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The Location of the Parasites in Muscle Cysticercosis

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Abstract. In muscle cysticercosis of swine and of bovine animals, the first caused by *Cysticercus cellulosae*, the latter by *Cysticercus bovis*, the parasites were proved to be located in the lymphatic capillaries of the muscle. The capillaries are gradually dilated by the cysticerci and later the original histological structure, surrounding the parasite, disappears in dependence on the developing inflammation.

Following our studies of the brain cysticercosis (Šlais 1960, 1965 a, b, 1967) we have started to study the reaction of the tissue to the parasites in muscle cysticercosis of swine and bovine animals. In this we succeeded to disclose a new fact, which substantially changes and completes the present ideas on this disease of domestic animals. However, the not decreasing frequency of human infection with the cestode *Taeniarhynchus saginatus* in Europe is calling for a more profound knowledge on cysticercosis of bovine animals and its diagnostics.

Up to date, only the connective tissue between the finer and coarser bundles of muscle fibers (perimysium internum) has been recorded in the literature as the site, where the parasites are situated in muscle cysticercosis. These descriptions are evidently basing on the assumption that the parasites are located directly in the connective tissue, where also the inflammatory process develops, which naturally, in a later phase, wipes off any histological structure of the tissue surrounding the cysticerci. Our findings of early stages of the infection have made it possible to prove unanimously the location of the parasite in the organs.

MATERIAL AND METHODS

Material for our studies has been obtained from the Prague abattoire. Until now, we examined 30 *C. cellulosae* in their original location in the musculature of a pig, imported from Hungary. For three years we have continuously been studying *C. bovis* in the skeletal and cardiac musculature of bovine animals and have so far examined over 600 bladders.

The material fixed in 10 % formol has been studied partly histologically in frozen and paraffin sections. Series of paraffin sections were made whenever necessary. These were studied by standard and special histological methods, especially histochemically.

FINDINGS

During our studies of young developmental stages of *Cysticercus cellulosae* from a massive cysticercosis of swine we observed that the growing larvae occur in spaces lined with a single layer of endothelial cells. This lining, sometimes placed only on a minimal layer of connective tissue, was found to communicate with bundles of muscle fibers or lobules of adipose tissue. However, in some places this connective tissue layer was more prominent and its cellularity larger than usual. Gradually, the growing larvae dilated these spaces so that their existence could be clearly proved. Places containing more connective tissue became less dilated than the other parts of the wall surrounding these spaces, which remained submerged into the growing bladder as a connective tissue septum. The histological character of the described spaces is typical for the lymphatic capillaries. (Plates II, III)

While removing fully developed cysticerci of the species *Cysticercus bovis* from the skeletal musculature of bovine animals, we noted the interesting fact that the cysticerci occurred always in the thicker membrane of connective tissue, dividing a greater number of primary bundles of muscle fibers, and that these parasites were found between those bundles. These membranes of connective tissue were formed by dense fibrous connective tissue and from them connective tissue bands were leading to the surface of the neighbouring bundles of muscle tissue (BENNINGHOF 1944). These membranes are interwoven with vessels and nerves and evidently with the terminal branches of the lymphatic vessels, in which the cysticerci develop. In the skeletal musculature of bovine animals the connective tissue is not reinforced at the crossing point of the lymphatic capillary network and, consequently, cysticerci with connective tissue septum cutting into their bladders were not found there. Cysticerci situated in the dilated lymphatic spaces until the occurrence of a more distinct development of the inflammation, are only surrounded by the connective tissue of the membrane, which, whenever these cysticerci are found, is misinterpreted for its fibrous encapsulation. (Plates I, IV)

The lining of even dilated lymphatic capillaries is formed by a thin layer of endothelial cells with oval elongated nuclei of 18—20 μ in length and 6 μ in width. Nuclei with less chromatin contain some coarser grains with staining properties of nucleoli; their thickness is minimal and, therefore, they appear as narrow short lines of condensed chromatin on the transverse section of the lining. Frequent findings of paired nuclei give evidence of apparently amitotic division, which seems to be connected with the excessive extention of the capillary lumina, caused by slow growth of the parasite. All nuclei are orientated in the same direction, the collagenous fibers of the first connective layer tissue, on which the endothelium is placed running in vertical orientation to the direction of the nuclei. (Plate V) In lymphatic capillaries extremely dilated by the adult cysticercus, the connective tissue of the interfascicular membrane is highly compressed and its stratified structure of fiber layers, placed vertically on each other, becomes clearly observable. A distinct alternation of the endothelial lining occurs when the developing inflammation starts to affect the metabolic products of the parasite; for a long time these changes are limited only to the portion of the capillary wall, which lies opposite the opening of the evaginated part of the cysticercus. Along all other parts of the periphery of the bladder no changes occur in the capillary wall up to the death of the parasite.

DISCUSSION

There is little information available on the existence of the lymphatic capillaries in the skeletal musculature. The few data on this subject found in the literature are contradictory and the only fact to be proved seems to be the absence of the lymphatic capillaries in the connective tissue enveloping the primary muscle fibers, i.e. in the endomysium, contrary to the blood capillaries. On photomicrographs in the paper by Kozma and Geller (1953), the lymphatic capillaries are clearly visible in the perimysium internum of the skeletal musculature of the dog, dilating after the strangling of the main lymphatic vessels. Their wall communicates directly with the muscle bundles. The wall of lymphatic capillaries consisting of endothelium only, is one of its typical features. Its endothelial cells are larger than those in the blood capillaries. The wall of the lymphatic capillary is, however, undividable from the surrounding connective tissue (Zhdanov 1952) and no valves are present in their lumina, a feature, differentiating the lymphatic capillaries from the lymphatic vessels of smallest dimensions. The lumina of the lymphatic capillaries are frequently much larger than those of these vessels, but often irregular, lacunary. In their wall there are often numerous small elevations, extending along the whole surface of the lumina and partly resembling valves. The first to give attention to them was von Recklinghausen (1871).

In cysticercosis of the heart we found parasites in the same lymphatic spaces not only in the myocardium, but very often in the endocardium and epicardium. After Zhdanov (1952), the subendocardiac network of lymphatic capillaries, situated in the connective tissue of the endocardium, is most irregular especially on the papillary muscles, where their loops are very elongated. Short capillaries of the endocardiac network fuse with the intramyocardiac network, the capillaries of which are three times larger in diameter than the blood capillaries. Because no larger draining lymphatic vessels are present in the myocardium, the lymphatic capillaries of the myocardium open mostly directly into the lymphatic capillary network of the epicardium. The subepicardial lymphatic network is highly developed and serves as a reservoir for the lymph of the endocardium and the wall of the heart.

The connective tissue of the myocardium is, in fact, more marked than that of the skeletal musculature and passes directly into the connective tissue of the epicardium and endocardium. FEDYAY (1961) confirmed lymphatic capillaries in the connective tissue between the primary bundles and the bundle groups of the cardiac muscle, in coarses connective tissue septa and around the adventitia of the vessels. Lymphatic capillaries in the myocardium are, therefore, occurring always in places with more connective tissue irrelevantly to their numerousness and also to irregularities in the thick connective tissue layer of the epicardium. This deviating character of the tissue, lying in close vicinity to the wall of the lymphatic capillaries is reflected by the moderately changed course of tissue reaction round the cysticerci located in the heart.

Especially marked is the location of the parasites under the endocardium, namely on the papillary muscles, at the fastening points of the chordae tendinae, where relatively large cysticerci are forming distinct lumps extending into the ventricle. According to DOBROVOLSKAYA-ZAYTSEVA (1961), the network of lymphatic capillaries is distributed in a deap collagenous elastic layer of the endocardium and is partly touching the myocardium. The longest capillaries are always extending along the bundles of collagenous and elastic fibers of the endocardium and the mechanical properties of the thin layer of endocardial connective tissue are supporting the marked arching of the parasites on the endocardiac surface without breaking through into the cardiac cavities.

The proof of the location of the cysticerci in the lymphatic capillaries in muscle cysticercosis brings up the question, along which routes the young larvae got there. The active penetration of the oncosphere through the intestinal wall and into the blood capillaries is a well known fact the same as their transportation with the bloodstream to the affected organs. In muscle cysticercosis it had to be assumed that the young larvae, while actively leaving the blood capillaries at the junction where they pass from the arterial to the venous part, are damaging their wall. This assumption may be affirmed by the minute hemorrhagies observed in experimental studies of the early phases of cysticercosis development, which have also been mentioned in older literature and emphasized by VON MEYENBURG (1929). We succeeded to prove hemosiderin in a minute focus in the connective tissue at the wall of encapsulation of one cysticercus in the musculature of a bovine animal. This indicates that the oncospheres penetrate actively the lymphatic capillaries of the muscle, in which they develop, washed by the lymph. Thus, they are in direct metabolic contact with the lymph and their metabolic products are distributed by the outflowing lymph into the blood far quicker than if they were growing directly in the connective tissue, as had been assumed so far.

A very interesting fact is also the variegated location of *C. cellulosae* in different hosts. In the swine, the most frequently attacked organs are the abdominal muscles, the diaphragm, the tongue, heart, masseters, the intercostal muscles, the pectoral and cervical muscles and some muscles of the posterior extremities. After OSTERTAG (1913), in a massive infection parasites are found also in the nodes, in the subcutaneous fat and in the brain; in even more massive infections, these parasites occur also in the liver and the lungs. Most authors considered the striking affection of the brain and the eyes of man apparent because of the relatively very easy findings of cysticerci in these organs. However, DIXON and LIPSCOMB (1961), basing on a complex of 450 cases of cysticercosis in soldiers of the British Army in India, which had all been most carefully examined and observed for a long time, confirmed unanimously the predilective affection of the CNS even in a generalized infection. On the grounds of literary data and our own observations (ŠLAIS 1965) on the exclusive location of the parasites in the region of the branches of the a. carotis externa in a generalized cysticercosis of the meninges and the brain, this location occurs in consequence of the embolisation of the larvae, which had entered the bloodstream. This depends also on the number of larvae, their weight and size, on the laws governing the bloodstream and on the anatomy of the principal arterial trunks of the attacked intermediate hosts, as pointed out by SCHWARZHAUT (1929). Only after the larvae have been passively carried into the capillary bed, they can move actively to the site of their definitive location in the organs. The embolisation of the larvae of *C. cellulosae* principally into the large arteries of man, arising from the arcus aortae, is also confirmed in a case of muscular and cutaneous cysticercosis, in which the upper parts of the trunk, the upper extremities, the neck and the head are almost exclusively attacked. Also our findings of cysticerci in the pectoral and brachial muscles in a muscle cystercosis (ŠLAIS 1965) confirm the foregoing statement.

Also the very limited location of *C. cellulosae* in bovine animals, where mostly the masseters and the cardiac muscles are affected, seems to be connected with the anatomical conditions of the circulatory system. This has been reaffirmed by CHABASSE and GENTHEN (1952) in France, who observed it in 83 % of all cases examined. Other sites of location of the cysticerci in cows are the cervical muscles, the muscles of the diaphragm, the pectoral and the intercostal muscles. Solitary parasites can certainly be found also in other organs, but that applies mostly to massive invasions and is very rare when the incidence of the parasites is low. However, this inconceivable location of individual *C. bovis* specimens in bovine animals is still one of the principal causes of human infection with adult cestodes even under most advanced hygienic conditions and compulsory meet control of the animals at the abattoire (DESPRÉS and RAUSCH 1961).

CONCLUSIONS

The proof that both cysticercus species with a primary location in the musculature, *C. cellulosae* and *C. bovis*, develop in the lymphatic capillaries of the muscles, may greatly contribute to a change of opinion on these two parasites. It has been found that the relatively high variegation of other possible sites, where the parasites may develop, lies just in the close relation with this adaption. While *C. pisiformis* and *C. tenuicollis* are found quite exceptionally outside their typical location in the abdominal cavity, the occurrence of *C. bovis* and *C. cellulosae* in other organs than the muscles is very frequent. For *C. cellulosae* this occurrence in some hosts such as man is almost typical. However, as these parasites are actually parasites of the lymphatic system, their varying location in the organs is not a reflection of lesser host specificity but depends principally on the way of embolisation of the oncosphere in various hosts.

The external environment of all mentioned cysticerci is liquid. While *C. crassiceps*, *C. pisiformis* and *C. tenuicollis* develop in the serous fluid of the body cavities, which are covered with a mesothelial lining, *C. cellulosae* and *C. bovis* adapt themselves to much narrower spaces of the lymphatic system in various organs principally in the musculature. This leads to certain changes in their morphology, the importance of the bladder increases, taking on even the role of protecting the scolex of the cestodes. Sufficient space in the body cavities is in favour of a quiet development of the cysticercus and the growth of its bladder; an important confirmation of this fact is the well known phenomenon that the bladder of *C. tenuicollis* changes its size according to the size of its host and can reach almost enormous sizes such as observed by ourselves in a cysticercus invasion of the body cavity of an elk. The closer conditions in the lymphatic capillaries are responsible for the fact that in *C. cellulosae* and *C. bovis*, which both, during their larval development pass through a similar stage as the cysticerci from the body cavities, their bladder grows in a later developmental phase round the scolex part so that this becomes finally completely enclosed by it, as we are able to illustrate (Šlais 1966). Thus, it protects it during the period, when the cysticercus increases its volume, from the pressure of the outlining tissue, which is certainly very high at this time. The distinctly larger sizes and oval shape together with the changed morphology of the scolex part in *C. cellulosae* when located in the subarachnoidal space and the cerebral ventricle prove very clearly that the shape of *C. bovis* and *C. cellulosae*, which they take on when growing in the muscles (so far this shape has been considered typical for them) is only a consequence of the environment, in which these cysticerci develop.

REFERENCES

Benninghof A., Lehrbuch der Anatomie des Menschen. Bd. I. Allgemeine Anatomie und Bewegungsapparat. 3. Aufl. J. F. Lehmanns Verlag, München-Berlin 1944.

Chabasse Y., Genthon H., Observations sur la fréquence et les localisations de la cysticercose bovine aux abattoirs d'Angers. Rec. Méd. Vét. 138: 1083–1093, 1962.

Després P., Rausch W., Diagnosis and the importance of bovine cysticercosis in Switzerland. Schweiz. Arch. Tierheilk. 103: 507–518, 1961.

Dixon H. B. F., Lipscomb F. M., Cysticercosis: An analysis and follow-up of 450 cases. London 1961.

Dobrovolskaya-Zaytseva E. A., (Lymphatic system of the endocardium of man). Arch. Anat. Histol. Embryol. 41: 76–81, 1961. (In Russian.)

Fedyay V. V., (Structure of connective tissue and the lymphatic system of the miocardia of man). Arch. Anat. Histol. Embryol. 40: 75–81, 1961. (In Russian.)

Kozma M., Gellért A., Das innere Lymphgefässystem der Organe. (Cit. from Rényi-Vámos F.), Budapest 1960.

Meyenburg v. H., Die quergestreifte Muskulatur Henke-Lubarsch, Handbuch der speziellen pathologischen Anatomie und Histologie, Bd. 9, Teil 1, Springer Verlag, Berlin, 1923.

Ostertag R., Handbuch der Fleischbeschau. Bd. II, 6. Aufl., Stuttgart 1913.

Recklinghausen v., Das Lymphogefässystem. Stricker Handbuch der Lehre von den Geweben des Menschen und der Tiere. 1: 214–250, 1871.

Šlais J., Histologischer Nachweis von Parasiten in nekrotischen und verkalkten Gebilden (Linguatula, Cysticercus). Zbl. allg. Path., path. Anat. 101: 200–207, 1960.

—, Průkaz parazita a tkáňové reakce při mozkové cysticerkóze. Čs. Parasit. 12: 263–297, 1965.

—, Morfologie původce mozkové cysticerkózy. Čs. Patol. 1: 65–76, 1965.

—, The morphology of Cysticercus racemosus and the determination of the cysticercus species. Folia parasit. (Praha) 14: 27–34, 1967.

Zhdanov D. A., (General anatomy and physiology of the lymphatic system). Leningrad 1952. (In Russian.)

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EXPLANATIONS TO THE PLATES

Plate I

Fig. 1. Live cysticercus in the muscle. The wall transparent at both ends, thickened and more opaque in the centre, where more fibrous connective tissue has accumulated. The outlining muscle bundles were taken out in order to show the direct communication of the envelope of the cysticercus with the interfascicular septa of the fibrous connective tissue. Fresh material of bovine animals (× 3).

Fig. 2. Half of the cysticercus protruding from the surface of the cut through the muscle. Note the communication of the formation with the system of thicker connective tissue septa of a higher order. The formation is not transparent throughout, but opaque to one half of its length because of more connective tissue accumulated there, in which filled vessels can be viewed. The yellowish colour on the top of the formation with a transparent wall is caused by inflammatory changes and the necrotic exudate inside the cyst. Fresh material of bovine animals (× 3).

Plate II

Fig. 1. Young stages of the cysticercus *C. cellulosae* with its bladder growing round the scolex part and dilating the lymphatic capillary in the musculature of swine. To the right of the parasite a dense occurrence of connective tissue can be seen in the capillary wall. A similar dense occurrence of connective tissue can be viewed to the left of the parasite in the musculature in the wall of another lymphatic capillary, which is collapsed and has a slit-like lumen. Goldner (× 12).

Fig. 2. An almost complete section through the lymphatic capillary in the musculature of swine; on the left, its dilation by the parasite *C. cellulosae.* Its bladder is also visible on the section. Above it, the thickening of the connective tissue in the wall can be viewed. Further along this capillary there are two more nodulous places, where other capillaries are crossing it, causing an accumulation of connective tissue. Goldner (× 12).

Plate III

Fig. 1. Longitudinal section through a fully developed parasite (*C. cellulosae*) in the musculature of swine; the growing parasite widened the lymphatic capillary in the place of a nodulous thickening of its wall by connective tissue, which had become little dilated and remained pressed into the bladder like a septum. Goldner (× 12).

Fig. 2. Tangential section through the wall of a connective tissue septum, dilated by a fully developed *C. bovis*. The cut has been carried close to the surface of the bladder, which is partly visible on the left and right margin of the figure. In the connective tissue, sections through the folds of the connective tissue wall are visible, which appear like horizontal slits covered with an endothelial lining. Goldner (× 140).

Plate IV

Fig. 1. Longitudinal histological section through the formation on Plate I, Fig. 2. Inside a collapsing cysticercus bladder with the invaginated part. This bladder is most deformed in its lower right part, where the inflammatory changes are most progressed. The thickening of the connective tissue in this part and along the right margin was caused by a fusion of the connective tissue septa following an atrophy of the respective muscle bundles. At the opposite end a thickening of the wall caused by the inflammatory proliferation, which had shown through the fresh material in yellowish-brown colour, can be seen. Weigert-van Gieson (× 10).

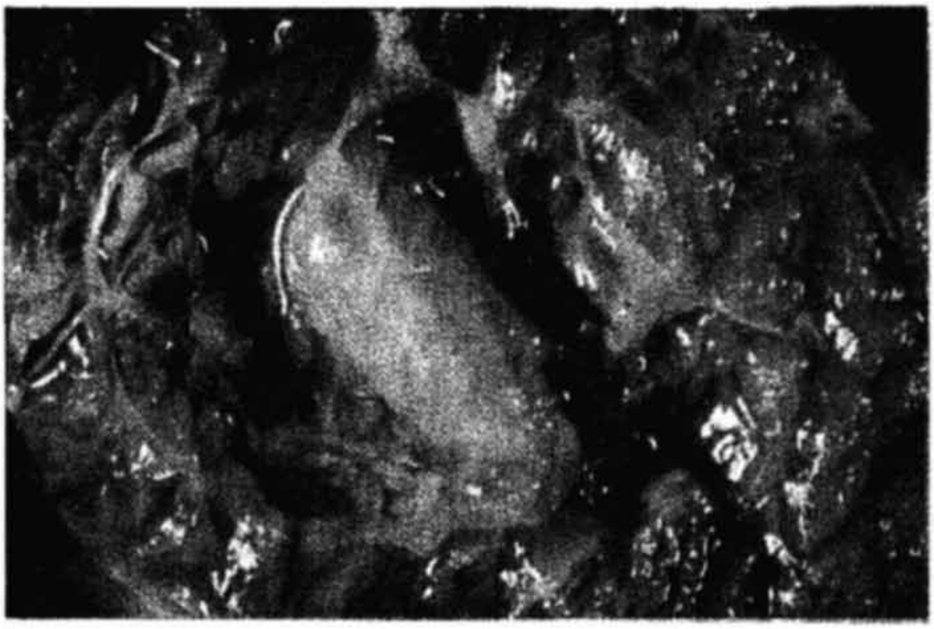
Fig. 2. Longitudinal section through the musculature of a bovine animal showing the interfascicular septum dilated by a fully developed *C. bovis*, lying close to the thicker tendonous septum. Inflammatory changes did not yet start around the parasite. Weigert-van Gieson (× 6.5).

Fig. 3. Shrivelled necrotic *C. bovis* in the centre of a distinct encapsulation area, filled with granulation tissue and necrotic exudate. The original histological arrangement of the tissue had been completely wiped out by the inflammatory changes. Weigert-van Gieson (× 6.5).

Plate V

Fig. 1. Tangential section, cut very obliquely through the tissue surrounding the developed *C. bovis*. On the right the endothelial lining, which appears like a wider strip covered with regularly distributed cell nuclei, all facing the same direction. Underneath it a strip of connective tissue fibers in differently crossed layers and then a tangential section through the vessel network on the border of the connective tissue septum and the musculature, the fibers of which are visible at the posterior margin of the figure. Hematoxylin-eosin (× 145).

Fig. 2. Tangential section through the endothelial lining, which had been lying closely to the parasite in the dilated connective tissue septum. The nuclei of the endothelial cells, all facing in horizontal direction, can be seen on the figure. The plasma of two endothelial cells is visible at the margin of the section. The collagenous fibers of the first layer of connective tissue, on which the endothelium lies, are running in vertical orientation to the direction of the cell nuclei. Goldner (× 1.165).



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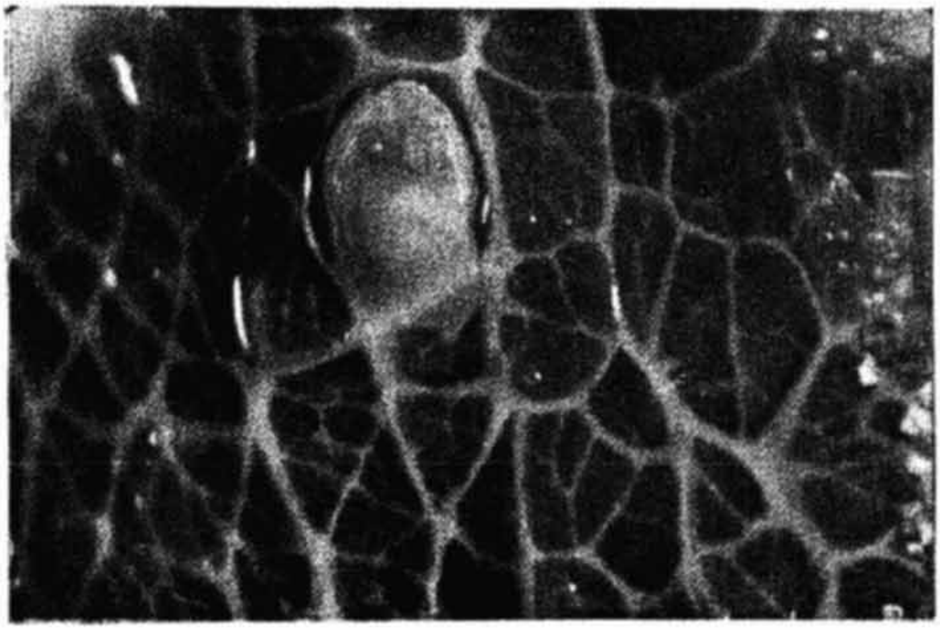

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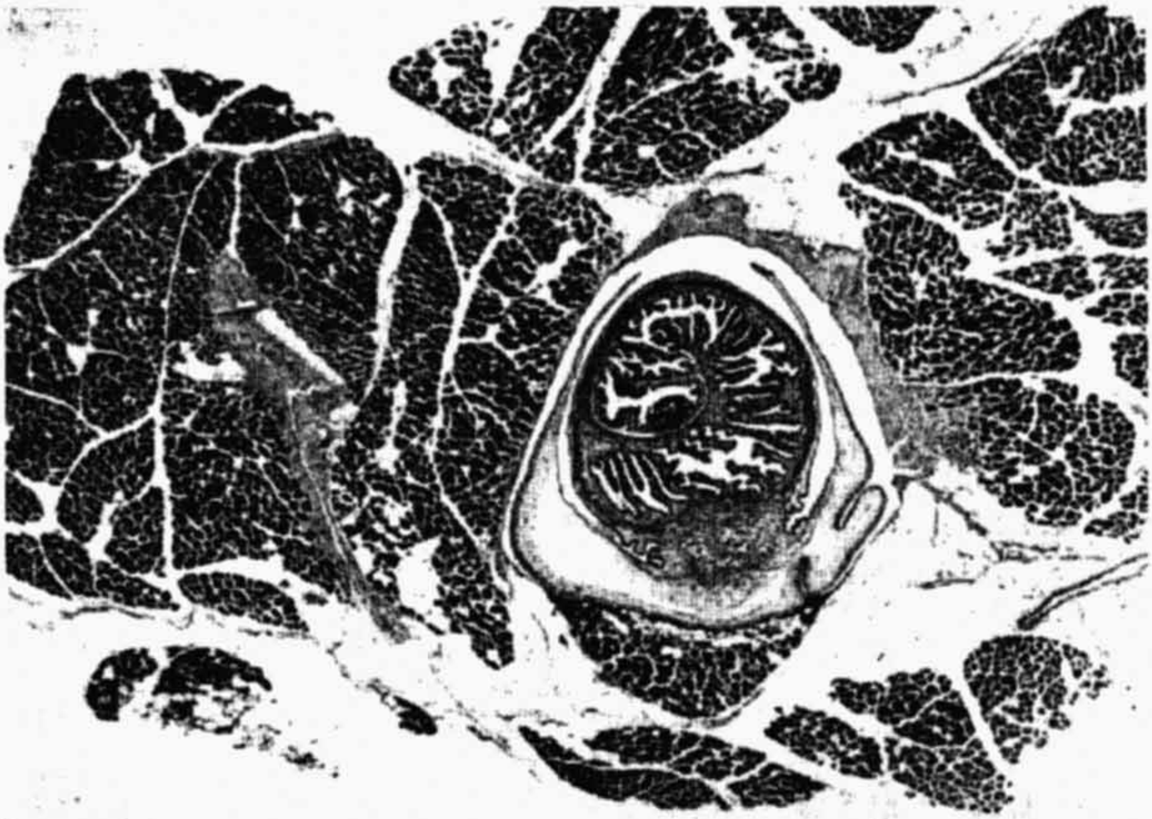

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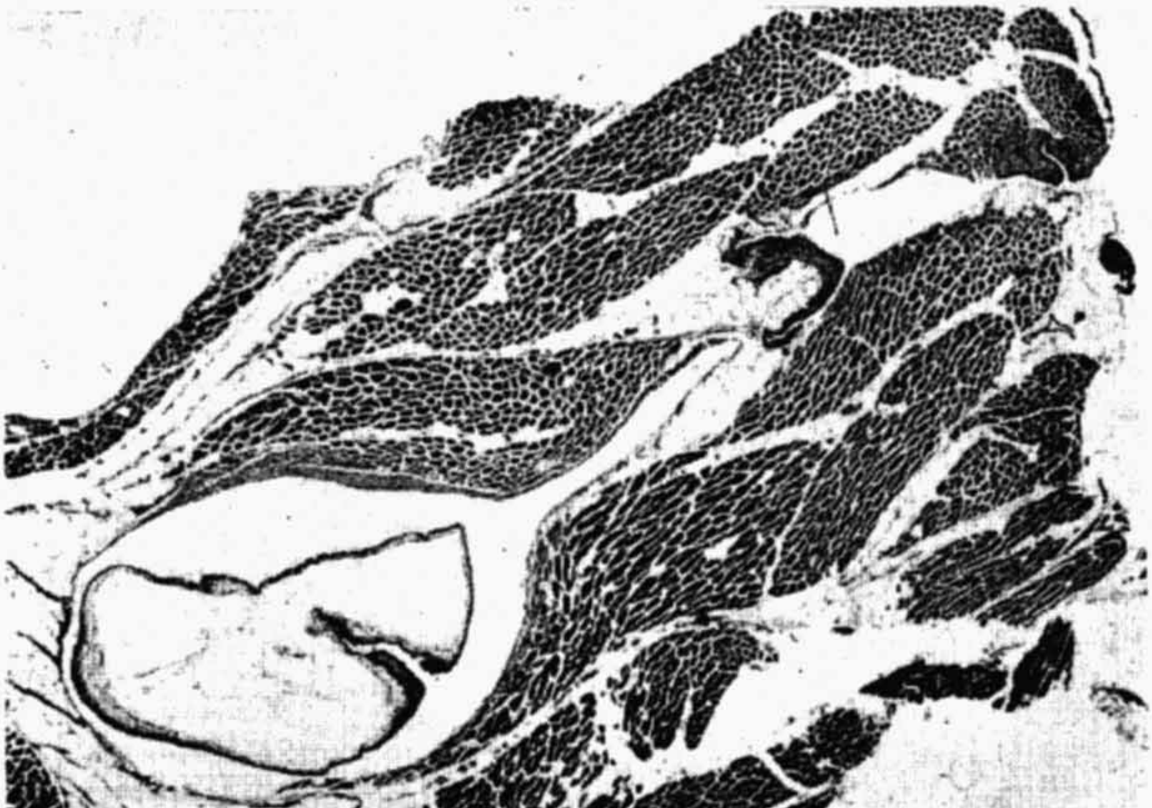

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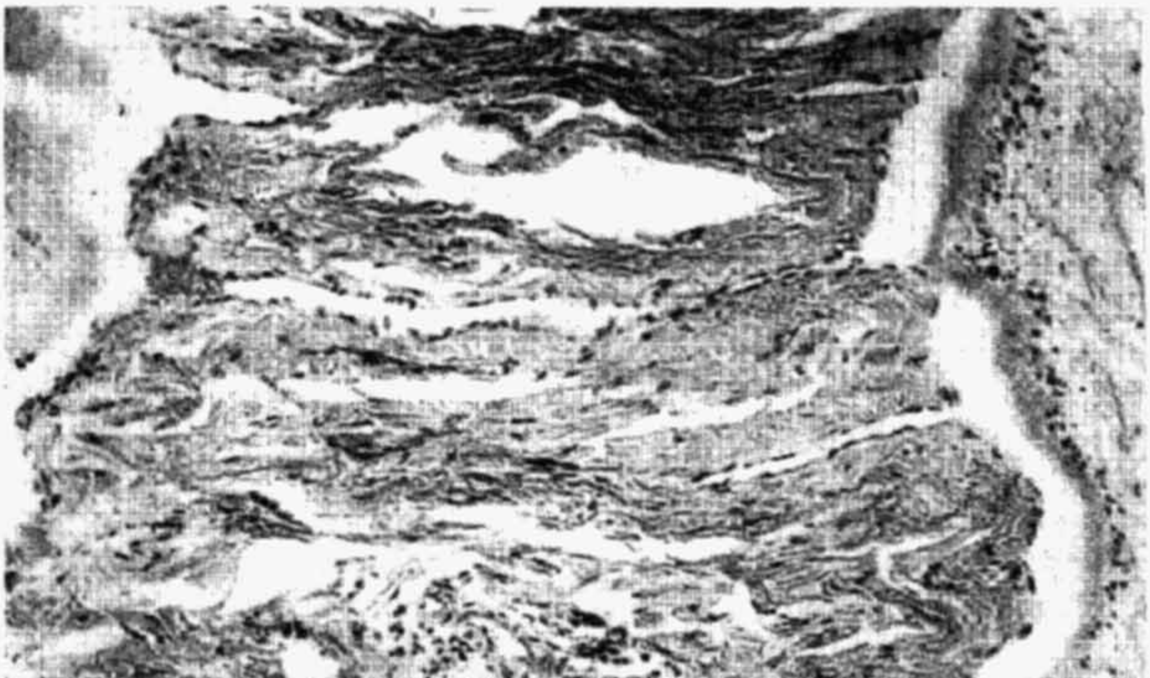

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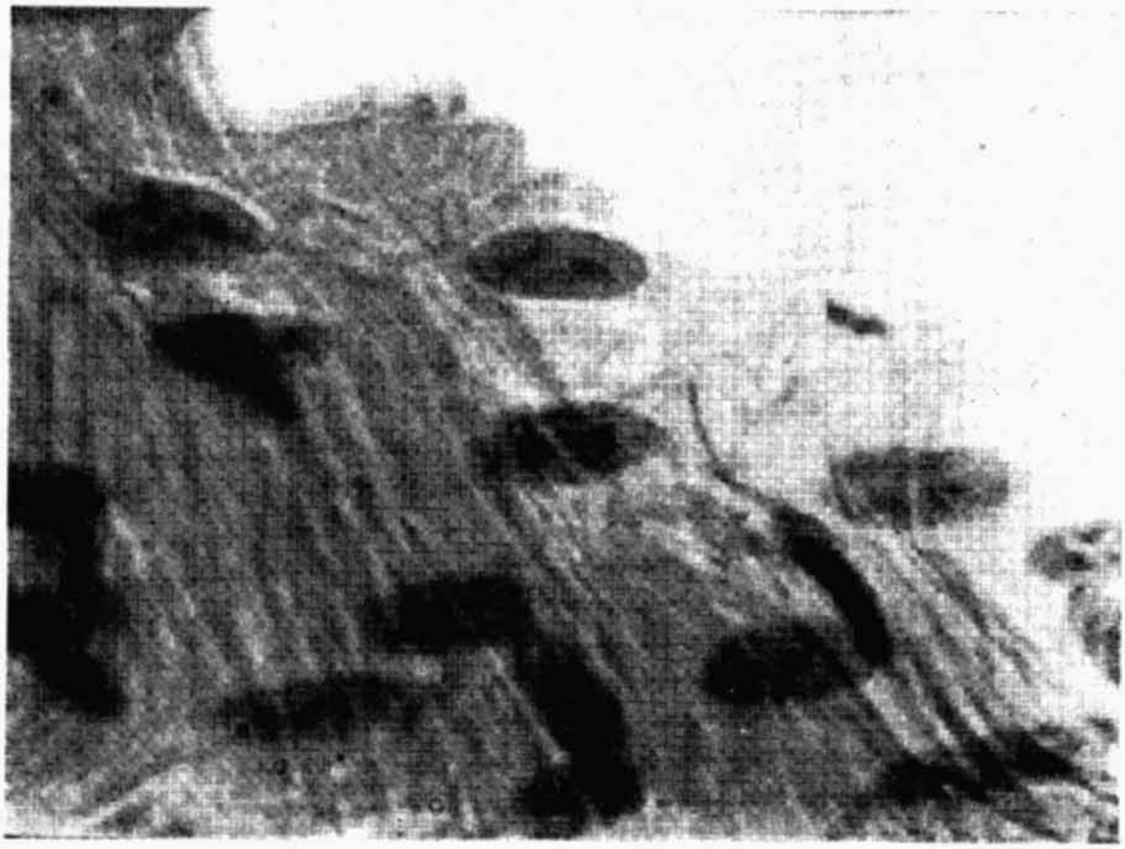

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