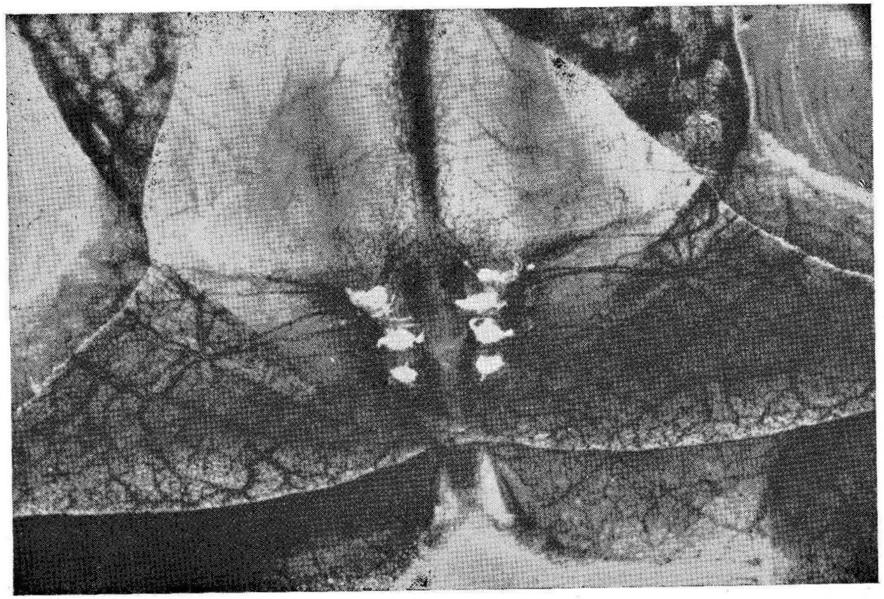

Xenopus lævis D. Male.

Injection of posterior lymph-hearts.

The opaque mass, injected intraperitoneally, filled three lymph hearts on the left and four on the right. The Saccus cranio-dorsalis has been opened and the skin flaps turned back to demonstrate the lymph-hearts.

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OBSERVATIONS ON THE LYMPHATIC SYSTEM OF THE SOUTH AFRICAN CLAW FOOTED TOAD

(*Xenopus lævis* Daudin)

by

E. ELKAN

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HISTORY

The argument on the question of the priority in the discovery of the lymph hearts in the frog was set forth, at great length, in papers by J. Müller and B. Panizza (1834). The two anatomists, one German, the other Italian, had, at the same time, become interested in the same subject and had published their discoveries almost simultaneously, Müller in 1832, Panizza in 1833. Müller's Archiv (1834) contains a résumé of both papers. Müller's contribution was immediately translated into English and appeared in the Transactions of the Royal Society in 1833. The posterior lymph hearts were the first to be discovered. The existence of the anterior pair was anticipated by Hall (1831) who discovered an 'artery' in the frog which, after the animal's death, continued to pulsate. The question whether arteries could or could not show any independent pulsation was then much discussed and Müller decided to follow up Hall's discovery. He found that the 'artery' was, in fact, a vein which derived its pulsation from its connection with the anterior lymph heart, hence its ability to continue beating after the death of the frog. The fact that Panizza was also acquainted with the anterior heart is shown by the excellent drawing accompanying his paper of 1833. Since this book is now rare and not easily accessible a reproduction of Panizza's illustration is here included (Fig. 1). The actual priority of the discovery must go to Müller who wrote (1832): "Ueber die Bewegung der Lymphe ist man noch völlig im Dunckel da man keine Kontraktionen der Lymphgefässe kennt; indessen habe ich bei mehreren Tieren und zuerst bei den Fröschen, ein kontractiles, rhymisch sich zusammenziehendes Organ gefunden, welches auf die Bewegung der Lymphe grossen Einfluss zu haben scheint, indem es mit den Lymphräumen der unteren Extremität und des Rumpfes in Verbindung steht. Das Organ ist doppelt vorhanden; es liegt auf jeder Seite hinter dem Hüftgelenk, zur Seite des Afters in der Regio ischiadica." (We know as yet nothing about the movement of the lymphatic fluid since we know of no contractions of the lymphatic vessels. I found however in several animals, and first of all in frogs, a contractile organ, moving rhythmically, which seemed to be of great influence on the motion of the lymphatic fluid being in communication with the lymphatic spaces of the leg as well as with those of the trunk. The organ is bilateral; it is, on each side, situated behind the hip joint, lateral to the anus in the regio ischiadica.)

The problems under discussion in those days were those connected with the motion of the blood and the lymphatic fluid. It was then doubted that the power of the heart was in itself sufficient to account for the movement of the blood in peripheral vessels and what Hall (1831) hoped to have found

was a peripheral pulsating artery. We know now that in all higher vertebrates the lymph moves without the aid of contractile vessels, aided through the contraction of muscles and guided by valves in the lymphatic ducts. The question why, alone among all the vertebrates only the amphibians and the reptiles should rely on lymph hearts to evacuate their lymphatic spaces, remains still unanswered. The presence of these hearts shows however that, where necessary, motor organs can develop in any part of the circulatory system.

The microscopic structure of the lymph hearts in *Salamandra* and in *Siredon pisciformis* (Axolotl) was first described by Weliky (1884) who drew attention to the structural similarity of the blood and the lymph heart, both having striated muscle fibres with large excentric nuclei.

The most extensive general description of the lymphatic system of *Rana* was given by Ecker & Wiedersheim (1889) in their standard work on the Anatomy of the Frog. The literature on the lymphatic system of *Xenopus* is as yet scanty. Bles (1905) mentioned the anterior—Beddard (1908), in the course of an investigation of the genus *Hemisus*, the posterior lymph hearts of *Xenopus*. Their innervation was described by Hotovy (1939).

The following pages report observations made with the object of increasing our understanding of the anatomy and the physiology of the lymphatic system of *Xenopus lævis* D., an anuran which, since 1930, has become a much valued laboratory animal. Some of the casualties occurring among laboratory stock are doubtless due to disease of, or injury to, the lymph hearts. A fuller understanding of their structure and physiology will help us to avoid damaging these extremely delicate structures in the course of experiments carried out on the toad.

Subcutanous Lymphatic Spaces

Xenopus lævis (Daudin) is a South African representative of the subfamily Xenopodinæ members of which occur in various parts of tropical and subtropical Africa. On account of their close anatomical relations the Xenopodinæ are grouped with the Surinam toads and their allies to form the family of Pipidæ. The family presents many unusual anatomical, ecological and physiological features and it has, since it was first discovered, remained of lasting interest to zöologists.

Contrary to the behaviour of higher vertebrates the amphibians do not drink. They receive the fluid they require partly with their food but mainly by absorption through the skin. The skin, in the live amphibian, is a selective membrane, permeable to water molecules in either direction. In a species which is exclusively aquatic, the part of the skin is that of a protecting envelope, a sensory receptor and an osmotic organ regulating the ion concentration of the body fluids. It is not geared to prevent a specimen from drying if accidentally it should find itself on dry land. Water then evaporates from the skin at such a rate that the animal dies within 24 hours—the speed depending on the temperature. While submerged, the animal constantly absorbs water through the skin and passes it on to the vascular system and the excretory organs.

The amphibian lymphatic system consists of large spaces, lined by a single-layer endothelium, between the skin and the superficial fascia and

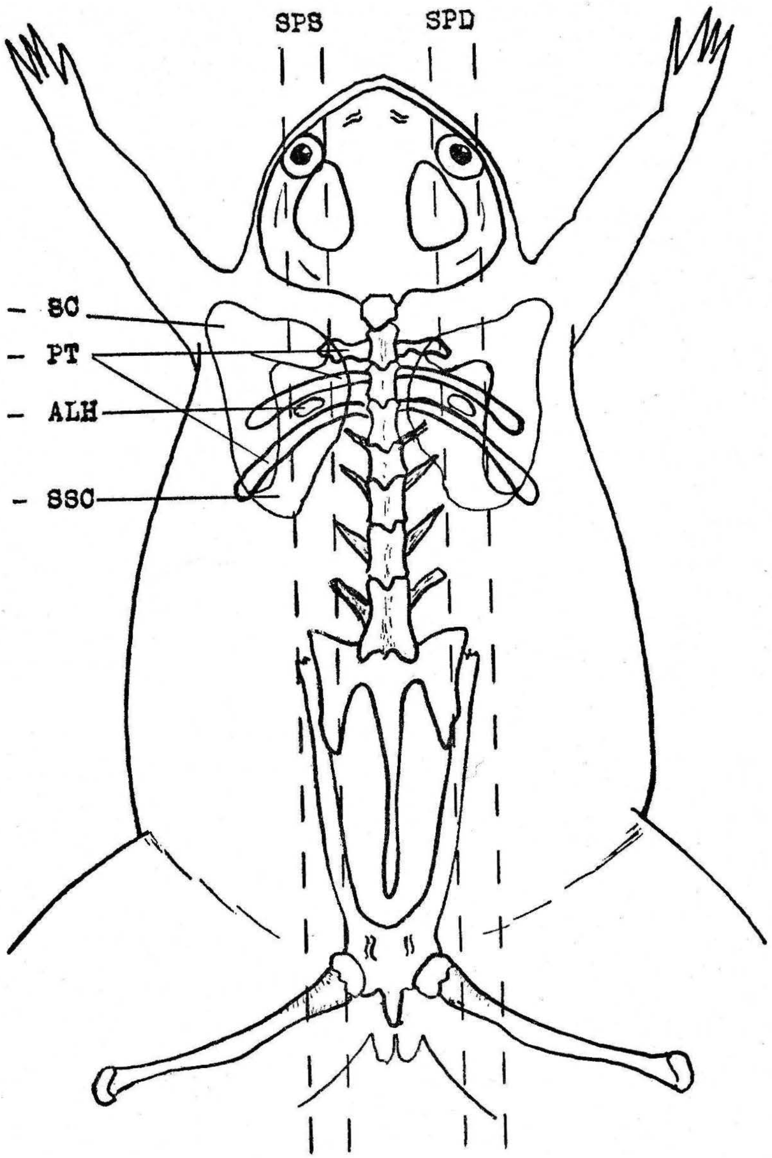


FIG. 5.

DIAGRAM, indicating the left (SPS) and right (SPD) sagittal planes in which the anterior lymph hearts may be found.

ALH anterior lymph heart; PT Processus transversus I-III; SC Scapula; SSC Suprascapula.

between the internal organs. The wide distribution of these lymphatic spaces gives the whole animal a remarkably loose structure. Hence the fact that amphibians can squeeze through gaps which often seem too small for their size. The lymphatic spaces represent one large communicating system, normally drained by two groups of contractile pumps, the lymph hearts. These organs are absent in the higher vertebrates. In amphibians and reptiles they return any excess fluid accumulating in the lymphatic spaces, to the venous system.

The extent and the disposition of the subcutaneous lymph sacs which envelop almost the whole animal, can be demonstrated by the injection of a solidifying mass into the Saccus cranio-dorsalis. A suitable formula for such a mass is :—

Colloidal kaolin	... ...	10 gm.
Gelatin	... ...	20 gm.
Aqua ad.	... ...	400 gm.

The animal should be killed in a 2% solution of urethane. The copious production of mucus, unavoidable if ether or chloroform are used, is hereby avoided. The kaolin-gelatin mass is injected through a wide-bore needle, in a water bath at 30°C. As the pressure gradually rises in the sac first injected, the mass gradually distributes itself to all the other lymph sacs. When all the sacs are well filled the specimen is put into a cold solution of 5% formalin which makes the injection mass permanently insoluble. When the specimen has remained in this solution for 24 hours the skin can be trimmed away to demonstrate the arrangement of the subcutaneous sacs (Fig. 2—4). The sacs which can thus be filled are :—

The Saccus cranio-dorsalis.
The Saccus submentalis.
The Saccus abdominalis.

The Saccus brachialis medialis, rt. and lt.
The Saccus femoralis, rt. and lt.
The Saccus suprafemoralis, rt. and lt.
The Saccus interfemoralis, rt. and lt.
The Saccus cruralis, rt. and lt.
The Saccus plantaris pedis, rt. and lt.
The Saccus dorsalis pedis, rt. and lt.

The mass also enters a thin appendix of the Saccus abdominalis lying between the Sacci interfemorales. The only lymph sac which remains unfilled on both sides and which seems to be without communication with the other subcutaneous sacs is the Saccus brachialis lateralis. All the other sacs are in communication through Ostia interseptalia. Valves were not seen in the septa dividing the subcutaneous sacs. They can however be demonstrated in the Sinus periproctalis.

In *Xenopus,* the Saccus lateralis, which is of considerable dimension in *Rana,* could not always be demonstrated. In some specimen, kaolin mass, injected into the Saccus brachialis lateralis, entered a narrow passage in the Septum laterale. By way of this passage the mass travelled in a caudal direction, entered the Saccus femoralis and gradually filled all the subcutaneous lymph sacs of the leg. This narrow passage may therefore be considered to be a vestige of the Saccus lateralis. Some specimens were found in which the Saccus brachialis lateralis was in communication with the Saccus cranio-dorsalis. The Saccus submentalis filled only from the Saccus abdominalis and not from either of the brachial sacci.

The Septum pectoralis and the Septum submaxillaris of *Rana* is, in *Xenopus*, represented by only one septum since the Saccus pectoralis is absent. Here again, directional valves as described by Kihara & Nosé (1931) for *Bufo japonicus* were not seen.

Histologically the subcutaneous lymph sacs of *Xenopus* differ in no way from those of *Rana*. They are lined by a single layer of endothelial cells; blood vessels and nerves, connecting the rest of the body with the skin, are carried in the septa. In the normal animal all these sacs are only potential spaces which when opened, present a moist surface but no appreciable amount of free fluid. Dehydrated frogs or toads, when offered a dish of water, enter this at once and remain in it for hours. Yet, their subcutaneous lymph sacs remain as empty as before. It must be assumed that the water absorbed by the skin is at once taken up by the dermal capillaries and carried into general circulation. There is no evidence to show that fluid absorbed through the skin passes directly into a subcutaneous lymph sac. These are probably kept moist by the activity of the endothelial cells with which they are lined.

The Lymph-Hearts

The multiplicity of these organs which, among the Salientia, is particularly marked in *Xenopus* (Fig. 15, Frontispiece), derives from the fact that, in the more primitive ancestors of modern amphibians, these organs followed, as they still do in urodeles, the segmental structure of the animal, one pair of lymph hearts being present in each segment. With the reduction of the body segments the number of lymph hearts has been reduced as well so that, in *Xenopus* we find one thoracic pair to a variable number (3—5) of coccygeal pairs of lymph hearts.

The student of anatomy, having dissected a number of animals of comparable age and size, expects to obtain gradually a definite picture of the morphology and topography of each organ, suitable to form the basis of a final description of the animal and all its parts. Such a result could not be obtained from the study of the lymph hearts in *Xenopus*. Their polymorphism defies any general description. The structural variability in *Xenopus* has already been noted by Millard (1941) who says (p. 437): "*Xenopus* shows a higher percentage of variations and abnormalities than any of the commonly dissected species of *Rana*." Some Zoologists regard *Xenopus* as a neotenic form. We have no other satisfactory explanation for its structural instability.

Only a few generally applicable statements on the lymph hearts can be made. They are found in only two regions of the body; the anterior pair is always single, the number of posterior pairs varies; they have an endothelial lining and their wall is made up of striated muscle fibres; they are uni- or multi-locular and are embedded in a network of fatty tissue and collagen fibres interspersed with few elastic fibres; by means of these fibres

they are firmly anchored within the 'pericardio-lymphatic space' (Wiedersheim) and they all drain lymphatic fluid from the deep and superficial lymphatic spaces into the venous system. Their beat is regulated by spinal nerves (Hotovy 1939). In specimens which have been used for pregnancy tests they may become subject to more or less severe pathological changes but their destruction does not, in itself, become a cause of the death of the animal.

The Anterior Lymph Heart

The anterior lymph hearts are much more inaccessible and much more difficult to study than the posterior group. They lie between the second and third transverse vertebral processes but have by no means always exactly the same relations to them. Ventrally they are covered only by the pleuro-peritoneum which their afferent valves perforate (Figs. 6—8). Dorsally they lie close to the M.longissimus dorsi and are separated from the suprascapula by the M.serratus medius (Fig. 6). If the viscera of a freshly killed animal are removed, the beat of the anterior lymph hearts can sometimes be observed with a lens. Photographically they can only be recorded when they are pathologically filled with blood and thrombosed (Fig. 11). Lateral to the lymph heart lies a small part of the M.intertransversari us connecting the second and third transverse processes.

Due to their extreme delicacy of structure and their close adherence to the surrounding structures, these organs cannot, in *Xenopus*, be dissected out for inspection or sectioning without gross distortion. To study them it is necessary to fix, decalcify and embed the animal *in toto* and to examine the hearts *in situ* from serial sections (Fig. 6). It is advantageous to inject fixing fluid when the animal is killed but in an investigation of the lymph hearts such injections must be made slowly and with great care since the hearts, and particularly the valves are easily distorted by abnormal pressures in the body cavities. After the specimen is embedded, orientation and the finding of the lymph hearts is necessarily difficult and much time is wasted in discarding sections containing no part of the structure. In the case of sagittal sections however, the eye provides a suitable guide (Fig. 5). In the normal, undistorted animal, sections which include part of the eye-ball usually also include parts of the anterior—sometimes even of the posterior lymph heart as well.

The polymorphism of these lymph hearts is such that no statement can be made either with regard to the number of valves to be found or on their location. In serial sections (Fig. 6) the number of afferent valves seen varied between two and ten. One valve, admitting fluid from the pleuro-peritoneal cavity, can always be found (Fig. 7), but there may, in addition, be numerous valves admitting fluid from lymphatic channels in the immediate neighbourhood of the lymph heart (Fig. 8). In some cases these pericardial lymphatics were found to be evaginations of the pleuro-peritoneum (Fig. 9) which transformed the immediate pericardial space into an almost spongy tissue. No evidence could be found to confirm the view that the anterior lymph heart is in direct connection with the Sinus subscapularis since no lymphatic channel could be demonstrated traversing the M.serratus medius which presents a barrier between the anterior lymph heart and this sinus. All the

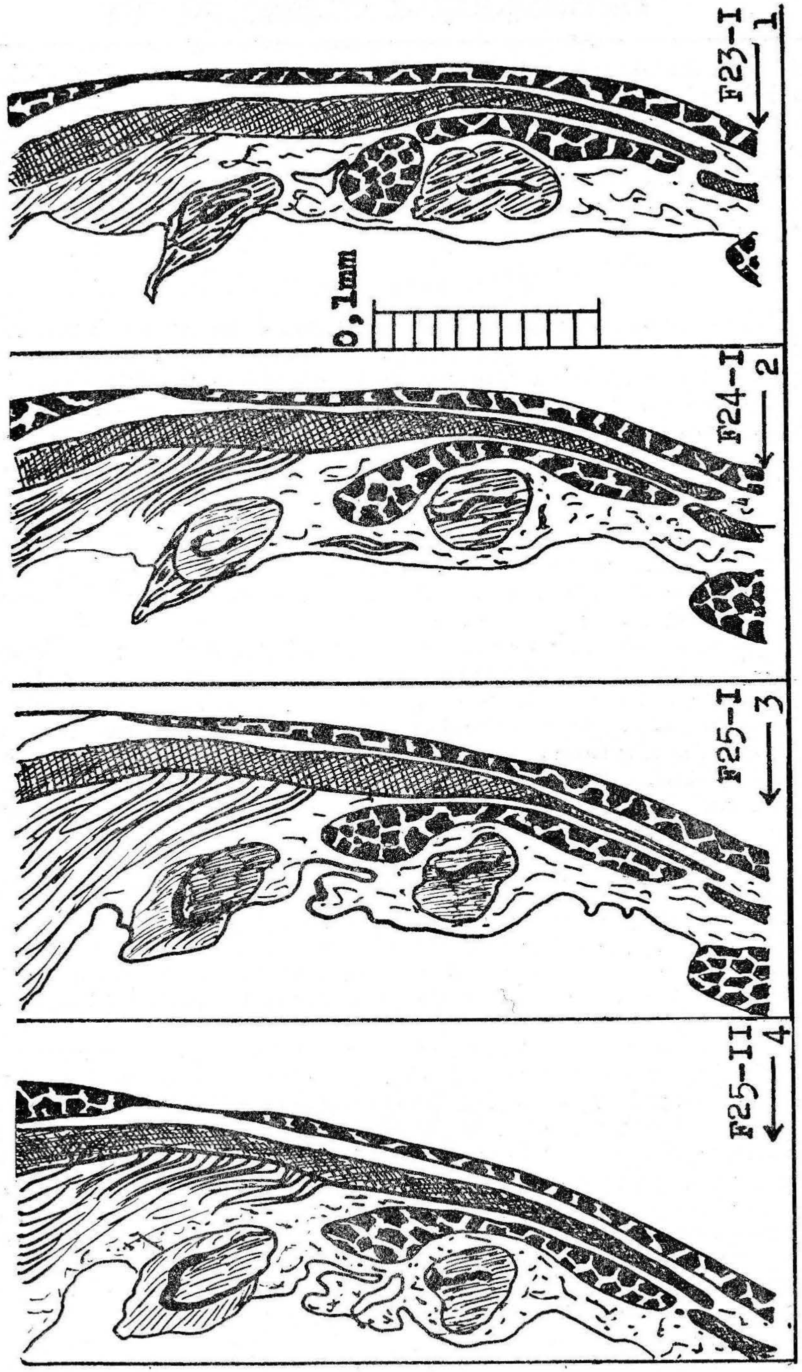
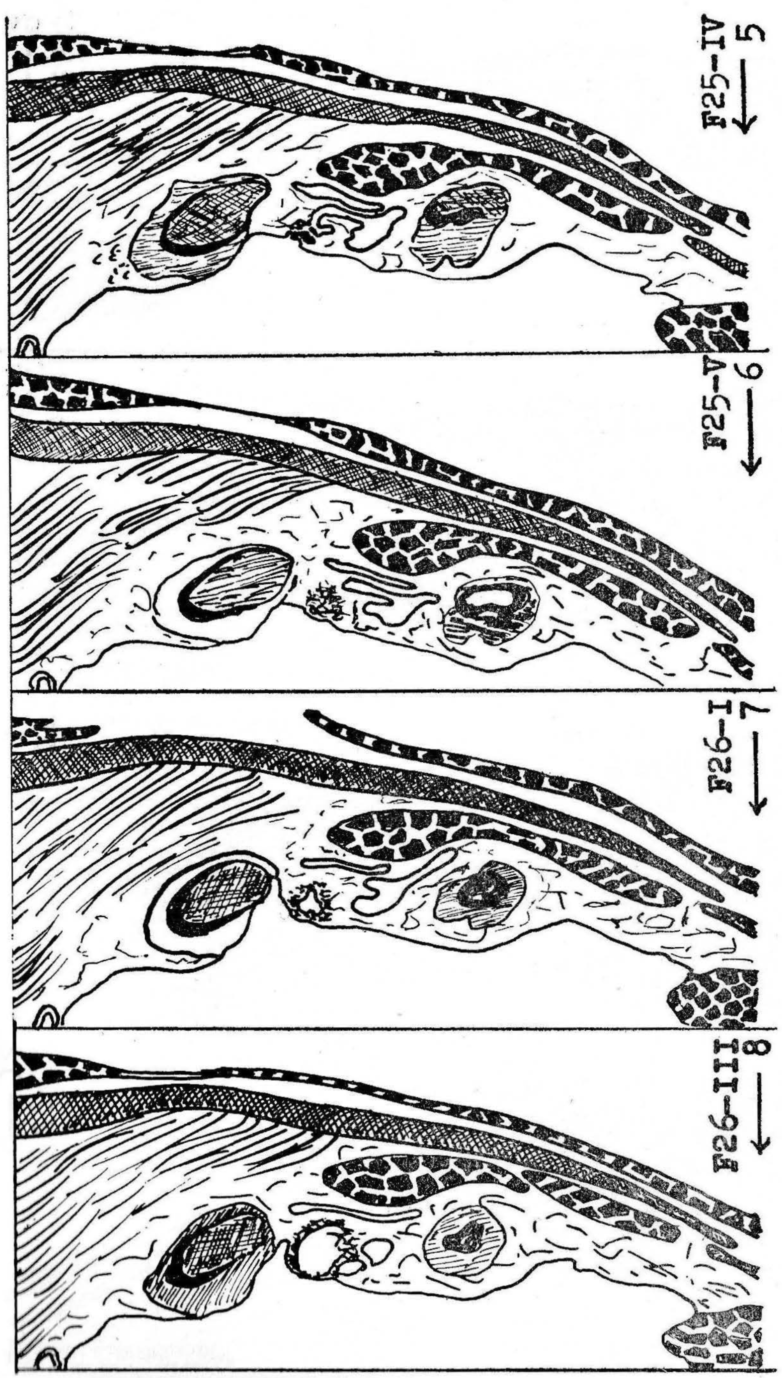


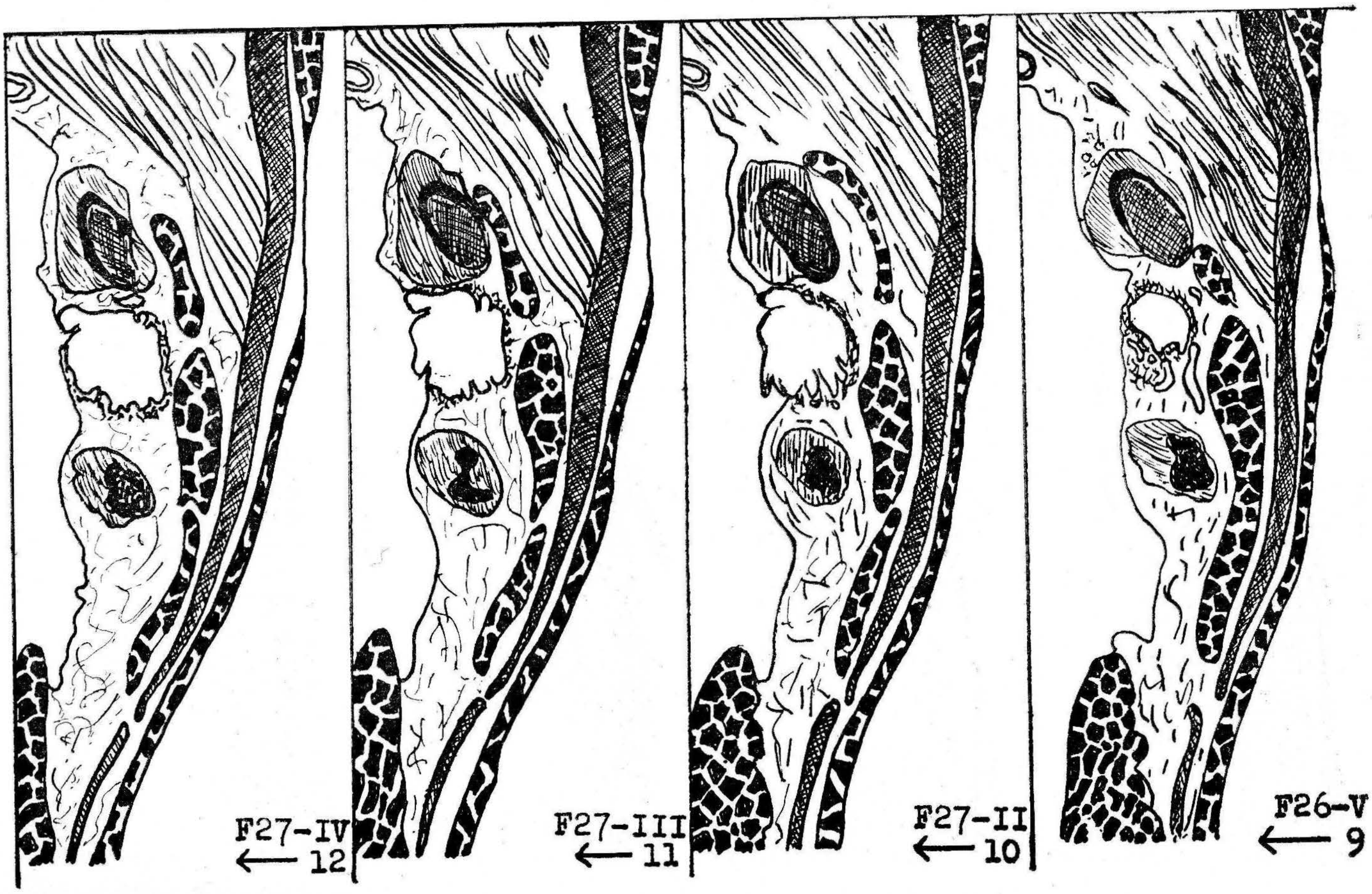
Fig. 6.

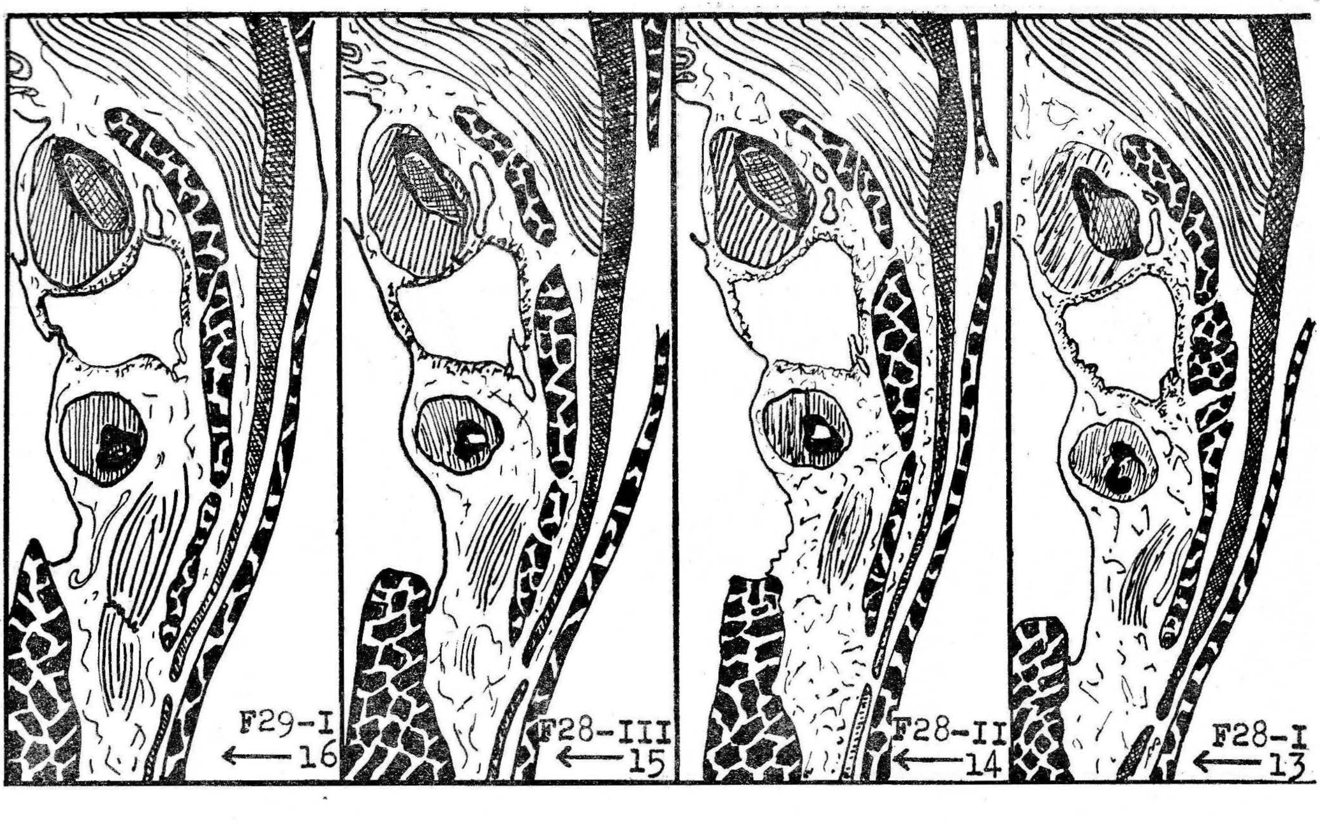
Diagrammatic tracings from sagittal serial sections through the thoracic lymph heart of *Xenopus lævis* D.

Each F figure indicates five sections of 12 mu, numbered I-V cut from lateral (1) towards the medial plane (18) the arrow indicating the direction towards the medial plane.



LH Lymph heart; MGD M.longissimus dorsi; MI M.ileolumbaris; MLD M.latissimus dorsi; MLS M.levator scapulæ; MSM M.serratus medius; PT2 Second Proc. transversus; PT3 Third Proc. transversus; PPC Pleuro-peritoneal cavity; SC Suprascapula; SCD Saccus cranio-dorsalis; VV Vena vertebralis.


F27-IV
12
F27-III
11
F27-II
10
F26-V
9


F29-I
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F28-III
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F28-II
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F28-I
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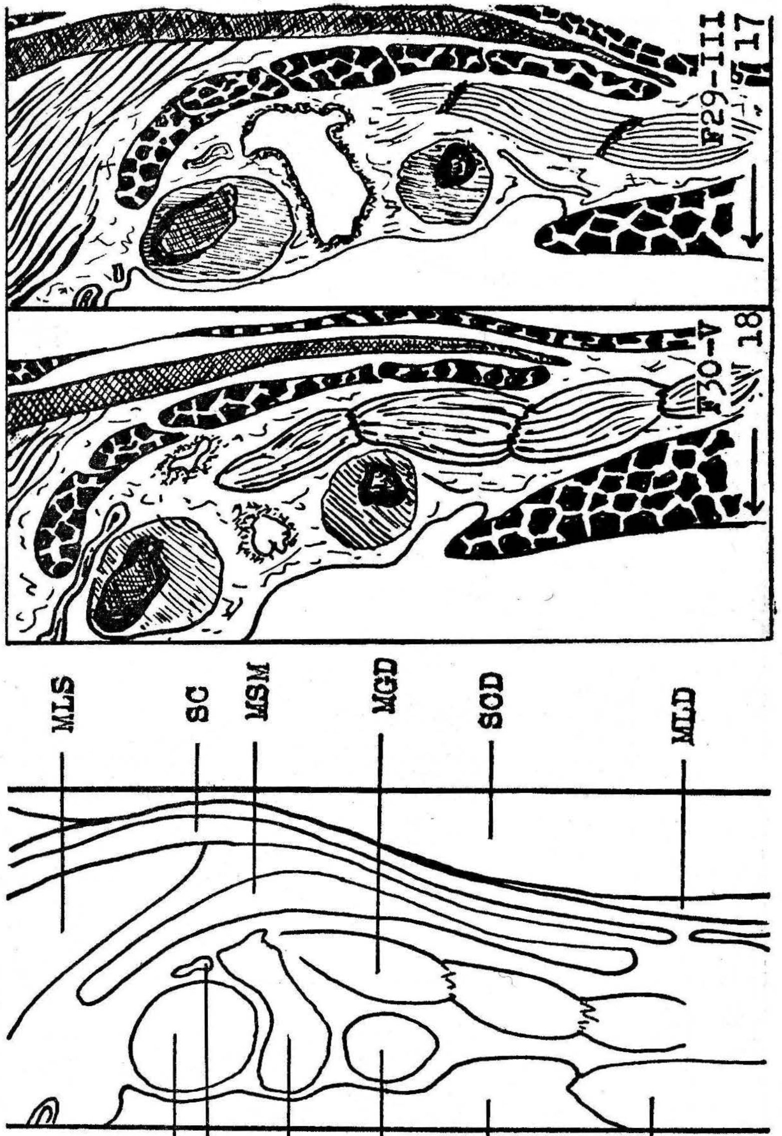


numerous lymphatic sinuses of the anterior half of the body can be assumed to drain directly or indirectly into the pleuro-peritoneal cavity whence the fluid is aspirated by the lymph hearts.

In contrast to the great variability in the numbers of afferent valves only one efferent valve could be found in each anterior lymph heart. This number is probably correct since only one branch of the V.vertebralis approaches the cephalic end of each heart. In some sections (Fig. 10) the lymph heart was separated from the vein by a short vessel, in others the vein appeared like an atrium to the heart (Fig. 7). The striation of the muscle fibres of the lymph heart makes it possible to distinguish between the wall of the heart and that of the adjacent vein.

The picture which the valves represent in sections can best be compared with that of a funnel. Valves of this shape were described by Kihara & Nosé (1931) and by Jolly & Lieurre (1929) for *Bufo bufo japonicus*, several species of *Rana* and for *Hyla arborea*. These authors describe the presence in and around the valves of fusiform, non-striated muscle fibres which would, presumably, take a part in the functioning of the valves. No muscle fibres of any kind could be seen to enter the valves in *Xenopus*. Mostly the valves are mere duplications of the endothelial lining of the heart, sometimes a few collagen fibres enter into them from the base but never further than halfways to the tip of the valve. The rim of the valve shows, in section, a bulbous thickening, which consists of endothelial cells only (Figs. 12 and 13). With a few exceptions, due, probably, to technical error, the valves were always found closed. Since no elastic fibres enter into their structure it must be presumed that the endothelium has, in itself, enough elasticity to keep the valve closed and to prevent any reflux of fluid. None of the valves in *Xenopus* were found to be supported by tendons as described by Jolly & Lieurre (1929) for *Rana*, *Bufo* and *Hyla*. The valves are extraordinarily delicate structures, sometimes bridging a considerable gap in the muscular wall of the lymph heart. It is very common to find erythrocytes on the 'wrong' side of the efferent valve and the frequent occurrence of lymphocardiac thrombosis is not surprising (Fig. 11).

The microscopical structure of the wall of the anterior lymph heart is not essentially different from the walls of the posterior group but it is always more delicate in appearance and the muscular coat is thinner. The endothelial lining is only one layer thick. The striated muscle fibres form a delicate network around the whole heart excepting the valvular gaps. Since very few elastic fibres are seen in the perilymphocardial tissue it must be assumed that the elasticity of the collagen fibres is in itself sufficient to bring about the diastole of the organ. Where the number of afferent valves exceeds that of the efferent structure it must be assumed that the rate of flow leaving the heart is much greater than that entering it. The muscular power of the lymph heart must balance that of the venous blood pressure.

The Posterior Lymph Hearts

The posterior lymph hearts of which, in *Xenopus*, there is always more than one pair, lie along cranially divergent lines on each side of the caudal end of the urostyle (Plate 1). The space between the fascia dorsalis and the dorsal femoral muscles is, in *Xenopus*, occupied by a well defined body of fatty and connective tissue. The lymph hearts are embedded in this fat and anchored by numerous strands of collagen tissue and some elastic fibres. The muscles at the base of this fatty tissue are the M.iliacus externus, the M.vastus externus (part of the M.triceps femoris) and the M.semimembranosus. Medially the fat body adheres firmly to the fascial aponeurosis around the urostyle, dorsally and laterally it is continuous with the superficial fascia which envelops the trunk and the legs. Ventrally however it is separated from the underlying muscles by the Sinus ilio-fibularis and the Sinus paraproctalis.

With regard to the anterior lymph heart it is not possible to ascertain its rate of action in the normal animal or to say whether the right and the left heart beat simultaneously or not. In the posterior hearts these details can be observed in the normal adult toad if it is taken out of the water, allowed to come to rest in a net and examined in a ray of incident light. The lymph hearts of one side will usually be seen to beat in unison at rates between 70—80/min. but the beats of the right and left group are not always synchronous. Violent sensory impressions—unavoidable while catching the toad and lifting it from the water—temporarily upset the rhythm of the lymph hearts.

For further examination the animal is killed with 2% urethane and the Saccus cranio-dorsalis opened. For a first examination it is advisable to make use of a normal, healthy toad, not of one which has died from disease. The beat which can be seen in the freshly killed or anæsthesized animal is not that of the lymph hearts themselves but the motion transmitted by them to the fat body which envelops them. The number of pairs of posterior hearts varies between two and five; the hearts in the middle of a row are usually larger than those at the ends. The number of hearts on either side is not always equal. Plate 1 shows an adult male toad with three hearts on the left and four on the right side.

It might be thought that all the posterior lymph hearts serve to drain the large Saccus cranio-dorsalis but contrary to this expectation dorsal afferent valves can usually be found in only one or two hearts on either side (Figs. 13 and 17). The remaining hearts have no communication with any of the subcutaneous lymph sacs. Their afferent valves (Figs. 12 and 14) lie on the ventral side of the lymph heart where they drain the Saccus iliacus, the Saccus ileofibularis and the Saccus periproctalis, also indirectly all the other sinuses of the posterior half of the body as well as the pleuro-peritoneal cavity. A feature rarely met with and peculiar to lymph hearts are the twin valves (Fig. 14). These are identical valves lying side by side in the ventral wall of posterior lymph hearts, draining the Saccus iliacus. Twin valves were also seen by Jolly & Lieurre (1929) in an unnamed species but which was certainly not *Xenopus*. It is difficult to visualise the need for twin valves but they must be considered normal structures in the Salientia.

The inspection of the coccygeal region of toads which have died from hydrops or from other conditions often furnishes the picture shown in Fig. 15. From this picture one obtains the very definite impression of the existence of communicating channels between the lymph hearts. The hearts themselves are so plainly visible because their valves have become incompetent and there has been a reflux of venous blood into the heart with subse-

PLATES

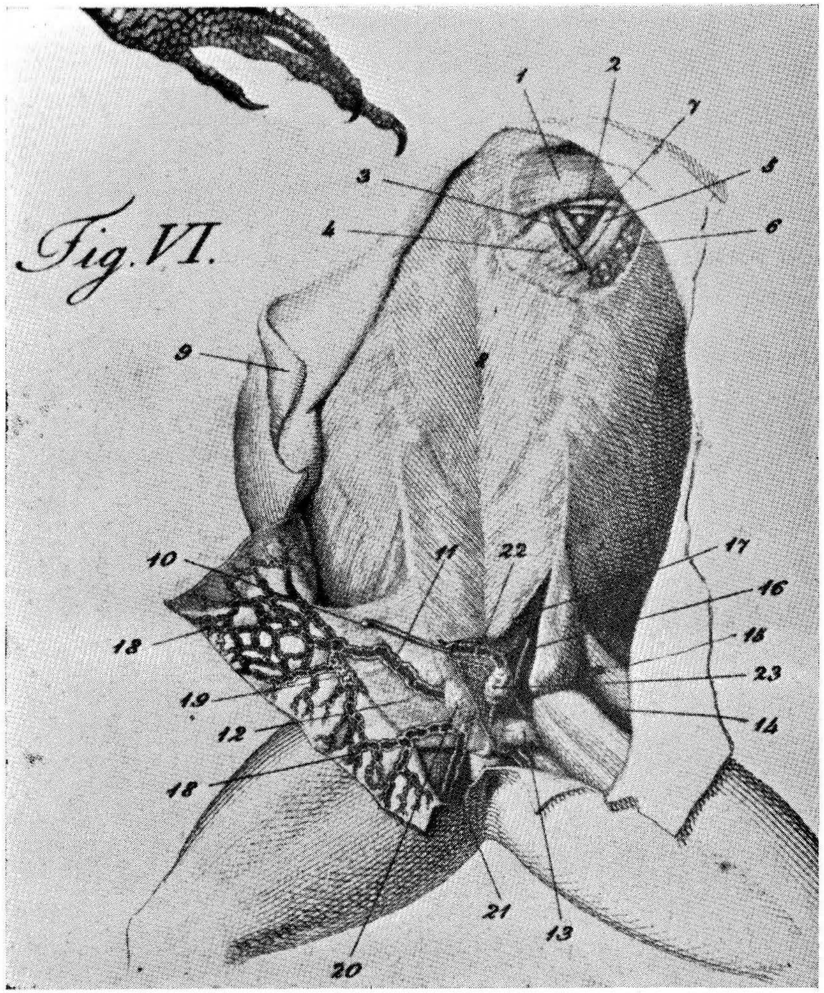


FIG. 1.

Fig. 1

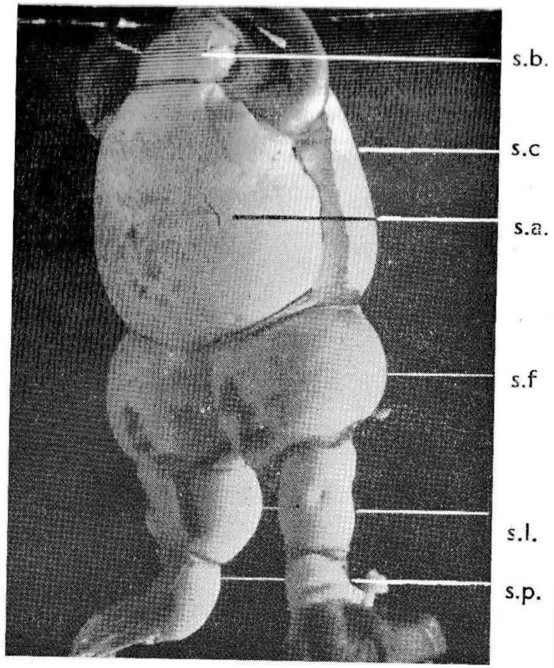
The anterior and posterior lymph hearts of *Rana*.
Panizza, B. (1833).
Sopra il sistema linfatico dei rettili.
Ricerche Zootomiche. Pavia.

TABLE VI. FIG. VI.

Dimonstra i vasi linfatici cutanei e le vesichette linfatiche pelviane e sottoscapolari della rana.

1. Muscula soprascapolare. 2. Scapola di cui fu portata vie la porzione che copriva la vesichetta. 3. Muscoli della spina. 4. Musculo sottoscapolare. 5. Legamento teso tra gli apici della apofisi transverse della terza e quatro vertebra, all'esterno del quale vedesi il musculo vertebro-scapolare o retraente la scapola. 6. Porzione di pulmone. 7. Vesichetta linfatica sottascapolare destra. 8. Muscoli latissimi des dorso. 9. Integumento rovesciati all esterno. 10. Vena che sorge della parte anteriore della vesichatta linfatica pelviana destra, circondete da troncolini linfatici, e rovesciata in un colla parete della cisterna linfatica iliaca. 11. Punto d'unione della vena della vesichetta linfatica pelviana sinistra con un ramoscello venoso proveniente dei tegumenti, nel qual punto s'infossa per metter foce nella vena crurale. 12. Cisterna linfatica iliaca. 13. Musculo piramidale o coccygeo-femorale. 14. Vena crurale. 15. Porzione della vena della vesichetta linfatica pelviana destra e suo sbocca nella vena crurale corrispondente. 16. Arteria iliaca. 17. Musculo ileo-coccygeo staccato in parte dall'osso ileo per meglio vedere la vesichatta linfatica pelviana. 18. Reti linfatiche integumentali. 19. Plesso linfatico che constantemente viene formato dall precedenti reti cutanei, e che s'inoltra nella cisterna linfatica iliaca. 20. Tessuto celluloso pinguedinoso che sta sopra la vesichatta linfatica. 21. Troncolini linfatici prosenienti dai tegumenti della parte interna e superiore della coscia. 22. Troncolini linfatici delle reti linfatiche cutaneo, che finiscono nelle vesichatta linfatica. 23. Vesichetta linfatica pelviana destra.

Species: Rana esculenta.



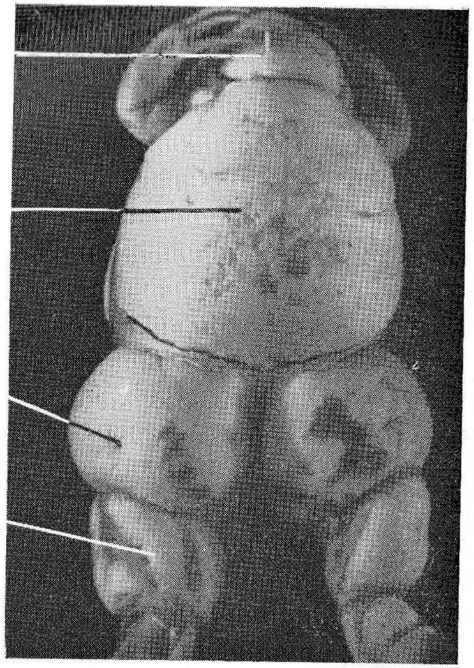


FIG. 2.
V entro-lateral view
Xenopus lævis D.

FIG. 3.
Ventral view

Injection of subcutaneous lymph sacs with kaolin-gelatine.
s.a. = Saccus abdominalis;
s.b. = Saccus submentalis; s.c. = Saccus cranio-dorsalis;
s.f. = Saccus femoralis; s.l. = Saccus cruralis;
s.p. = Saccus pedis plantaris et dorsalis.

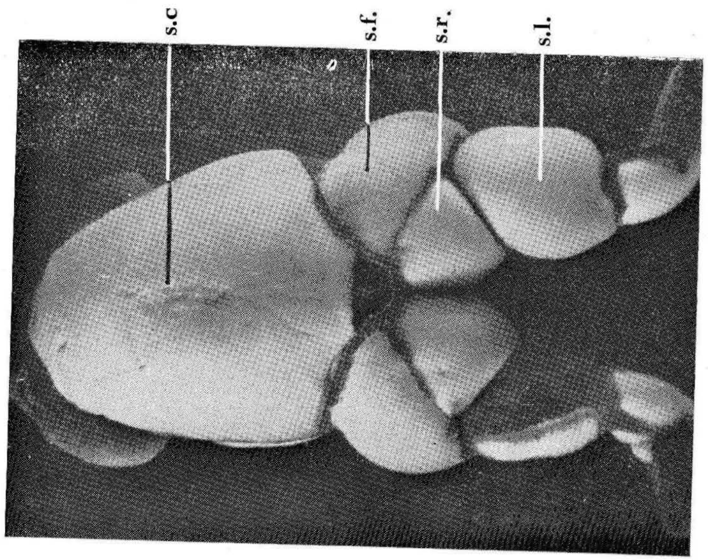


FIG. 4.
...*opus lævis* D.
...ction of subcutaneous lymph sacs with kaolin-gelatin.
Dorsal view.

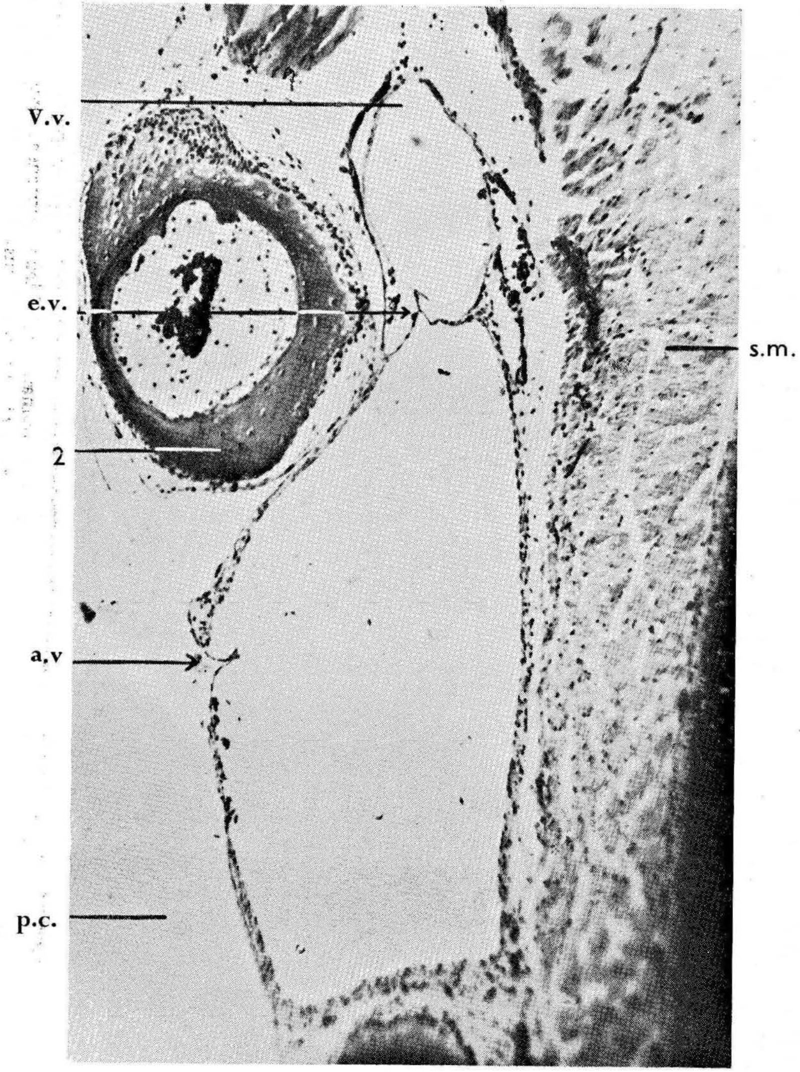


FIG. 7.
Xenopus lævis D.

Sagittal section through thorax showing anterior lymph heart with afferent and efferent valve.
a.v. = afferent valve; e.v. = efferent valve;
p.c. = pleuro-peritoneal cavity; s.m. = M.serratus medius;
V.v. = Vena vertebralis; 2 = Second transverse process.

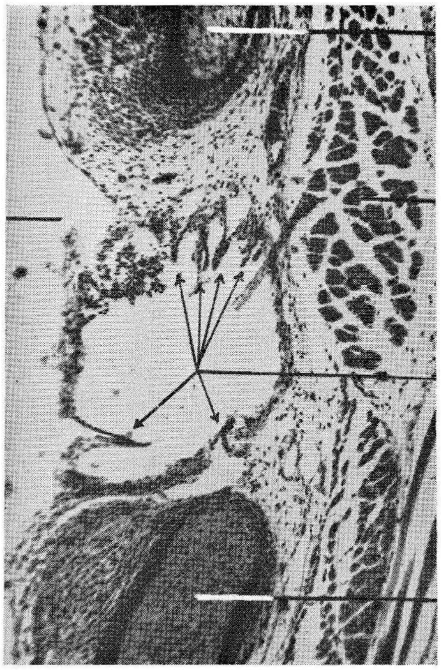


FIG. 8.

nopus lævis D.

Sagittal section through the thorax showing an anterior lymph heart th six afferent valves.

2, 3 = Transverse processes. a.v. = Afferent valves.

p.c. = Pleuroperitoneal cavity. s.m. = M.serratus medius.

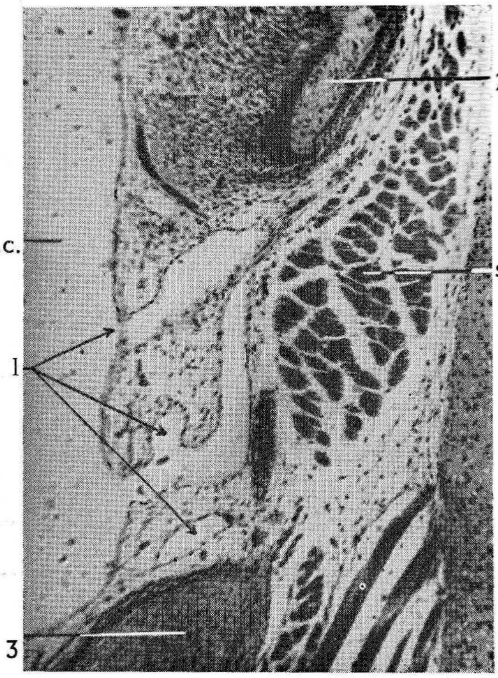


FIG. 9.

Xenopus laevis D.

Sagittal section through the thorax close to the anterior lymph hea showing lacunæ in the wall of the pleuro-peritoneal cavity between th second and third transverse process.

2, 3 = Second and third transverse process.
1 = Lacunæ; p.c. = Pleuro-peritoneal cavity.
s.m. = M.serratus medius.

V.v.

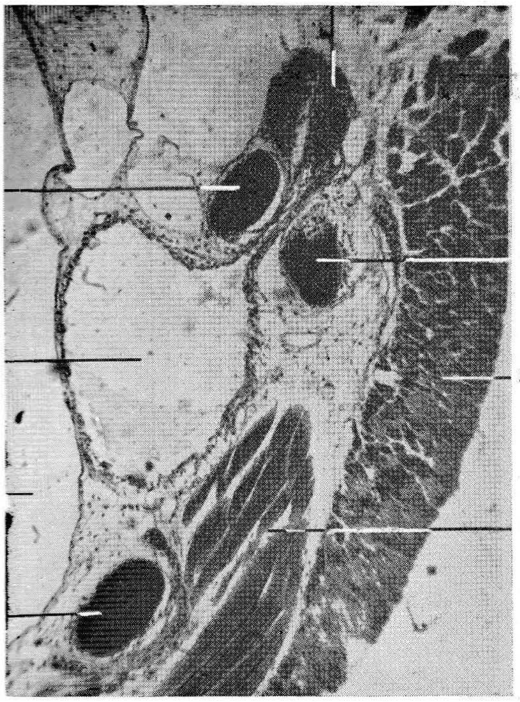


FIG. 10.

nopus lævis D.

Sagittal section through thorax showing the anterior lymph heart conted with the vertebral vein by way of a short canal.

, M.longiss. dorsi. p.c., pleuro-peritoneal cavity;
., M.serratus medius; L, anterior lymph-heart;
2, 3, Proc. transversus I-III.

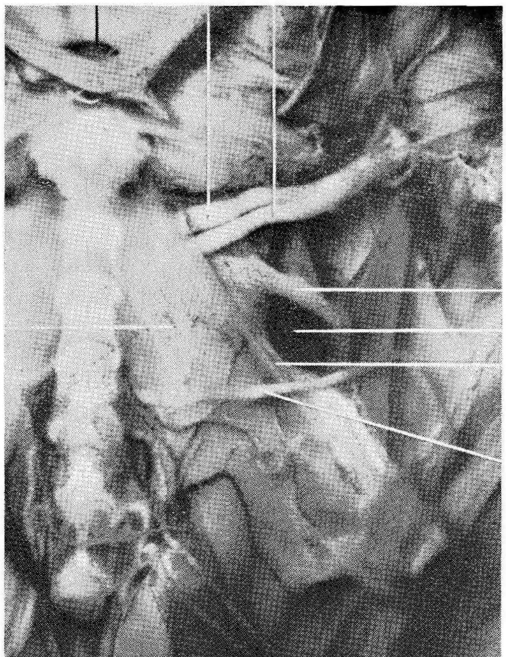
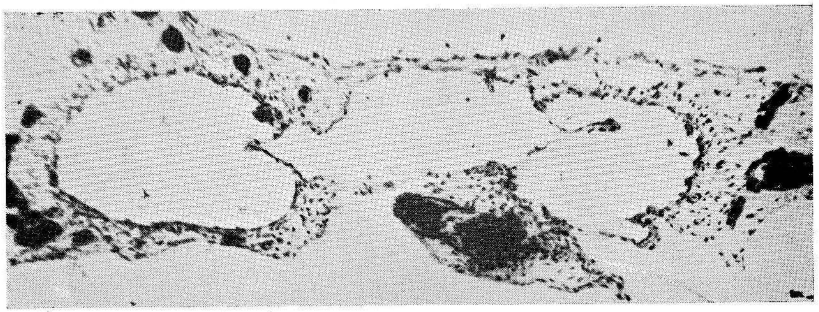


FIG. 11.

Xenopus lævis D.

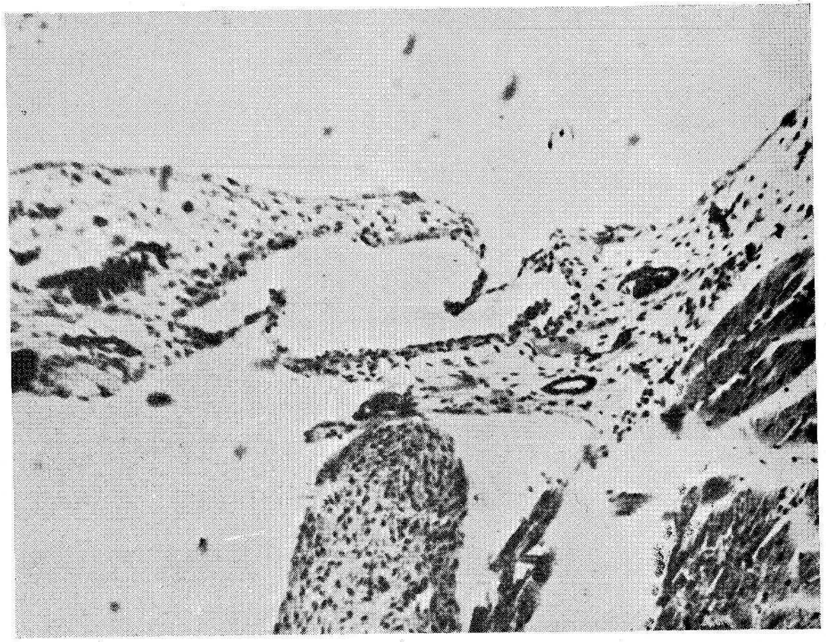
Dorsal aspect of thoraco-abdominal cavity in a case of thrombosis c the left anterior lymph heart.

i = M.ileolumbaris;
o = oral ostium of the Eustachian tubes;
III, IV, V, third, fourth and fifth spinal nerves;
2, 3, second and third transverse processes.
L = Lymph heart.



Xenopus laevis D. Fig, 12

Transverse section through coccygeal region, showing two lymph-hearts draining the saccus iliacus.



Xenopus lævis D. Fig. 13

Transverse section through coccygeal region, showing lymph-heart with external and internal afferent valves.

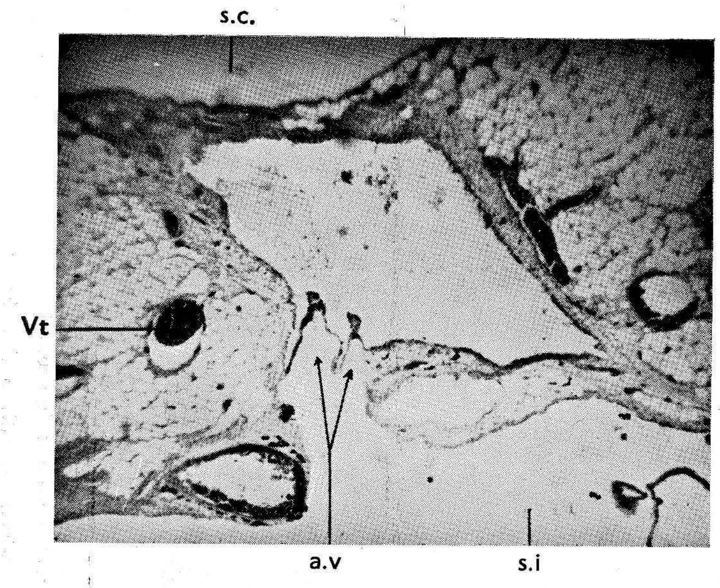


FIG. 14.

Xenopus laevis D.

Transverse section through coccygeal region showing a posterior lymph-heart with afferent twin valves. Twin valves have also been seen in other salientia and, though they are only rarely seen, are not considered to be abnormal.

a.v. = Afferent twin valves.
s.c. = Saccus cranio-dorsalis.
s.i. = Saccus iliacus.
V.t. = Branch of V.iliaca transversa.

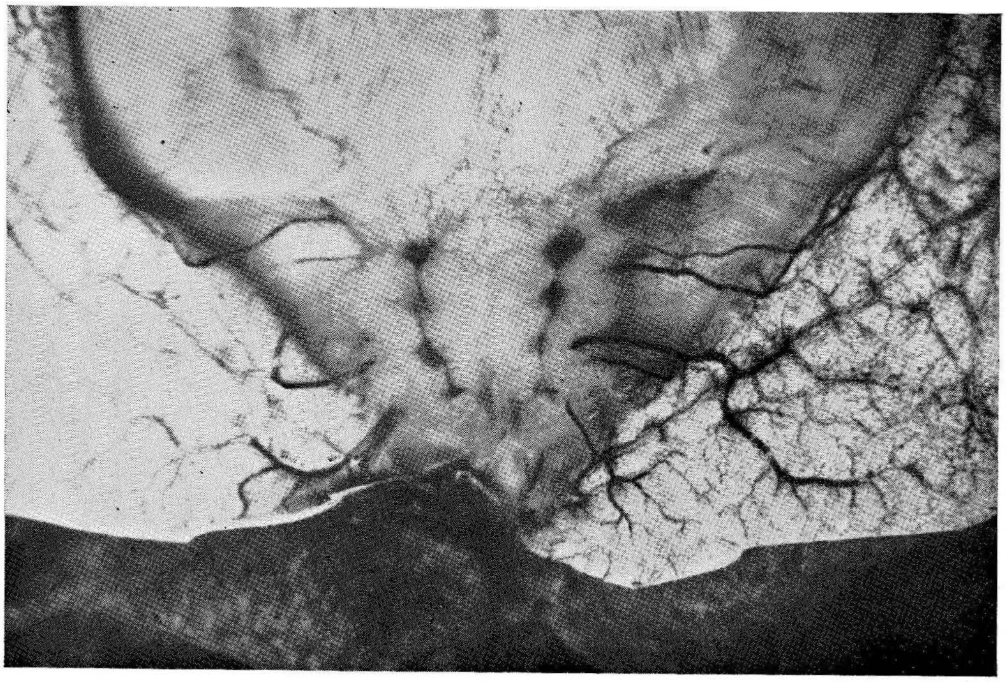


FIG. 15.

Xenopus laevis D.

Adult female. Saccus cranio-dorsalis hydropic, opened over coccygeal region, shows four pairs of lymph hearts filled with blood and thrombosed.

The branches of the V.iliaca transversa which normally drains the lymph hearts take the appearance of connecting channels. The cutaneous veins communicate with the V.coccygea.

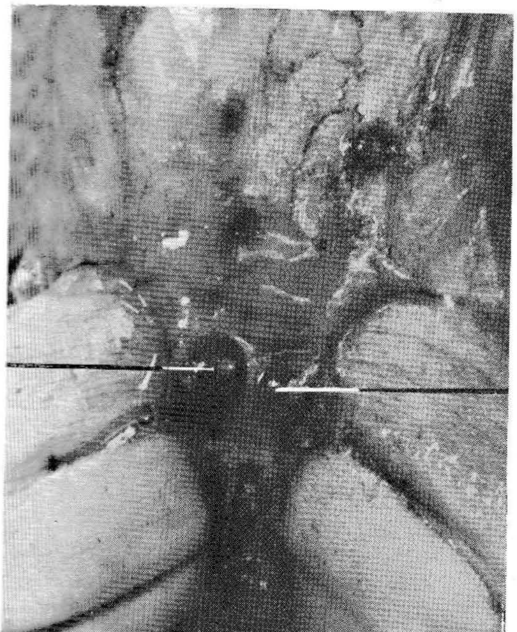


FIG. 16.

...pus laevis D.

... female. Coccygeal region with thrombosed and dilated posterior ... hearts.
...ymph heart.

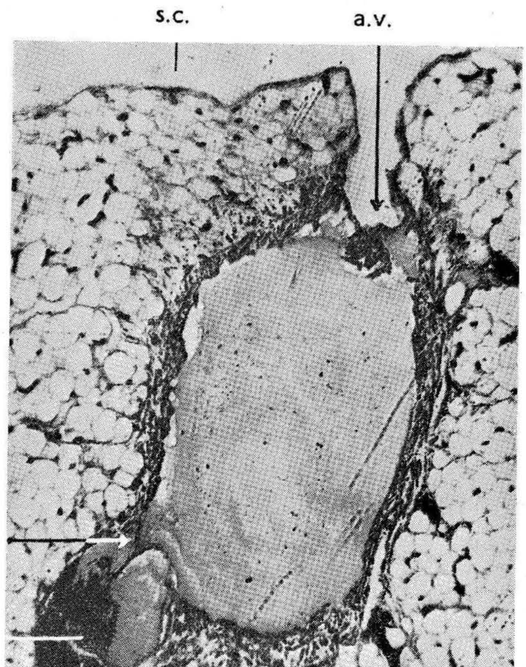


FIG. 17.

Xenopus lævis D.

Transverse section through the posterior lymph heart showing an afferent valve draining the Saccus cranio-dorsalis and an efferent valve connecting with a branch of the V.iliaca transversa. x 60.

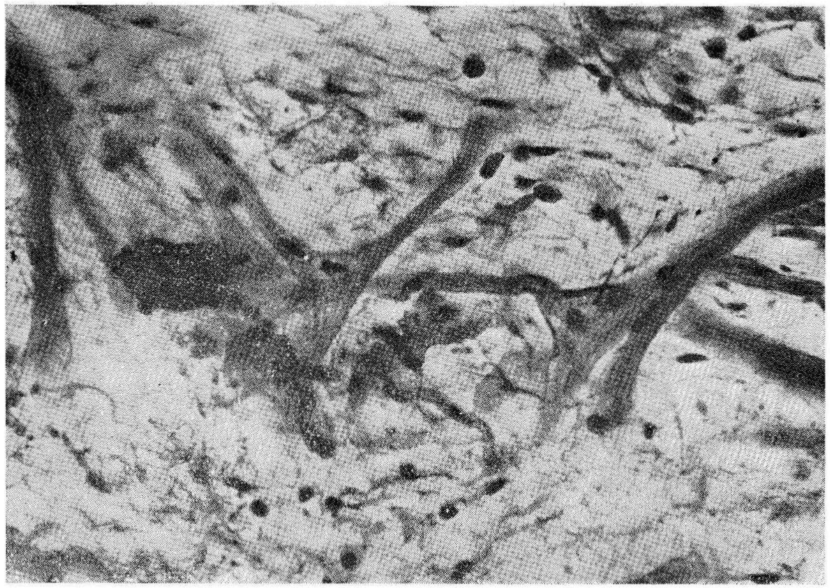


Fig. 18.

Xenopus lævis D.

Tangential section through the wall of the posterior lymph-heart showing bifurcating muscle fibres. x. 450.

quent thrombosis. Sections through such lymph hearts show the deep afferent valves and the efferent valve in various stages of destruction. The thrombosis does not limit itself to the lymph heart but also affects the small branches of the V.iliaca transversa which normally drains the hearts into the V. ischiadica and the V. femoralis. Just as in the anterior hearts the posterior ones have only one efferent valve each (Fig. 17) situated usually in the lower third of the lateral wall of the heart. The absence of channels communicating between the lymph hearts could further be demonstrated in a normal adult male toad which was killed and immediately peritoneally injected with a suspension of lead chromate. The specimen was then fixed, bleached and cleared in glycerin. The mass has filled the lymph hearts to capacity but communicating channels are not seen. (Frontispiece)

The microscopical structure of the posterior lymph hearts is essentially that of the anterior ones with the exception that the posterior hearts are often found to be trabeculated and that their afferent valves are larger and not quite as delicate as those of the anterior hearts. This is particularly true of the valves draining the Saccus cranio-dorsalis whose rim is more reinforced than that of the other valves. It cannot be stated how many afferent valves a lymph heart has since no two lymph hearts are ever found identical in shape, size or number of valves. Some of the afferent valves do not derive their fluid directly from the large sinuses but connect with lymphatic channels which lie in the coccygeal fat body, an arrangement similar to that encountered in the neighbourhood of the anterior hearts. In the normal animal these hearts are found to contain nothing but lymph but in one of my sections a lymph heart was seen to contain a large fatty polypus which, in turn, had a metacercarial cyst in its centre. Cysts of this kind are no rarity in imported toads which can survive some of these trematode infestations for years.

In analogy with the blood-heart one would expect the muscle fibres of the lymph hearts to form a general network. In actual fact the wall of the lymph hearts consists mainly of isolated striated fibres of very unequal length and direction. The longest fibres seen were ten times as long as the shortest. The long fibres lie deep in the wall, the short ones nearer the lumen. In a few, very exceptional cases however, bifurcating fibres with central nuclei could be demonstrated (Fig. 18).

Hydrops in *Xenopus lævis* D.

For the purpose of this paper "hydrops" describes the accumulation of unabsorbed fluid in one or several of the deep or superficial lymph spaces of *Xenopus*.

In normal, healthy toads these spaces, and in particular the subcutaneous lymph sacs are only potential spaces, comparable with the normal vertebral pleural or peritoneal cavity and no fluid can be aspirated from them. The deep lymphatic spaces which envelop single organs and groups of organs allow the structures a degree of mobility relative to one another unknown in higher vertebrates. The superficial lymph sacs too envelop almost the whole body so that there is no animal easier to skin than a frog. It can be assumed that this peculiarly loose structure plays a great part in the animal's remarkable ability to escape and has a commensurate survival value.

There are no signs to indicate that the lymphatic spaces play an important part in the metabolism of the animal. The largest and most accessible lymph sacs which are most often used for giving injections, are most likely to suffer from such interference and in a large laboratory animals are from time to time found in which these lymph sacs, particularly the Saccus cranio-dorsalis, have become completely obliterated through adhesion. In others an excess of lymphatic fluid accumulates in the sacs, giving the toad the appearance of a blown-up balloon, yet neither of these conditions is, in itself incompatible with the life of the toad. Animals in this condition do not feed well but they live on for many months although they never recover.

Noble (1931) suggests that the lymph sacs "collect blood (devoid of erythrocytes) which seeps through the capillaries and return it to the veins." It is not clear however how this acellular blood—or lymph—normally gets into the lymph sacs. Amphibians regulate their requirements of water not by drinking but with the aid of their skin which is permeable both for fluids and for gases dissolved in them. The skin is abundantly vascularized by branches of the pulmo-cutaneous and other vessels and thereby drained of any surplus water. The destruction of the lymph hearts by thrombosis—and this can happen both to the anterior and the posterior group—is not, in itself a necessary cause of hydrops. It is doubtful if the endothelial lining of the sacs excretes any fluid without the stimulus of a chemical or bacterial irritating agent. In such a case the narrow openings of the afferent valves become speedily blocked, the equilibrium of pressure necessary for the normal functioning of the lymph hearts is thrown out of balance, the efferent valves become incompetent, a reflux of blood into the lymph hearts occurs and thrombosis ensues (Fig. 16). Such thrombosed hearts can become considerably enlarged and although the animal so affected may live on, no repair process ever takes place.

In the routine *Xenopus* test for pregnancy neither the instruments used nor the extract injected are sterilized. This practice seems to be reasonably safe. In this laboratory where approximately 12,000 tests are done annually on a stock of about 1,600 *Xenopus* there are never more than 20—30 specimens suffering from the consequences of injections. Hydrops is never seen in freshly imported *Xenopus* though it was seen in a recently imported specimen of *Rana ornata* in the collection of the Zoological Society of London. It has also been shown that subcutaneous hydrops may occur in very young *Xenopus* tadpoles, bred in captivity (Schwabacher & Elkan 1952). Such tadpoles do not survive and they would therefore never be found in imported material. In the absence of a full explanation of the appearance of hydrops in tadpoles only a few days old, it was assumed that excessive dosage of gonadotrophin overstimulated the toads used for breeding and that this led to the production, at excessive speed, of masses of immature and genetically unfit eggs which, in so far as they developed at all, showed many other abnormalities besides that of hydrops. The expectation of life of such larvæ could be improved by the addition of sodium chloride to the water.

Attempts at treating hydrops have so far not been successful. Seven out of a batch of 200 *Xenopus* kept for some time in captivity were found to suffer from varying degrees of hydrops and cultures were made from fluid aspirated from the dorsal sac. One toad (No. 1) yielded a growth of *paracolon* bacilli; another (No. 3), a growth of *Streptococcus hæmolyticus* belonging to Lancefield's group A. Five other toads (Nos. 2, 4, 5, 6 and 7), had blood-stained fluid which remained sterile. Toad No. 3 received 10,000 u. of penicillin into the Saccus cranio-dorsalis for seven days. This animal recovered. Toad No. 1 received 0.5 gm. of Streptomycin for seven days. It failed to recover although the aspirated fluid became sterile. The remaining toads received Streptokinase-Streptodornase in doses of 0.2, 0.4 and 0.6 ml., representing 2,400, 4,800 and 7,200 Cathie units. These injections were repeated at weekly intervals for four weeks. None of the animals so treated recovered from their hydrops.

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REPTILES AND AMPHIBIANS OF THE HIGHLANDS OF ECUADOR

By

Robert Copping

The district concerned is the narrow plateau between the two chains of the Ecuadorean Andes. The city of Quito is more or less in the centre of the area, and is the locality of all the species mentioned with the exception of those specifically referred to other regions. Quito is at an altitude of 9,500 feet—a critical height, it would appear, as a very few hundred feet makes a difference to the fauna. The region being equatorial the weather is more or less constant, although June, July and August are usually very dry and the rest of the year rather wet. The yearly rainfall is about 58 inches. The air is cool, although the sun is very bright and scorching. Nights are cold, but without frost. There is never snow, but fairly frequent hail. All specimens live well at English room-temperature.

Reptiles

The only snake is *Leimadophis albiventris*. This is a rather pretty bright green snake reaching a length of perhaps 3 feet. It has much the habits of *N.natrix*. Like this species, it incubates its eggs in heaps of rotting vegetation. It does not appear to reach the city of Quito itself, but is found in valleys only six or seven hundred feet lower.

There are three lizards. *Pholidobolus montium* is a Teiid of the size and rather the appearance of *L.vivipara*. It is very active and can stand considerable changes of temperature, but I have not found it higher than 10,000 feet. In the natural state it eats flies—which it may stalk from a distance of two or three feet—and other small insects. In captivity it readily eats small worms, woodlice, etc. It drinks dew in the early mornings and will drink out of puddles or in captivity out of a shallow vessel. It gets very tame, but is always alert in the sun. It is the only lizard known to me that habitually sheds its skin absolutely entire. It deposits a few rather large white hard-shelled eggs.

Proctoporus unicolor is a Teiid of much the same size as the preceding. It lives just below the surface of the ground, and the limbs are reduced though still functional. The tail is long and as stout as the body. The colour is dark olive green (almost black) dorsally, and bluish black below with bright red stripes on the under surface of the tail. It is not very active, and is extremely susceptible to high temperatures, dying if exposed to the sun for even quite a short period. In captivity it will eat maggots and any very small insects. It may be partly nocturnal, but in a light and shady cage it will walk about and eat and drink freely during the day.

There is also a *Leiocephalus*. I am not certain of the species. It is greyish brown with cream stripes and reaches a length of about twelve inches, although it is possible there are two species, a larger and a smaller. It is very active and very aggressive. It lives among the large sharp-leaved agave plants and seems to be found only where these grow. Like some other iguanids it can run for several feet across the surface of water.

There are five frogs. *Gastrotheca marsupiata* (or possibly a variety of this species), is extremely interesting and offers much scope for study. After the female has deposited the eggs they are returned to a dorsal pouch where they remain until they are small tadpoles, at which stage they are deposited in the water. The tadpoles grow very rapidly, and reach the considerable size of eight centimeters although the frog itself is no larger than *R.temporaria*. The young frogs are of course very large and grow very fast, and it is possible that they are mature at the end of the first year. The species shows remarkable colour-variation. The basic colour-pattern is greenish-brown with two rather wide irregular darker dorsal lines. There is a black band along each side, edged with white. However, it is by no means unusual to find specimens bright leaf green all over, or green with dorsal lines in brown, or brown with dorsal lines in green, or fawn with no markings at all—and every possible variation between. The means by which the eggs are introduced into the pouch has not yet been observed.

Eleutherodactylus unistrigatus is small—a large female is about 3.5 cm. The colour is pinkish brown evenly mottled with black, though there is a considerable variation in the ground-colour, some (especially the males), being a bright brick red. Some have a median white line and others have several parallel lines as well. The individuals also vary considerably with humidity and temperature—going light in warm, dry surroundings and very dark when cold and wet. It has a call like the clicking that can be made by bending a thin piece of tin. This is a very bright little frog, calling even when shut in a specimen bottle, and catching bluebottles as actively as a hylid. It shows many hylid characteristics, climbing easily and often being found in low bushes. To the best of my knowledge nothing is known of its breeding habits. Although I have handled many females with eggs apparently ready to be deposited, I have never seen a male in the position of clasping nor have I seen the tadpoles. The young frogs are extremely minute—about half a centimeter long.

I have found only one hylid—and only one specimen at that. It does not correspond to *Hyla quitoi,* described by Fowler in 1913. It is a small green slender frog with very long hind limbs. The yellow ventral surface is just visible from above, giving the appearance of a narrow lateral yellow line. It is nocturnal and lives in low bushes near water. I have not heard it call.

The last species is a small leptodactylid, which perhaps is a new species. A large specimen is four cm. long. The upper surface is dark greenish brown mottled with blackish and the ventrum bright orange with the anterior abdominal vein visible as a purple streak. It appears to have a highly specialised environment. I have found it only in streams two or

three feet wide that repeatedly pass under ground for twenty or thirty yards and then reappear, although completely hidden by vegetation. The bottom of the stream is invariably of very thick mud. The frogs appear never to leave the streams and never to come into the light. They are killed by a very slight increase of temperature (the temperature of the stream is between 12 and 13°C.) or by exposure of a very few minutes to the sun. They are very active but never hop more than a few feet without stopping. Their call is absolutely characteristic, being a low plaintive whistle. The tadpoles are long and thin and sluggish, with the organs clearly visible through the skin. An interesting feature is an apparent tendency to deformity—out of some seventy specimens in my possession, fifteen show marked deformities, several having three fore-limbs on the left-hand side, others two, others two feet on the same limb. These specimens are possibly a little weaker than the others, as out of nine that died in an over-crowded collecting-bag, five were young frogs with deformities. A possible explanation is that the tadpoles are nibbled by the gastrotheca tadpoles—a few of which are always to be found in the same water. These latter are very aggressive, often biting the developing frogs of their own species. It would of course be interesting to raise a number from spawn, keeping some with gastrothecas and some by themselves.

Before concluding I should like to point out that this probably exhausts the species in the Quito district, but certainly not in the whole Interandean region. For example, from Ambato, 90 miles to the south, comes another *pholidobolus* characterised by a red tail and a difference in scalation. It is in process of being described by Dr. Orcés of Quito. Farther south still is to be found the very showy *Leiocephalus rhodomelas*—the males a delicate rose pink ventrally (or bright red in some specimens) with a bold black line along the belly and crossing the girdles.

I should also like to take this opportunity of mentioning that I have found *B.blombergi* (first described from Colombia in December, 1952) in some numbers in the north of Ecuador. A large female measures 20 cm. It lives in shallow ditches and is not very active. It is essentially tropical, and much more dependent on moisture than the much smaller *B.marinus.*

SOME NOTES ON THE BREEDING OF AMPHIBIA, 1952.

Green Tree Frog (*Hyla arborea*)

By

R. Bushnell

Some 18 of this species were kept in an all glass tank measuring 18″ x 18″ x 36″ high. The bottom was liberally covered with moss, under which was six inches of broken stones and pieces of rock, allowing plenty of room for the frogs to crawl under during cool spells of temperature.

The frogs had been kept under these conditions during the whole of the winter 1951-2. The tank being situated in a lean-to greenhouse facing due south received the maximum of sunshine, and as the greenhouse was heated with an oilstove the temperature seldom went below 50°F. During the cool spells it was noticed that the frogs retired under the moss, making their reappearance if the temperature rose much above 60°F. Feeding during the winter was very spasmodic and only during relatively warm periods, and it was also noticed that the frogs liked to enter a dish of water occasionally.

About the end of March, 1952, the tank was overhauled and fresh moss introduced, also a small Portugese Laurel in a pot was sunk into the middle of the tank, and a species of creeping plant commonly known as "Wandering Jew" was introduced. This gave a good cover, in addition a glass dish approximately 8″ x 6″ x 7″ deep was placed to one side of the tank, the top rim being level with the moss; this was filled with water and heavily planted with *Elodea.* A small brick was placed at one end to help the frogs to get out of the water if they wished.

Towards the end of April an extra six male *Hyla* were obtained and placed in the tank. At this time all the males which now numbered over 20, spent the whole of their time during the day sitting in the undergrowth around the pool, but in the late afternoon and evening became very active calling and jostling each other in and around the pool. The females during this period could be seen on the far side of the tank away from the pool, but they appeared to approach nearer during the height of calling periods, and two pairs were observed in amplexus in a similar embrace to our common frog on 29th and 30th April.

For some days the temperature had been quite warm, during the daytime 70°F. and during the day of 1st May it was showery although warm. The temperature in the greenhouse was 60°F. during the night and spawn was found in the morning (no actual spawning act was observed). The eggs were quite small, the total size including jelly being approximately the size of a common frog's egg minus the jelly. They were laid in a cluster in the water weed and appeared brownish in colour.

The eggs were removed on the 2nd May from the pool and placed into a 18″ x 10″ x 10″ tank in front of the greenhouse window facing due south.

They left the egg case on 8th May.

During the day the tank was shaded by a piece of plywood to keep down the extreme temperature that would have occurred through the sun's rays beating through the glass. They appear to do well under a semi-shaded condition.

They commenced to metamorphose on 2nd July, 1952. Their colour was a greenish brown and it was remarkable that they could run up the side of the tank before their tail had disappeared (it appears that the sucker pads on their feet develop at once).

During the whole of the tadpole stage they were fed on fresh lettuce crushed and worms slightly chopped, and after leaving the water (in about a week) as soon as the tail had disappeared they commenced to eat *Drosophila* (fruit fly).

Notes of Doubt

Lack of hibernation does not impair the fertility of the female frog as proven by the above record. But does it affect the fertility of the male? Some doubt of this is in the writer's mind because no check was made of which males mated. (It is hoped to answer this question this year.)

Does lack of natural sunshine cause structural weakness in the bone formation of the young?

Does high temperature artificially created impair the development of the young frog? In one case it is known that six specimens all died after a short period of time at a temperature of 70°F.

Does too large a food (blue bottles) given to the young frogs cause internal rupture? Some small frogs have died with what appears to be their intestines extruded from their anus.

Why is it that the tadpoles seem to develop at such largely differing periods? As stated above, the first tadpole metamorphosed on 2nd July, 1952, but others continued to change right through to December and tadpoles were still alive in March, 1953, and all appeared to grow to the same size.

Does this occur under natural conditions?

A point to bear in mind—the adult frogs will eat their young, so be warned!

PAINTED FROG. *DISCOGLOSSUS PICTUS*

These frogs are very retiring and if plenty of cover is given in a tank, they will very rarely be seen. Early in May is their normal time for spawning, and the males will then become more active whilst the nuptial pads on their front legs will quite clearly be seen.

It has been found best to set up a tank with about 3 inches of water and a pebbly bottom, with two large bricks placed so that the frogs can get out of the water if they wish.

The male clings to the thighs of the female for a short period of time. The eggs, which are quite small, are laid thickly over the bottom of the tank; they are not in a clump as with our common frog, but appear to be separate.

Spawn laid on the 6th and 7th May hatched from the eggs on 12th May (temperature of water approximately 70°F.). Tadpoles were fed on crushed lettuce and chopped worms. These tadpoles were quite rapid in growth and metamorphosed from 25th June onwards. The young frogs were very small and were fed on *Drosophila* flies.

Experience has shown that these tadpoles winter under mild conditions quite easily. Some of them did not metamorphose until March of the following year.

CLAWED FROG. *XENOPUS LAEVIS*

These frogs are entirely aquatic and are normally kept during the winter in an all-glass aquarium with a depth of some 8 to 10 inches of water. This is placed in an unheated greenhouse at an air temperature of about 50°F.

Early in June when the weather is rather hot (particularly if thunder is in the air), the male is heard giving his peculiar underwater trill. Both sexes are then removed from the tank and placed in a new one freshly filled with cold tap water to a depth of 10 inches, containing plenty of water weed (*Elodea densa* and *crispa*). This is not planted, but a layer of stones is placed on the tank bottom because it is thought to give a purchase to the frogs when mating.

Almost immediately the male frog should commence to clasp the female around her thighs, and it it noticed that his head appears to be continually jerking up and down. (This may be a stimulus to the female.) He appears to double his rear legs under him and upwards, bringing his vent almost touching that of the female, which has become somewhat elongated. The eggs, which are laid in small batches of three or four at a time, are quite small and have a brownish tinge to them. They are covered by very little jelly but are adhesive and stick to the water plants. The eggs on plants should be quickly removed when seen as the female *Xenopus* will otherwise immediately commence eating the eggs, scooping them up with her front legs.

The eggs do well in deep water in full sunlight, but the water must not get too warm (90°F. would be fatal). The eggs hatch very quickly and the tadpoles leave them in about three days. These are quite white at first and cling head-first to the water plants, absorbing their yolk sacs. After about a further three days they become free swimming and take up the familiar head-down position; at this stage they appear to do best in green water and grow rapidly. Although they grow rapidly, owing to the very large size they reach before they metamorphose, they have never been reared to small frogs before the winter has set in, about October.

When the cooler weather sets in they are brought into the greenhouse and placed in a tank with a small heater which maintains a temperature of about 65°F. During the tadpole stage the most satisfactory food is dried and crushed stinging nettle leaves. The tadpoles are very liable to drown at the time of metamorphosis, so the tank is thickly planted. When their tail has disappeared the frogs are fed on *Tubifex* worms and White worms, gradually increasing the size of them until garden worms are taken.

CORRESPONDENCE

THE SPECKLED BAND AND ALL THAT

I read with a frown of disapproval reference in an article in your June number to Conan Doyle's "famous story", "The Speckled Band", claiming that this referred to Russell's Viper. Such a claim is only permissible if

the term "Swamp Adder, the deadliest snake in India" be an alternative name for this reptile, if it be able to crawl and bite about an English house in a temperature at which any other ectothermic reptile would be moribund, if it be able to wriggle up and down bell-ropes, hear a low whistle, make a bee-line for a saucer of milk and inflict instantly fatal bites so cunningly that the victim shows no signs of snake-bite. If Russell's viper cannot do these amazing feats, we must look elsewhere for the principal actor in this farrago of egregious drivel which has done more harm to the cause of intelligent ophiology than any other story; forming an excellent example of the nonsense a really talented writer can produce when he starts to write on a subject of which he is completely ignorant.

Yours, etc.,
WALTER ROSE.

SHEEPS' HEARTS AND BULL FROGS

Although I have kept frogs off and on for over 60 years, I was of the opinion that, though they could be induced to eat meat by agitating pieces in front of them to simulate insects, they would not take it otherwise; an opinion I expressed in "Reptiles and Amphibians of Southern Africa." However, a batch of baby Bull Frogs (*Rana adspersa*) has shown me that, for this species at least, this is not the case.

For some time now it has been my habit to feed eight juveniles on sheep's heart, cut into pieces about the size of my thumb and scattered about their vivarium. These pieces may be snapped up as they drop, or they may be eaten hours later. The feeding powers of these youngsters are enormous and it is not unusual for a 1½ oz. frog to weigh nearly 3 ozs. after a good meal. The eight of them will consume a large sheep's heart, fat, cartilage and all, in about a week.

Yours truly, WALTER ROSE.
"Oaknook," Protea Road, Newlands, C.P., South Africa.

EDITORIAL NOTES

The British Herpetological Society has among its members not only experienced writers of scientific papers, but also many who have never written anything for publication. It is certainly not true that only experienced people have anything to contribute to herpetology, and these notes are written to guide, encourage and, in places, to warn intending contributors.

THE GENERAL OBJECTIVE. All papers, unless they are intended as reviews of existing work, must be accounts of your own original work. It is your job to convey this unique knowledge into the heads of other interested persons with the least effort to them. In order to do this, you must use the language and style appropriate to the subject. If your paper is, for example, anatomical, you will have to use the technical terms of anatomy. If, however, your paper is a description of the behaviour of an animal, it is often possible to use the terms of everyday life with equal precision. Choose for yourself the most suitable style, and do not try to copy other scientific papers. Some of the usual features of papers have real value, but others are mere fashions. (Kapp, 1948.)

PREPARATION. It is almost impossible to write a paper on a subject no aspect of which has ever been studied before. Anyone can, by inadvertance, overlook previous work, but papers should not be submitted so innocent of references that they give rise to the suspicion that the author does not know what has been already written on his own subject. The work of searching the literature is often long, but for anyone enthusiastic enough to write a paper, it is certain to increase his interest. The first step is usually to look through the past volumes of the *Zoological Record*, for there you will find the titles of practically all zoological papers systematically indexed. From this, you will be able to compile a list of papers which look as if they might be relevant. Your next task is to see these papers. This is often supposed to be more difficult than it is. The simplest method is to join the Zoological Society, for the subscription of £3 covers the use of the most comprehensive zoological library in the country, and the papers are almost certain to be there. If you cannot join the Society, you may be able to find the *World List of Scientific Periodicals* in the local library. In this book, you will find the names of the libraries in which the journals you seek are to be found. The Science Library at South Kensington is open to all free of charge. The British Museum Library is only open to holders of tickets, but these are available free to anyone who can show good enough reason for having one. There may be a library in your own home town which has the journal, and even if the library belongs to a university or to some apparently private institution, there may be provision for the admission of the occasional reader. The librarian of your local public library will tell you the powers he has for borrowing journals. Our own Society has a library, which increases in value with time. See what the librarian has to offer. In one way or another, persistence will usually yield a result.

THE TITLE OF THE PAPER. From the foregoing paragraphs, it will be clear that the title of a paper is an important thing, for it acts as a guide to the contents for people who begin by knowing nothing else about it, and may have to make a journey to see it. It must be full enough to

perform this function, and if you cannot make it so in a few words, then you will have to use some more. A title such as "Observations on *Gastrotheca marsupiata*" may head a paper on anatomy, breeding in captivity, distribution, or almost anything else. It is as important to prevent uninterested people from reading your paper as it is to attract those who are interested.

ILLUSTRATIONS. Graphs and drawings must be in Indian Ink on Bristol Board, or in some other medium which is equally good for reproduction as a line block. This is a process with a photographic basis, and the rule is not the Editor's whim. Ordinary ink and paper just will *not* do. Every mistake or imperfection will be faithfully copied, so your drawing must be as good as you can make it, and should be much larger than the final reproduction. Make it almost like a poster, with every line and dot in proportion. (Cannon, 1936.) Photographs can only rarely be accepted, because of the cost of reproduction, and colour photographs are almost impossible for us.

SUMMARY. This is often the only part of your paper to be read, for unless the reader is likely to be interested, it is a waste of his time to go on. You are not writing a best seller, but are aiming at a small circle of interested people, so summarise your paper so that anyone can see whether the contents are likely to interest him.

REFERENCES. The accepted method today is to insert the author's name and the year of publication in brackets at the appropriate place in the text, and then to list all the references in alphabetical order at the end. There are good reasons for this method, but it causes the most interruption to the reader, and any equally clear method may be used if desired. All references must have been actually seen, unless this is quite impossible, when the fact that it has not been seen should be indicated by some such phrase as "Not seen, reference from Smith, G. 'Sea Snakes of the Arctic Ocean,' Methuen, London, 1888, p. 156." References to books must include author's name and initial, title of book, publisher's name and town, date, page number. References to journals must include author's name and initial, title of paper, journal name, volume and page number, date. Remember that books are rarely the first place of publication, and the original source should be cited if at all possible.

PROOF READING. The printer pays for his own mistakes but charges for alterations you make which are not in the copy. If you make too many of these alterations we may have to charge you. On the other hand, very careful reading is necessary to find mistakes, as there is a natural tendency to read incorrect things as correct. An alteration in the first lines of a long paragraph may involve resetting the whole. Deletions and additions can often be balanced so as to affect as few consecutive lines as possible; e.g., change "alterations you make which are not in the copy" to "alterations and additions not in copy."

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