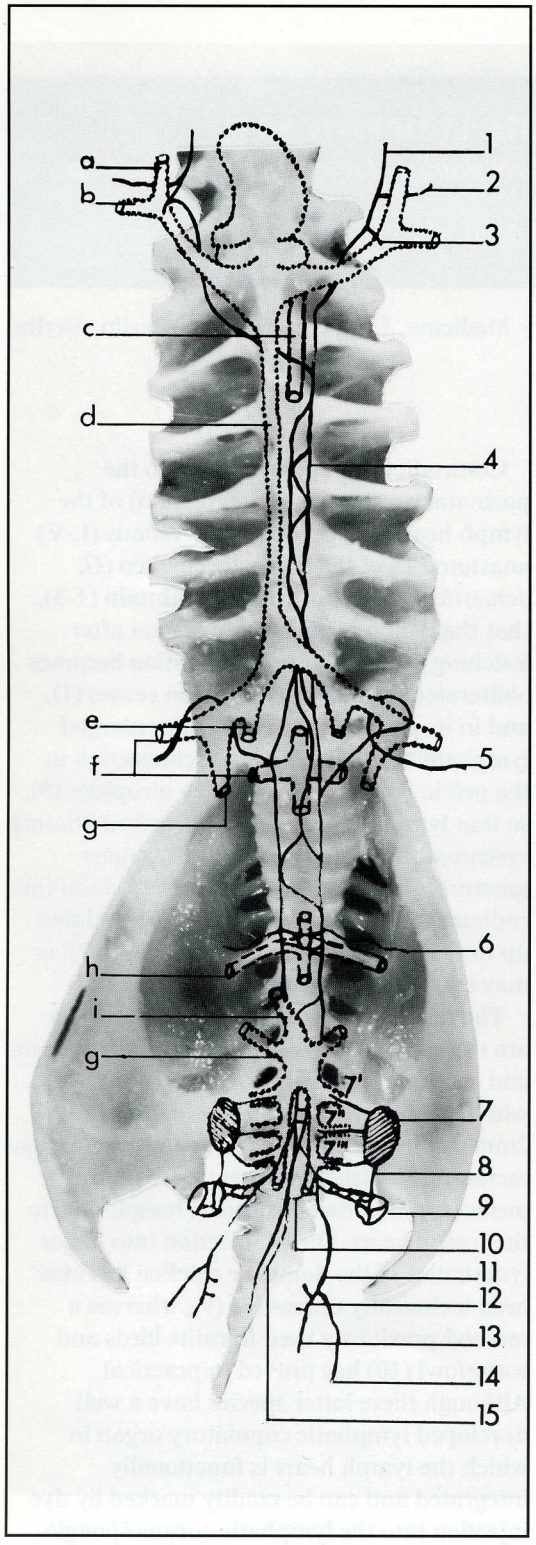

Fig. 1. The lymphatics of the body cavity of the chicken (G. domesticus) projected onto the skeleton (ventral view). Arteries—dashed, veins—dotted. The lymph heart, located dorsally on the transverse process of the first two free caudal vertebrae, is shaded.

a. Jugular vein
b. Subclavian vein
c. Aorta
d. Superior vena cava
e. Caudal renal vein
f. External iliac artery and vein
g. Caudal portal renal vein
h. Ischial artery
i. Caudal mesenteric vein

Lymphatic-venous anastomoses:
7'. The cranial efferent cordis lymphatic enters the cranial intersegmental vein.
7''. The ventral intermediate efferent cordis lymphatic joins the intermediate intersegmental vein.
7'''. The caudal efferent cordis lymphatic connects to the caudal intersegmental vein.

1 .Jugular lymphatic
2. Subclavian lymphatic
3. Lymphatic-venous connection between the thoracoabdominal trunk and the superior vena cava
4. Thoracoabdominal trunk
5. External iliac lymphatic
6. Ischial lymphatic
7. Lymph heart
8. Afferent cordis lymphatic
9. Lymph heart plexus
10. Ventral lymphatic arch
11. Internal iliac lymphatic
12. Pudendal lymphatic
13. Cloacal lymphatic
14. Lateral caudal lymphatic
15. Median sacral lymphatic

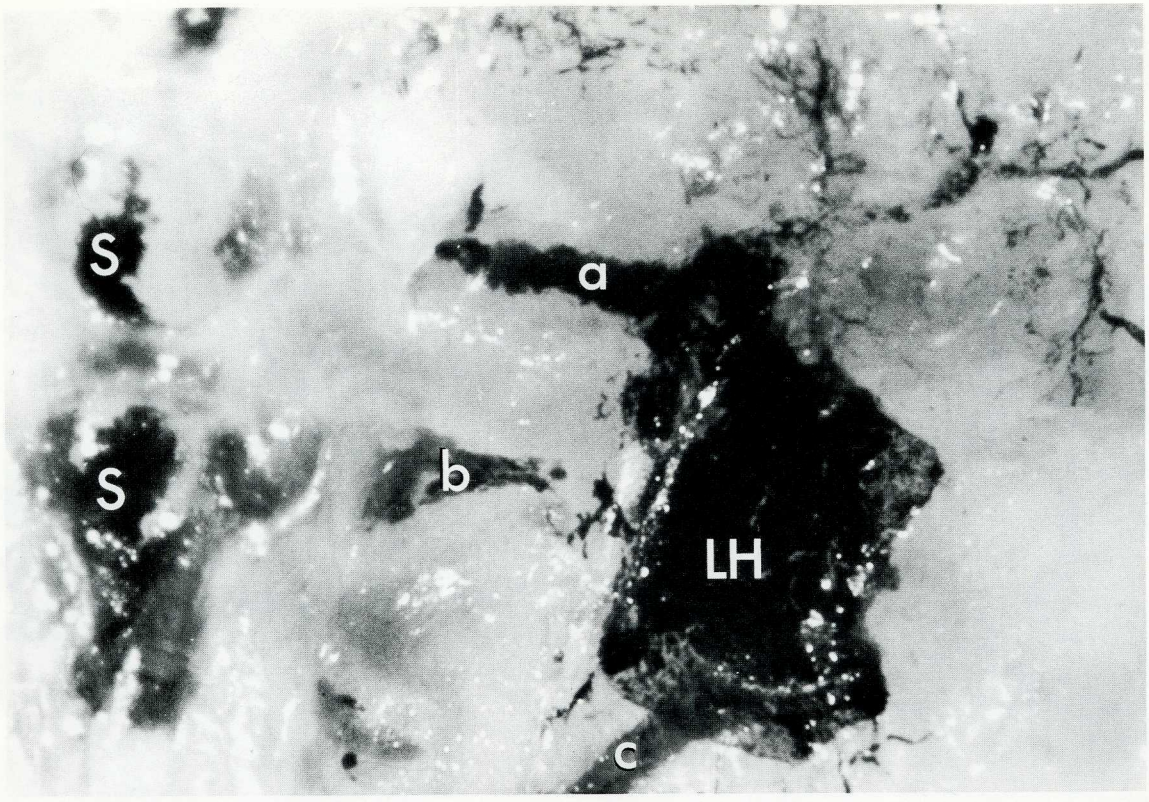

Fig. 2. Dorsal view of the right lymph heart (LH) which has been highlighted by blue dye injection. The major efferent lymphatics which emanate from the lymph heart medially are the cranial (a), intermediate (b) and caudal efferent cordis lymphatics (c). The dorsal branch of the intermediate efferent cordis lymphatic continues into the vertebral canal as the lymphatic-venous sinus of the dura mater (S). (original magnification=38x)

sum of the copulatory organ, this structure is inconspicuous in the domestic chicken. Accordingly, in this study we examined the lymph heart of domestic chickens (roosters) within 24 hrs after birth and up to 28 weeks using a combination of indirect and direct injection of a Berlin-blue solution.

MATERIALS AND METHODS

Thirty-nine roosters (*G. domesticus*) were studied. These included 2 chicks of each of the following age groups: 1-9 days, 2, 3, 6, 9, 11, 12, 13, 15, 17, 23 weeks and one at 28 weeks. The chickens were anesthetized, decapitated, then bled, skinned, and the body cavity opened.

The injection fluid used on the chickens up to 9 weeks of age was a solution of 3% watery Berlin-blue (B-B). After 11 weeks old, another injection fluid was used, namely GEROTA-mass after GEROTA (11). It consists of following percentages by weight: 75% Ether, 15% turpentine oil and 10% Prussian-blue.

Up to the second week, an indirect fluid injection was made into the metatarsal footpad subcutaneously by means of an infusion pump ("Precidor", Infors AG, Basel).

In 2-15 weeks old chickens the indirect injection was combined with a direct injection into the thoracoabdominal lymph trunk, a structure equivalent to the mammalian thoracic duct. For this purpose, a self-made glass needle with a very thin lumen of about 0.2-0.4mm was provided with a tube and a syringe. The syringe was fixed to the infusion

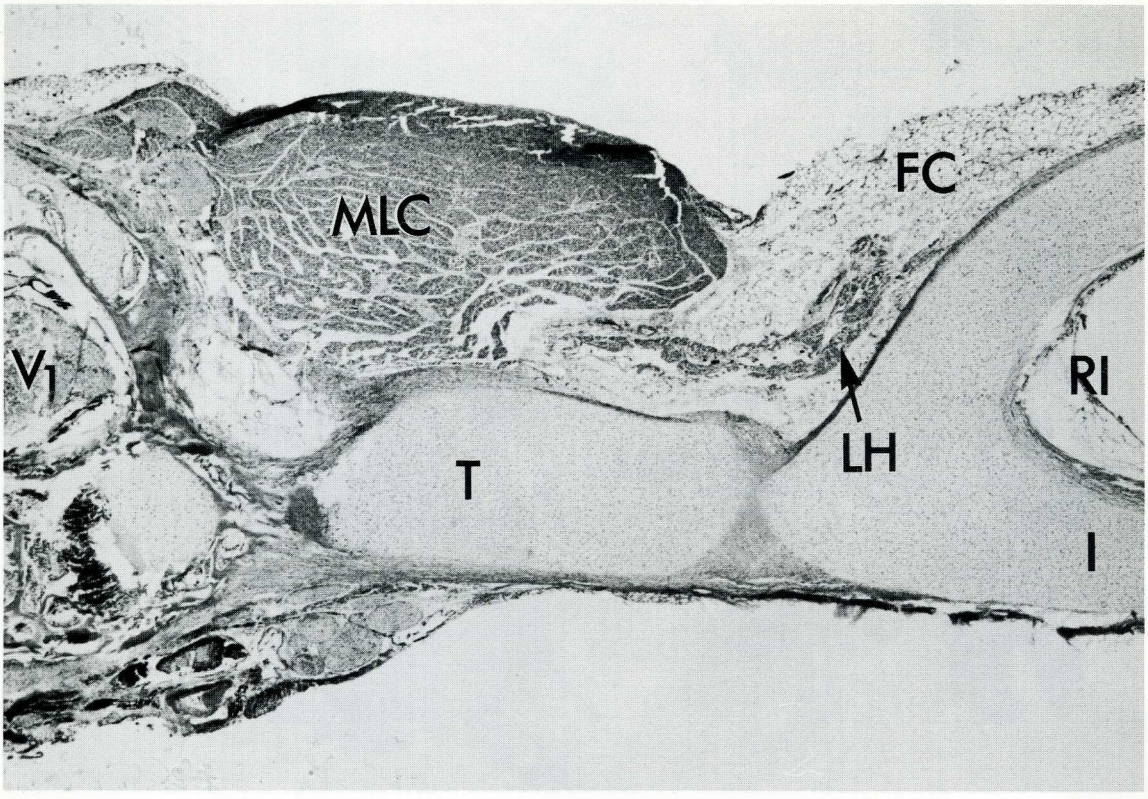

Fig. 3. Cross-section between the synsacrum and the first free caudal vertebra (V1), viewing the right body side. The lymph heart (LH) lies in a fatty capsule (FC), dorsally on the transverse process (T) of the first free caudal vertebra, and is covered by the levator caudae muscle (MLC) in young chicks. I=Os ilium, RI=Recessus iliacus. (original magnification=43.5x)

pump. Under a stereoscope (Carl Zeiss, Germany) the puncture needle was introduced into the marked thoracoabdominal lymph trunk via a prepared puncture hole and adhered by tissue adhesive (Histoarcryl Blau, B. Braun, Melsungen). The injection fluid was injected by the infusion pump. Where GEROTA-mass was used it was necessary to place the infusion pump with the syringe about 10 inches below the table with the specimen to obtain a certain ether vapor tension according to the fluid column. When the injection was completed, the needle was removed and the puncture hole closed with tissue adhesive.

Beyond the 16th week, a combination of the indirect fluid injection and a direct fluid injection into the caudal tibial lymphatic (medial to the tibiotarsal joint) was utilized.

Dissection and visualization of the lymph heart with its afferent and efferent lymphatic connections was done with a stereomicroscope (Tessovar, Carl Zeiss Co., Oberkochen, Germany).

RESULTS

The topography of the lymph heart and its relationship to the skeletal system is shown in *Figs. 1 and 2*. The elongated, dorsoventrally flattened lymph heart lies parallel to the long axis of the body, on both sides of the vertebrae, dorsal to the pelvis. The superior two-thirds of the lymph heart lies on the transverse processes of the first free sacral caudal vertebra *(Fig. 3)*. The inferior one-third covers

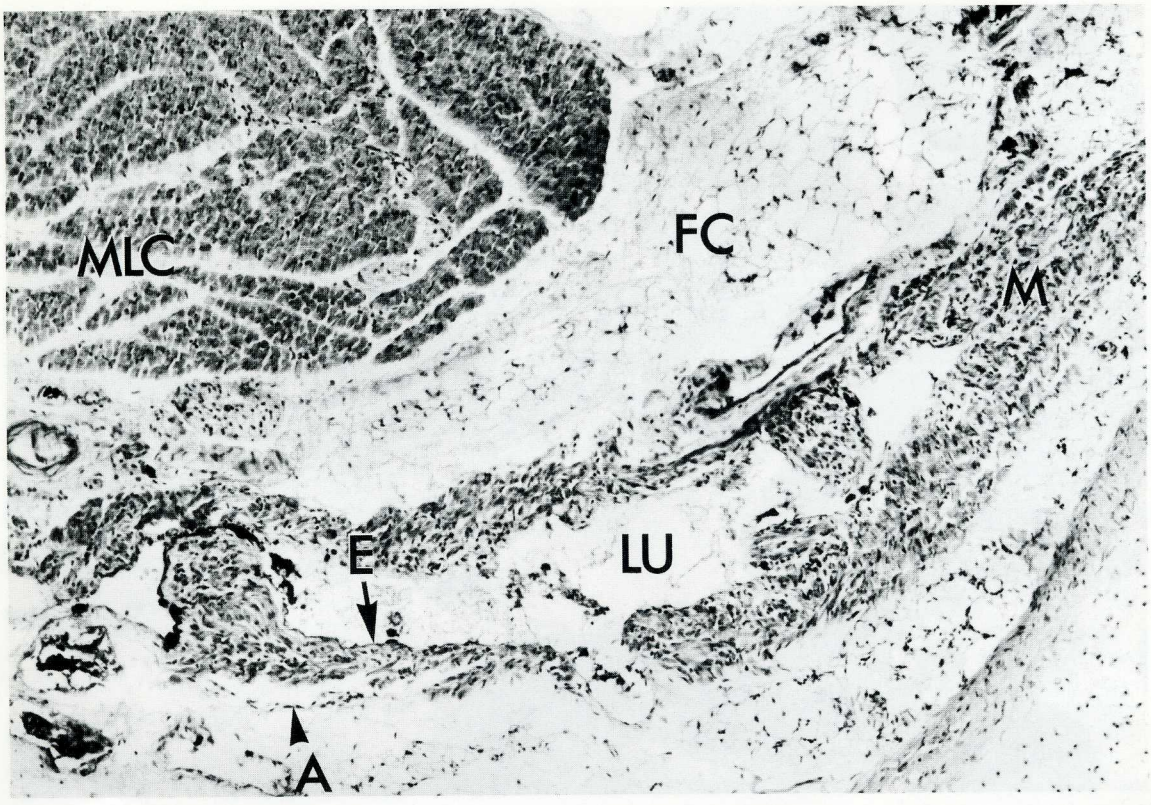

Fig. 4. The wall of the lymph heart consists of endocardium (E), which is composed of endothelium and the stratum myocollagenosum with smooth muscle cells, myocardium (M) containing striated muscle tissue, and the connective tissue of the adventitia (A). LU=lumen, FC=fat cells. (MLC=Levator caudae muscle) (original magnification=200x)

the incisura intertransversaria, located between the synsacrum and the first free caudal vertebra.

After removal of the skin and the fatty layer, the lateral half of the lymph heart is readily recognized in young chicks after blue dye injection. Unstained, the muscular wall of the lymph heart is too pale for macroscopic differentiation from adjacent fat and connective tissue *(Figs. 3,4)*. The levator caudae muscle covers the medial half of the lymph heart and its efferent lymphatic vessels. With increasing age, larger portions of the lymph heart are obscured by growing muscle.

As depicted in *Fig. 1*, the lymphatics of the hindlimb connect with the thoracoabdominal lymph trunk via the external iliac lymphatic, and to a lesser extent, via the ischial lymphatic. At age 1-9 days, the comparatively large lymphatic lumen and the many anastomoses between both thoracoabdominal lymph trunks, which cross the aorta ventrally, are particularly prominent. At the confluence of the external iliac lymphatic, lymphatic continuation divides in two directions from the thoracoabdominal lymph trunk. The cranial lymph continuation reaches the superior vena cava through the thoracic part of the thoracoabdominal lymph trunk. The caudal lymph connection from the lumbar region of the thoracoabdominal trunk, joins the internal iliac lymphatic near the terminal division of the aorta.

The plexus cordis lymphatici, so named because of its position near the lymph heart, lies lateral to the above described anastomoses,

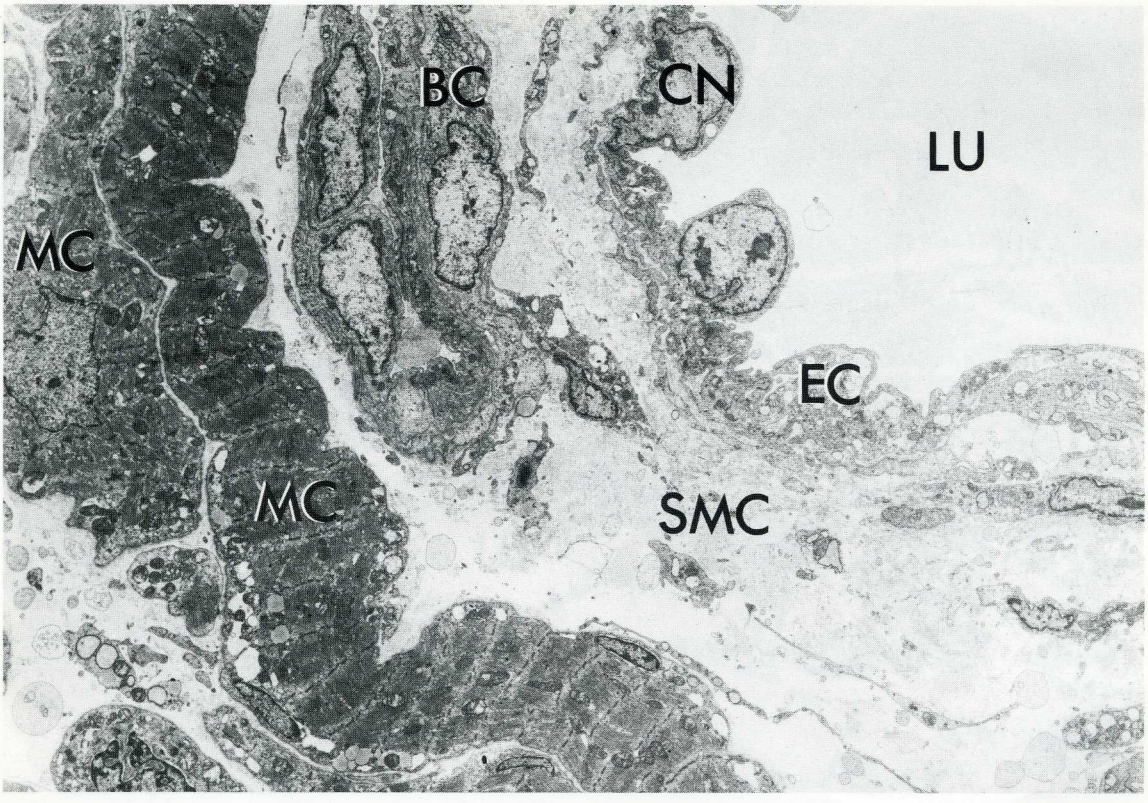

Fig. 5. The lymph heart wall with the endocardium, consisting of endothelial cells (EC) and the stratum myocollagenosum (SMC) with smooth muscle cells, and the myocardium, containing striated muscle cells (MC). LU=lumen, BC=blood capillary, CN=cell nucleus of an endothelial cell. (original magnification=4140x)

halfway along the internal iliac lymphatic and adjacent to the first free caudal vertebra. Both plexi (right and left) connect through a network of anastomoses, which lie ventral to the medial sacral artery. As in waterfowl and ratite birds, it should correctly be termed the ventral lymphatic arch.

A single afferent lymphatic channel from the lymph heart plexus emanates from the pelvic cavity through an opening between the synsacrum and the first free caudal vertebra. After displaying an intact intraluminal valve, this lymphatic continues via a lateral opening to gain access to the lymph heart, situated dorsal to the transverse process of the first two free coccygeal vertebrae.

With increasing age (2-15 weeks), the degree of branching of the thoracoabdominal lymph trunk decreases. The right and left trunks lie dorsolaterally to the aorta and are completely enveloped by fatty tissue. The lymphatics become finer and more thin-walled. The delicate afferent cordis lymphatic leaves the thoracoabdominal lymph trunk either just before the origin of the internal iliac lymphatic, or after the origin of the median sacral lymphatic and reaches the lymph heart without forming a plexus.

During the 17th through 28th weeks, the lymph heart can no longer be visualized clearly by dye injection. The caudally directed lymphatic continuation from the thoraco-abdominal lymph trunk, reaches only as far as the branching of the ischial artery from the aorta. Further demonstration of the lymph heart in chickens of this age is possible only by using refined light microscopy and histology.

Usually three main lymphatics leave the

lymph heart *(Figs. 1,2)* medially and join intersegmental veins (lympho-venous or L-V anastomoses). The superior efferent cordis lymphatic leaves the lymph heart superomedially, courses through the transverse foramen between the last two synsacral vertebrae and joins the cranial intersegmental vein, which, in turn, joins the internal iliac vein inferior to the origin of the caudal mesenteric vein. The caudal efferent cordis lymphatic is not consistently developed. When present, however, this lymphatic leaves the lymph heart inferiomedially through the transverse foramen between the first and second caudal vertebrae, and joins the caudal intersegmental vein, which later joins the median sacral vein. Just after its origin, the intermediate efferent cordis lymphatic divides into a dorsal and a ventral branch. The ventral branch runs ventromedially through the transverse foramen between the synsacrum and the first free caudal vertebra, and joins the intermediate intersegmental vein, which crosses the lymph heart posteriorly and joins the internal iliac vein. The dorsal branch of the intermediate efferent cordis lymphatic follows a similar path into the transverse foramen, but instead of joining an intersegmental vein, the lymphatic joins the sinus of the dura mater *(Fig. 2)*, thereby creating a third type of L-V anastomosis.

DISCUSSION

The differences between our findings and earlier conclusions may relate to the minute size of the lymph heart of *Gallus domesticus*. Thus the lymph heart of the domestic chicken with its afferent and efferent vessels, is barely 2mm long, cannot be seen with the naked eye, and requires the aid of a stereomicroscope. In comparison, the lymph heart of ratite birds is about 25mm long, that of the swan is about 15mm, and that of the duck is about 5mm (10). Another technical difficulty in studying the chicken is that the pale muscle color after hatching, and the prominent fatty tissue surrounding the lymph heart make it extremely difficult to delineate the lymph heart without dye injection. An important corollary of this research, therefore, was development of a proper injection technique for depicting the lymph heart.

The lymph heart is easily marked in waterfowl and ratite birds (10), by dye injection into the lymphatic corpus spongiosum or metatarsal footpad, because both the cranial and caudal lymphatic continuity to the lymph heart remain intact throughout the lifespan of these fowl.

This simple coloring method is not feasible, however, in domesticated chickens. Here, the lymph heart is delineated using different injection fluid or different coloring techniques depending on age.

In chicks up to 9 weeks of age, the used injection fluid was a solution of 3% watery B-B. This watery solution had the following disadvantage: after introducing the glass needle into the lymphatics, it became apparent that the air pressure inside the needle was lower than the capillary pressure. A small amount of lymph fluid moved retrograde into the needle forming clumps in the needle lumen after mixing with the watery B-B fluid. In chickens younger than 9 weeks, this factor was not a problem because the lymphatics had a comparatively larger lumen and did not require such an extremely fine glass needle.

In chickens up to 11 weeks in age, the glass needle had to be no wider than 0.2mm because the lumen of the lymphatics became extremely fine. Clumping stain particles obstructed this very fine glass needle and the lymphatics were not color marked. Accordingly, we used GEROTA-mass (11) which consists of 10% oil Prussian-blue, 15% turpentine oil and 75% ether. In the short period from introducing the needle into the lymphatic until switching on the infusion pump, the capillary pressure was lower than the ether vapor tension and no lymph fluid was "sucked" into the needle lumen. To obtain a particular pressure according to the fluid column, the infusion pump with the syringe was placed about 10 inches below the table with the specimen. This

adjustment was necessary to avoid accumulation of ether vapor bubbles in tube sections disturbing the continuous infusion process. Beginning the injections, a gas "cushion" preceded the oil Prussian-blue injection fluid. One important reason for the successful color-marking of the very thin-walled lymphatics is the fact that the ether vapor tension dilates the lymphatic lumen for a short period. On the other hand, the advantages of the GEROTA-mass is partially offset by the difficulty in handling and its incompatibility with electron microscopical preparation techniques.

Regarding the different coloring techniques in chickens under two weeks of age, indirect dye injection subcutaneously into the metatarsal footpad is best. Massaging the footpad causes the interendothelial junctions to open and close more actively, allowing the blue dye to migrate into adjacent lymphatics, thereby highlighting them within the body cavity. At this stage, lymphatic connections from the thoracoabdominal trunk are possible in both a cranial and caudal direction with the right and left trunks connecting through a network of anastomoses. After the second week of age, however, the thoracoabdominal lymph trunk and the lymph heart can only be detected in part. There are several reasons for this difficulty. First, the colored tracer is poorly absorbed by the lymph vessels, which are extremely small and thin-walled in proportion to the body size; second, the number of anastomoses and collateral lymphatic vessels gradually decrease, thereby resulting in a decrease in the total volume of the blue dye flowing through the system; and third, the distance between the injection site and the pelvic region is increased as the chicken grows. Thus, a combined approach whereby an indirect footpad dye injection is followed by a direct dye injection into the thoracoabdominal lymph trunk was found to be the best coloring method to depict the lymph heart and lymphatics from the 2nd to the 15th week. In chickens older than 15 weeks, after injection into the metatarsal footpad, the blue dye remained in the thigh, the thoracoabdominal lymph trunk was not displayed and, therefore, could not be directly injected. Accordingly, at this age the best method proved to be a combination of the indirect injection into the metatarsal footpad followed by a direct injection into the caudal tibial lymphatic just medial to the tibio-tarsal joint. In chickens older than 15 weeks, caudally directed lymph flow within the body cavity to the lymph heart was increasingly hindered by now well-developed intraluminal valves within the thoracoabdominal lymph trunk. Moreover, a bypass via valveless anastomoses and collateral vessels was no longer possible because these channels had degenerated by this time. On the other hand, the superior lymph flow to the great veins at the entrance of the thorax was still intact.

Due to the anatomy of the domestic chicken, as contrasted with waterfowl and ratite birds (10), blue dye from an indirect injection into the metatarsal footpad does not gain access to the lymph heart via the dorsal lymphatic arch but rather through the internal iliac lymphatic. The right and the left lymphatic plexi are interconnected by a ventral network of anastomoses, which mostly atrophies by the 15th week of age. There is no dorsal network of anastomoses as seen in waterfowl and ratite birds (10). In chickens as in waterfowl and ratite birds, however, the afferent cordis lymphatic courses dorsally, between the synsacrum and the first free caudal vertebra, through a lateral foramen to gain access to the lymph heart. The efferent cordis lymphatics, on the other hand, differ in that they are only singular in chickens, and the caudal efferent cordis lymphatic may be absent in chickens.

Whereas in water fowl and ratite birds (10) the afferent cordis lymphatic and the three efferent cordis lymphatics can be marked throughout their lifespan, in chickens after 16 weeks old, the lymph heart can longer be marked by dye injection and accordingly neither can the afferent and efferent cordis lymphatics be visualized. Nonetheless, by use of refined light microscopy during the age of

17 through 28 weeks, it is demonstrated that the afferent and efferent cordis lymphatics and the lymph heart are indeed intact.

At this age, lymphatic continuity to the lymph heart changes: the continuity is only functional from the caudal lymphatics of the pelvic area (median sacral lymphatic or internal iliac lymphatic to the afferent cordis lymphatic and from there to the lymph heart); lymphatic continuation from the hindlimb into the lymph heart via the thoracoabdominal trunk is now gone.

The functional importance of the lymph heart differs according to age and species. For example, lymph hearts have a vital function in amphibia, and their destruction leads to progressive hemoconcentration and death within a few days (12). The lymph heart of avian fetuses probably has the critical function of propelling lymph flow in the body since there are no rhythmic pressure changes in the body cavity as long as the fetus remains relatively motionless in the egg and does not begin breathing until just a few days before hatching.

In adult waterfowl and ratite birds the lymph heart takes on a distinctly different function. Rather than a pump for the body's lymphatic system, the lymph heart is integrated into the copulating organ system (corpus spongiosum) for preservation of the species (10). Other avian species, including *G. domesticus*, have either an extremely small corpus spongiosum or none at all. This finding has led to the widespread notion that the lymph heart of these latter species is of little or no importance (3), and that it probably regresses soon after hatching (1-5). Although the lymph heart stops developing at a very early stage, and its function after hatching is apparently minimal, a very small organ nonetheless remains intact, including a lumen, endocardium, myocardium, and adventitia *(Figs. 4,5)*. What function, if any, lymph drainage into the dorsal sinus has is unclear. Perhaps because the pressure in the sinus of the spinal canal is so low, a propulsive force from a retained lymph heart facilitates spinal fluid circulation.

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