Onderstepoort Journal of Veterinary Science and Animal Industry, Volume 7, Number 1, July, 1936.

A Contribution to the Study of the Pathology of Oesophagostomiasis in Sheep.

By P. J. J. FOURIE, Sections of Hygiene and Pathology Onderstepoort.

INTRODUCTION.

Oesophagostomiasis is perhaps the most serious parasitic disease of sheep in the Union of South Africa at the present time. A true understanding of the lesions produced by these parasites and especially a clear conception of the pathogenesis of the lesions may assist in evolving simple and efficient measures of control of the parasite, which is making sheep farming very difficult in many parts of the Union.

Weinberg (1909) gives a detailed microscopic description of twenty-three cases of oesophagostomiasis in various species of apes. The lesions of these apes infested with different species of oesophagostomum are all essentially the same. The author believes that the larvae reach the tissues of the intestinal wall through the blood stream. They rupture the blood vessel and cause a haemorrhagic nodule. Cysts were never seen in the small intestine, most of them are present in the submucosa, some are in the muscular layers and others are rarely found subperitoneally. From the photomicrographs the cysts described are mostly older nodules and would not, strictly speaking, conform to what can be regarded as young cysts which form when the larvae penetrate the mucous membrane in the first instance. He never found larvae in the walls of the intestine, but the adult stage only. Sometimes ruptured cysts become infected with bacteria from the intestinal flora and ulceration resulted. No worm nodules were found in other organs of the body. Those monkeys having large numbers of adult worms in the large intestine had diarrhoea. The author believes that substances secreted by the worms were not responsible for death. He believes death to be due to a septicaemia, although he could not cultivate any bacteria from the blood, but he states that the organisms probably did not grow on the media used. He found an adult female worm and a number of eggs in the morula stage in a nodule. In direct communication with this nodule was another, which possibly held a male worm which could have fertilised the female, before leaving its own nodule for the intestinal lumen.

Walferston Thomas (1910) describes what, according to him, is the second recorded case of oesophagostomiasis in the human subject, the first being described by Brumpt in 1905, quoted by him.

He has never seen a cyst above the level of the muscularis mucosae. This is interpreted to mean in the mucosa proper. He describes cysts in the submucosa and external muscular coat. The cyst consists of the worm and red cells in varying states of disintegration. Eosinophiles, neutrophiles and mono-nuclear cells are present in varying numbers in different cysts. Giant cells are also described, and in some cases there is calcification.

Hall (1920) in a popular article on parasites and parasitic diseases of sheep, mentions the presence of cysts in which the parasites may be present. These cysts contain necrotic material, yellowish or greenish in colour, and may be of a caseous or calcareous nature. Larvae which migrate to the mesenteric lymphatic glands, the omentum and the liver, probably die there and never get back to the intestine to complete their development. In severe cases there is emaciation and diarrhoea. He ascribes the bad effects produced by the worms to: (1) large portions of the intestinal mucous membrane are rendered functionless by the lesions produced therein by the parasites; (2) absorption of toxic substances from the worms themselves and from necrotic material from lesions produced by the parasites.

Theiler (1921) describes the post-mortem appearances of oesophagostomiasis in sheep as those of a pronounced anaemia, hydraemia and serous atrophy of fat. Nodules are present in the small and the large intestine. These he classifies arbitrarily as (1) the reddish nodule which is the young nodule; (2) the green nodule, which contains green pus and (3) the hard nodule in which calcification has taken place. Theiler further states that two serious complications may take place as a result of nodular worm infestation:—(1) Reksiekte* (Intussusception) and (2) septic infection of the serous cavities.

Joest (1926) describes the young nodule as consisting mainly of lymphocytes, together with the oesophagostomum larva in a relatively small amount of broken down tissue in which eosinophiles are present. Fibroblasts surround this central mass but a well defined connective tissue capsule has not yet formed. These young nodules are situated immediately under the muscularis mucosae. The larvae may migrate into the submucous tissues and the nodules may undergo caseation and necrosis. Sometimes ulcers develop.

Mönnig (1934) states that in lambs or in older sheep which have no resistance to the parasite the larvae cause practically no reaction, by their migration into the mucosa. In such cases a large number of adult worms can be found in the colon, without any nodules in the walls of the intestine. The fact that in some cases larvae pass into the submucosa, with the development of nodules, he ascribes to some degree of immunity which the mucosa appears to possess. The larvae according to this author may stay in the nodules for about three months when the contents become caseous and calcified, the parasites either die or leave the nodules to wander about between the muscle fibres. Some larvae may enter the blood and lymph

* An Afrikaans word, literally translated " stretching disease ".

vessels or pass into the abdominal cavity, producing nodules in the liver, lungs, myocardium and abdominal fat. The nodules are usually sterile, but larvae may carry bacteria into the walls of the intestine and produce acute inflammation, peritonitis or even abscesses. The worms are not blood suckers but feed on the intestinal contents or the predigested mucosa, after subjecting the mucosa to the action of secretions from the oesophageal glands. The worms secrete a toxic substance which has a harmful effect on the host.

Wetzel (1934) believes that a glandular secretion from the nodular worms, causes a chronic inflammation of the intestinal mucous membrane of the sheep and that the parasites actually feed on the inflammatory exudate produced in this way.

LESIONS OF OESOPHAGOSTOMIASIS IN LAMBS.

Most of the material used in this study was the same as that used by Veglia (1923) when he was investigating the life history of this parasite. He infected lambs with large numbers of third stage larvae and killed these lambs at intervals of 12 hours, 24 hours, 36 hours, 2 days, 3 days, etc. Specimens of intestines were collected in formalin and these were kindly placed at my disposal by Veglia.

In addition to this material obtained from Veglia several other two-tooth merino sheep were experimentally infected with pure faeces cultures of Oesophagostomum larvae.

Serial sections of the intestine were cut and stained in the ordinary way with haemalum-eosin and van Giesen. In some cases the sections were specially stained with Giemsa, etc., for bacteria.

Lesions in the Intestines of Young Lambs Experimentally Infected with Oesophagostomiasis. (Veglia's material.)

Control Lamb No. (2). *Specimen No.* 6569.

This lamb was not infected. The intestine had a normal appearance micro- and macroscopically. A careful examination was made for the presence of oesinophiles in the mucous membrane. Very few of these cells could be identified therein.

12 *Hours after infection.*

Lamb No. 31. *Specimen* 6570.—Small intestine—no parasites are seen either in or on the mucous membrane. Large intestine—no parasites are seen in the mucous membrane, but in some sections the parasites are seen lying on the mucous membrane. The larva in this case must be just on the point of entering the mucous membrane (see Plate I) or what is more likely is that a portion of the parasite has already entered the mucosa and that which is seen as apparently lying on the mucosa is merely a cross section of that portion of the larva which has not yet passed into the mucosa. If the larva is merely lying free on the mucous membrane it would probably not have remained there during the process of cutting and the manipulation of the sections during staining etc.

PLATE I.—Specimen 6570. 12 hours after infection

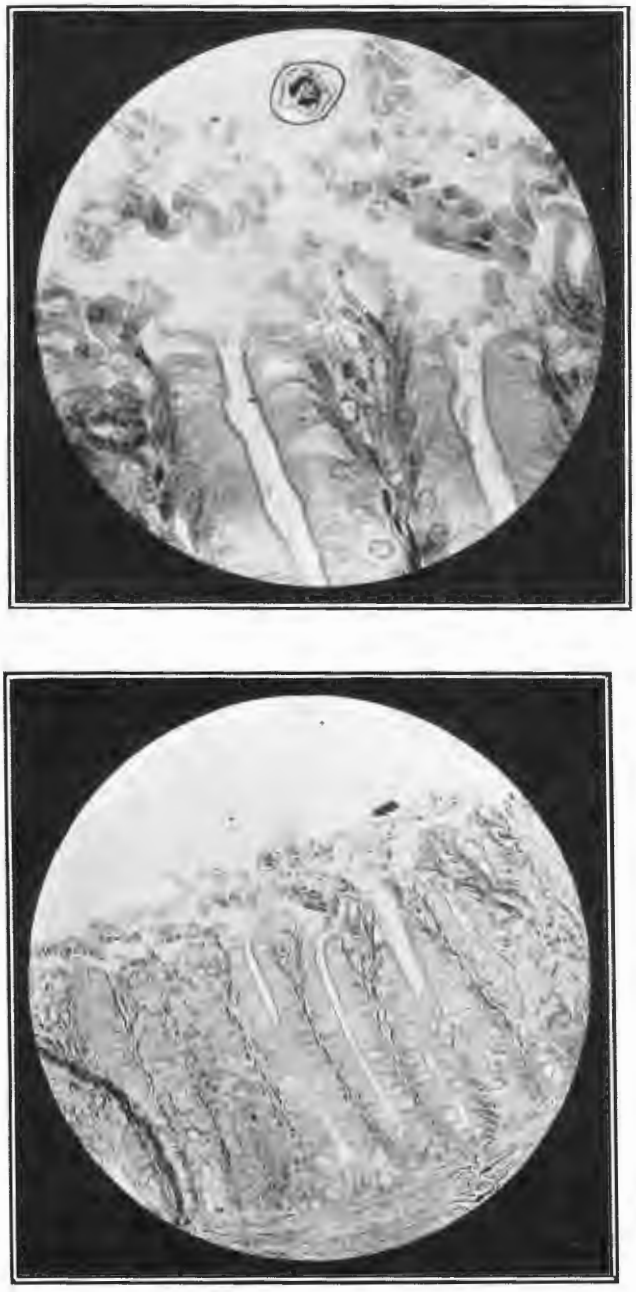


Fig. 1 (×150) and Fig. 2 (×450).—Larvae are just on the point of entering the mucous membrane of the large intestine.

As far as can be made out from a very careful microscopic examination of serial sections from the small and large intestine no evidence of any disturbances could be found.

24 *Hours after infection.*

Lamb No. 39. *Specimen* 6571.—Small intestine—Duodenum. There is very marked hyperaemia. The vessels are distended with blood. In some places the villi are markedly infiltrated with red cells. There is in addition a certain amount of desquamation of the cells of the mucous membrane. These lesions are possibly the early stages of a haemorrhagic enteritis (Plate II, Fig. 1 and 3), and probably directly or indirectly due to the effects of what can be conveniently described as primary parasitic migration, that is migration which is taking place when third stage larvae enter the mucous membrane and until they become encysted under the muscularis mucosae (i.e. against the muscularis mucosae and on its mucous membrane side). Many larvae are present. These are coiled up against the muscularis mucosae. This lesion has already appropriately been described by Veglia (1923) as a cyst. A very fine membrane forms the cyst wall which surrounds the larva. In addition to the larva one can recognise well preserved red cells in some cysts. (Plate III, Figs. 1 and 2) whilst in others there is a structureless (pink staining with eosin) fluid (Plate IV, Figs. 1 and 2). Evidence of inflammation or of bacteria was never seen within the cysts.

The question now arises as to how the cyst wall is formed. Goodey (1922) points out that the sheath of ensheathed larvae is produced by the old cuticle which is replaced by a new one developing underneath it. The possibility must therefore be considered that the old sheath of the larva actually forms the wall of the cyst. This would be the most economical means of supplying itself with this apparently protective covering. It has already been shown that the parasites are on the point of entering the mucous membrane 12 hours after infection and that many larvae are encysted 24 hours after infection. If a certain amount of time is allowed during which the parasite is making its way through the mucous membrane to become encysted under the muscularis mucosae, the cyst wall must in many cases be completed within 12 hours' time. It would be remarkable if the tissues would undertake this service so promptly on behalf of the parasite against its own interest. From the available histological evidence it would nevertheless seem, that this is actually what takes place. The cyst wall stains with 1 per cent. methyl-green and with haemalum eosin. With van Giesen it stains a light pink colour. Under oil emersion and with the light slightly cut off, the cyst wall has a granular appearance in places. Striation which is characteristic of the structure of the skin of, amongst others, nodular worm larvae, was never seen in the cyst wall, although it should be mentioned that in sections, one frequently fails to identify striation in the skin of encysted nodular worm larvae. On structural grounds one cannot differentiate the two on clear-cut lines. In some cysts there are numerous red cells between the coils of the larvae. If the cyst wall is formed by the outer skin of the parasite, then these red cells must be between the two skins, which would seem to

PLATE II.—Specimen 6571. 24 hours after infection.

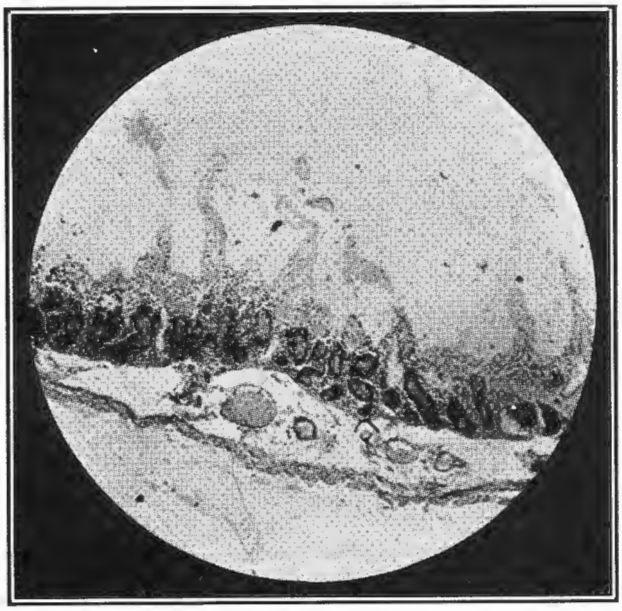


Fig. 1 (50×).—Duodenum haemorrhagic infiltration of mucous membrane.

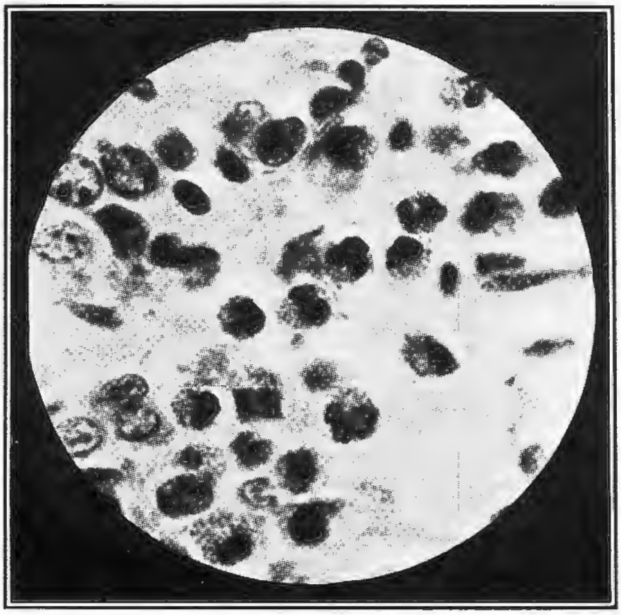


Fig. 2 (1200×).—Eosinophile infiltration of mucous membrane.

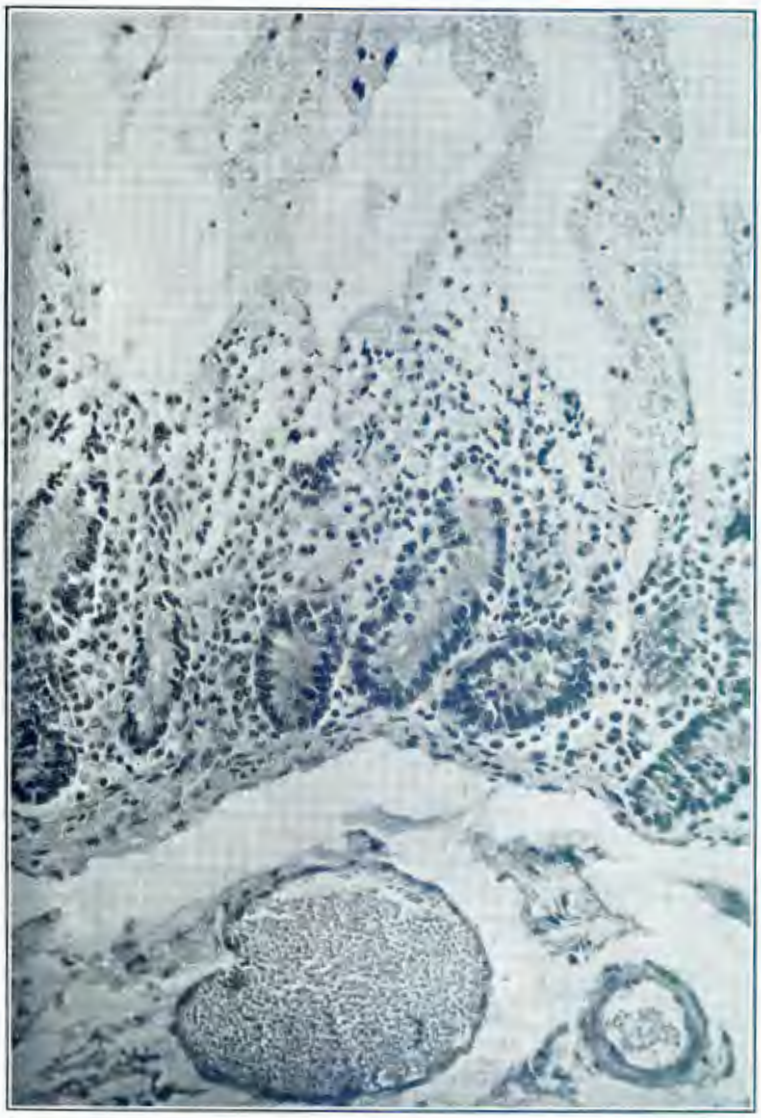


Fig. 3 (270×).—As Fig. 1, showing hyperaemia and haemorrhagic infiltration of villi.

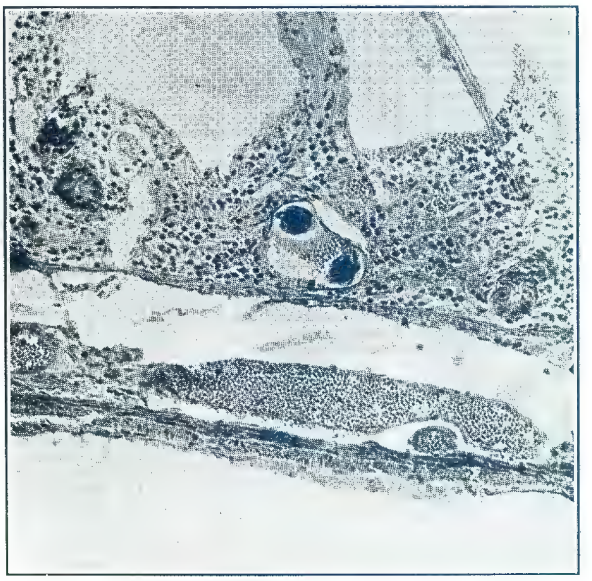
be an exceedingly unlikely, if not an impossible eventuality. Even if the red cells should have been ingested by the parasite, they would not be present between the two skins, but in the alimentary canal of the parasite. Veglia (1923) further points out that the second ecdysis which occurs in the first parasitic stage of the socalled third stage larva is completed in the lumen of the alimentary canal of the sheep, very soon after the parasite is ingested. Thus having cast

the old skin, it seems very unlikely that it could, within 24 hours, release the new skin to form a cyst wall. All the available evidence points to the formation of the cyst wall by or from the tissues.

Feng (1931) believes that in *Physaloptera clausa* infection of hedgehogs and *Physaloptera caucasica* infection of monkeys, the worms produce a secretion which causes liquefaction of the tissues. Spindler (1933) believes that such a liquefaction or more precisely a coagulative necrosis of the tissues may also be caused by the *Oesophagostomum longicaudum* in pigs and that this homogeneous tissue actually forms the cyst wall. As the parasite only remains encysted for a short time (4-5 days according to Veglia) it may be advantageous to have a protective covering which is not too strong. If such an envelope consists of dead tissue, this may automatically disintegrate in a few days time and release the parasite to complete its development. The great objection to this view is the fact that the cyst walls consist of such well-defined, albeit delicate thread-like structures, that it is hard to believe that a process of coagulative necrosis can produce them. Histological evidence which may to some extent support Spindler's contention is furnished by a cyst which is reproduced in Figs. 1 and 2, Plate VI. From the walls of this cyst one sees in places what appear to be hooklike processes projecting into the cyst cavity. If there was a mass of liquefied tissue which the parasite pushed out in the form of a cyst wall, one can well imagine that some of this substance could flow or be forced between the coils of the larva and so produce these hooklike processes.

However on careful examination with the higher magnifications one can differentiate what can be described as the membranous cyst wall, as a well-defined delicate thread running round the hooklike process. In addition the two stain differently with van Giesen, and the structureless material inside the cyst cavity can similarly be differentially stained. However too great importance should not be attached to this, as the material inside the cysts may be present as a thin layer, as a result of which it may stain more lightly than the thicker cyst wall. The exact nature of the structureless material which is present in some cysts (Plate IV, Figs. 1 and 2) has not been determined. If it is not the result of a process of liquefaction, is it secreted by the parasite, or by the wall of the cyst? Some cysts as already clearly shown undoubtedly contain red cells and it seems very likely that the structureless material is the remains of these red cells after they had become haemolysed. In view of the foregoing the question of the formation of the cyst wall must for the time being remain open. In the case of one cyst (5 days after infection, Fig. 3, Plate IV) a nucleus resembling that of a fibroblast seems to be present in the cyst wall and the possibility that it may consist of some form of connective tissue cannot be entirely excluded. It is rather remarkable that in all cases the larvae become encysted immediately against the muscularis mucosae and that encysted larvae are never seen in the wall of the intestine deeper than the muscularis mucosae. It seems very likely that this structure merely offers a convenient mechanical barrier to the progress of the larvae and as there is no particular reason why they should go further, they become encysted there.

PLATE III.—Specimen 6571. 24 hours after infection.



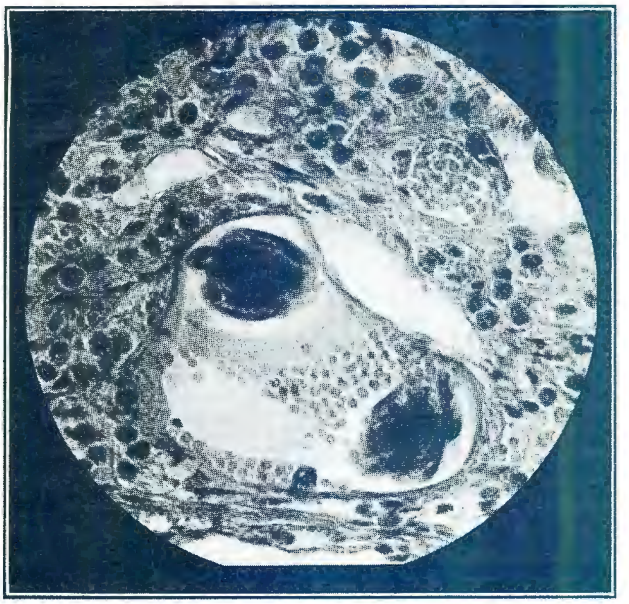


Fig. 1 (×150) and Fig. 2 (×450).—Duodenum. Larva encysted under muscularis mucosae.

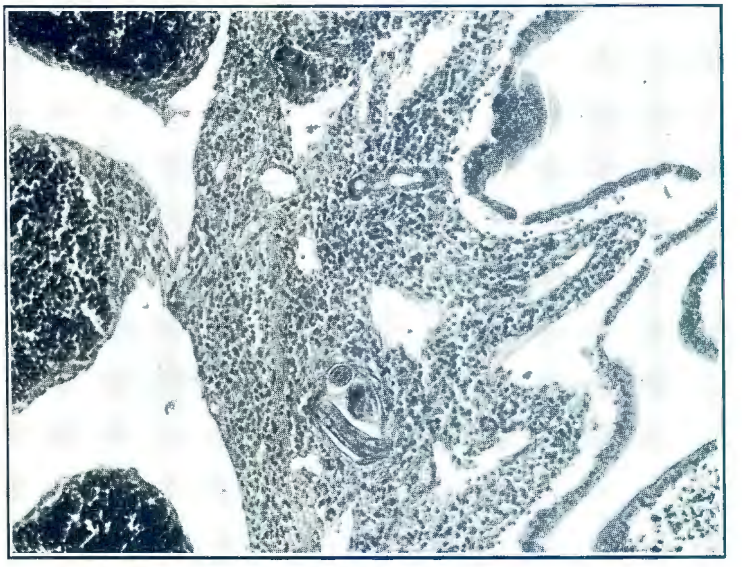
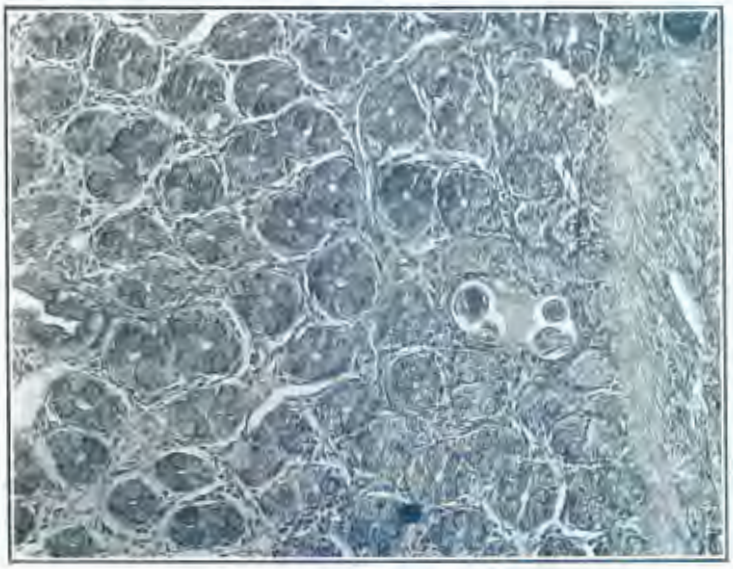


Fig. 3 (150×).—Encysted larva under muscularis mucosa—terminal portion Ileum.

Already at this early stage abnormal numbers of eosinophiles are present in the mucous membrane. Plate II, Fig. 2. Many of these cells seem to have round nuclei, but one hesitates in making a definite statement to this effect, as the cells may be lying in such a way that one may not be able to see the other lobes of the nuclei. If one is actually dealing here with eosinophile myelocytes, they must have developed locally from lymphoblasts (hemocytoblasts of the unitarians). Maximow 1923(b) quoted by Maximow (1928) showed that lymphocytes may even in tissue culture be induced to differentiate into amongst others eosinophile myelocytes. It seems very unlikely that the bone marrow could react so quickly that these cells could function in the intestine within a period of 24 hours. If it is merely a question of the mobilization of the eosinophiles, one can understand their rapid withdrawal from the blood, but then one could not be dealing with myelocytes. These cells occur free and not in epithelial cells and were therefore not confused with the " Schollenleukozyt " described by Weill, quoted by Keasbey (1923).

These eosinophiles are diffusely present almost throughout the mucous membrane but seem to be more numerous in the neighbourhood of larvae. Seeing that the animal was exposed to a gross infection one can expect that parasites are entering the mucous membrane almost along the entire line of the intestine, this probably explains their diffuse distribution. In the control uninfected lamb, hardly any eosinophiles are present. Similar lesions are present in the Ileum, (Plate III, Fig. 3). Here also numerous eosinophiles are seen to be diffusely present throughout the mucous membrane. This portion of the intestine does not contain lesions like those of the early stages of a haemorrhagic enteritis to the same extent as the duodenum.

PLATE IV.—Specimen 6571 (Figs. 1 and 2). 24 hours after infection.



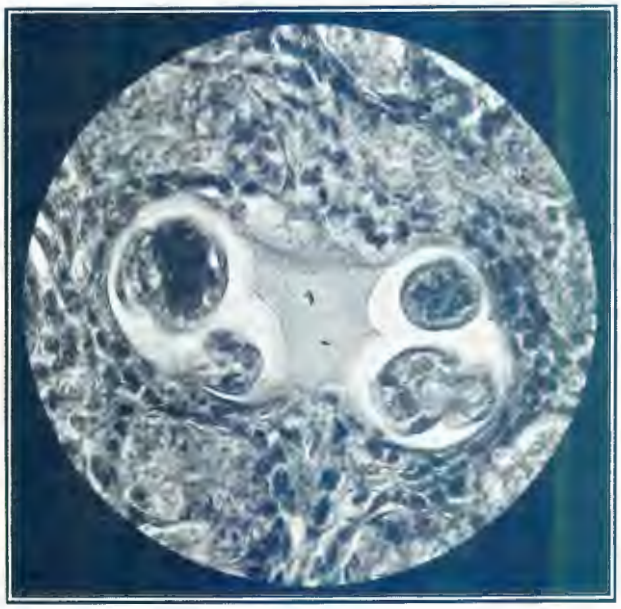


Fig. 1 (150×) and Fig. 2 (450×).—Large intestine. Larva encysted under muscularis mucosae.

Fig. 3, Spec. 6578. 6 days after infection. (1500×).—1 nucleus fibroblast, 2 cyst wall, 3 cyst cavity, 4 portion of encysted larva.

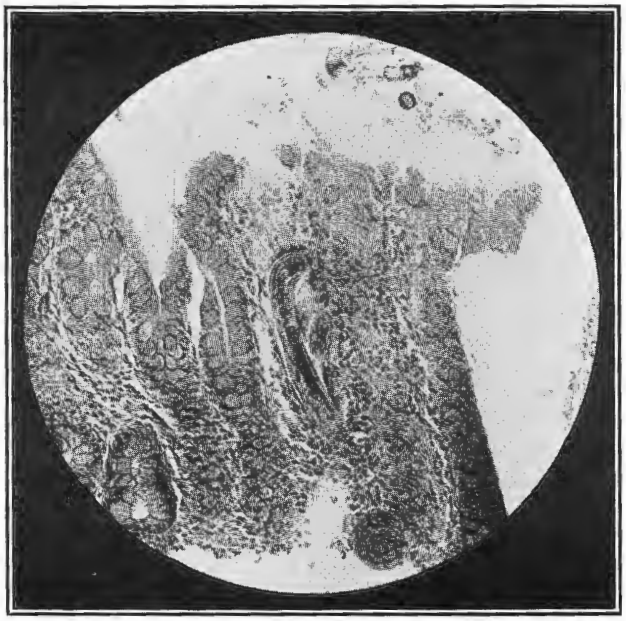
Large Intestine.—As the encysted larvae lie coiled up against the muscularis mucosae, they are cut in several places in cross section. This is clearly seen in Plate IV, Figs. 1 and 2. Immediately around the cyst wall there are quite numerous eosinophiles. This would seem to be quite remarkable if it is remembered that the larvae had probably only ventured into the mucous membrane during the previous 12 hours. In Plate V a larva is seen more or less in longitudinal section. It is not possible to make out definitely which is the cranial portion of the parasite. However, it would seem that the larva is entering the mucous membrane between two glands. The presence of a considerable number of eosinophiles is very definite evidence that the tissues are resisting this invasion; not only immediately around the parasite are numerous eosinophiles, but these cells are also prominently present in the tissues some distance away from the parasites. Similar lesions are present in the small and large intestine of another lamb No. 36 destroyed 24 hours after infection.

48 *Hours after Infection.*

Specimens from three different lambs were available for examination.

No. (1). *Lamb No.* 129. *Specimen* 6572.—Encysted larvae are present under the muscularis mucosae, whilst others are still migrating apparently on their way to the muscularis mucosae. In this particular lamb the eosinophile reaction, although present, is not so well marked as in the previous case.

PLATE V.—Specimen 6571 (Figs. 1 and 2). 24 hours after infection.
Specimen 6574 (Fig. 3). 3 days after infection.



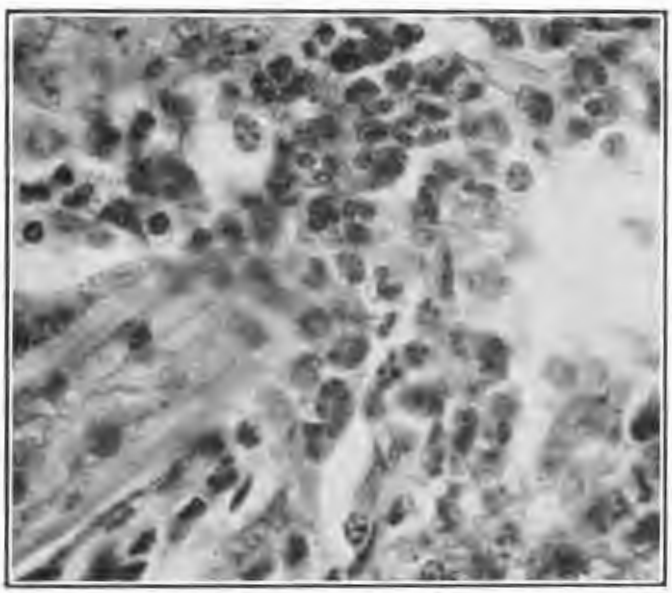


Fig. 1, 6571 (150×) and Fig 2, 6571 (850×).—Larva entering mucous membrane between two glands. Eosinophile infiltration around parasite.

Fig. 3, Spec. 6574 (250×).—Empty cyst 3 days after infection.

No. (2). *Specimen* 6912—*Additional Lamb.*—Whilst no parasites are seen in sections examined from the jejunum, ileum, caecum and ansa spiralis, parasites are found on the mucous membrane apparently on the point of entering the duodenum, the junction of the caecum and ansa proximalis and the rectum. Eosinophiles in appreciable numbers are present throughout the mucous membrane of the small and large intestine. This eosinophile reaction is probably due to the presence of larvae which had already entered the mucous membrane on their primary parasitic migration, although no such parasites could be demonstrated in the depth of the mucous membrane and no encysted larvae were found in hundreds of sections examined. The method of infection will probably influence to a considerable extent the rapidity with which the parasites pass through their various developmental stages. If the larvae must first of all enter the rumen, it will take them longer to become encysted than if they should pass through into the abomasum and this may possibly explain the apparently delayed development in this lamb.

No. (3). *Lamb No.* 38. *Specimen* 6573.—No parasites were found in any of the serial sections examined. With the exception of a catarrhal enteritis in the ileum no lesions were recognised in the small or large intestine.

3 *Days after Infection.*

Lamb No. 18. *Specimen No.* 6574.—Encysted larvae are present against the muscularis mucosae of the large intestine. In the vicinity of the cyst are scattered eosinophiles. In some places empty

cysts are seen (Plate V, Fig. 3). The ruptured end of the cyst is immediately opposite the muscularis mucosae. On following a sort of track which is present between the mucous membrane and the muscularis mucosae, portions of a migrating larva, which could possibly have migrated from this cyst, are found. The larva was unfortunately cut in such a way that it could not be definitely identified as a fourth stage larva. From this it would appear that what will be described as secondary parasitic migration in this paper, can sometimes take place considerably earlier than 5 days, which according to Veglia (1923) is the time when the fourth stage larvae usually emerge from their cysts.

4 *Days after Infection.*

Lamb No. 36. *Specimen No.* 6575.—No parasites were found in the sections cut from the first portion of the small intestine. In the ileum numerous encysted larvae are present under the muscularis mucosae. The cysts are now increased in size and can readily be picked out under an ordinary dissecting microscope with a magnification of 16. The larvae can only grow if they have sufficient available food material. It seems unlikely that they can build up sufficient reserve material during the short time of their primary parasitic migration, for their growth while they are encysted. If such reserve materials are not available the larvae probably make use of red cells and possibly other substances present within the cysts for food. In some of the sections photomicrographs of which are reproduced (Plate VI) the cyst wall is clearly seen to consist of a

PLATE VI.—Specimen 6575. 4 days after infection.

Fig. 1 (150×).—Encysted larva. Ileum.

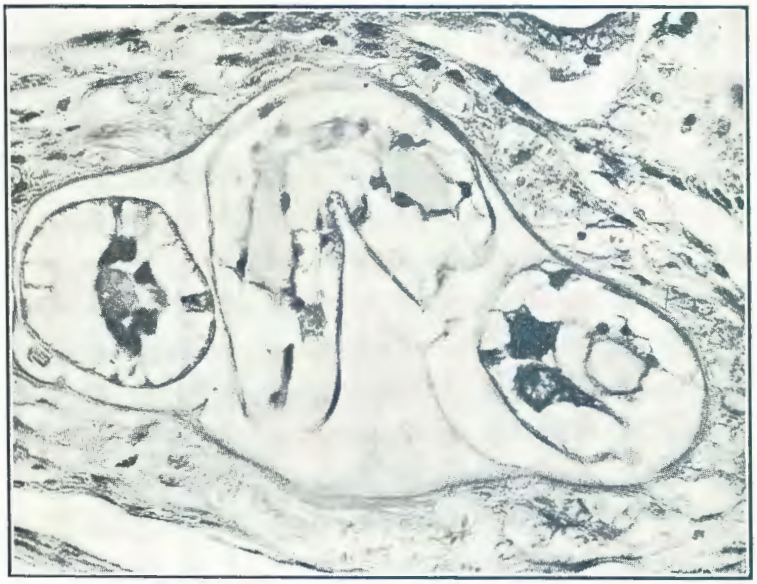


Fig. 2 (450×).—As Fig. 1.

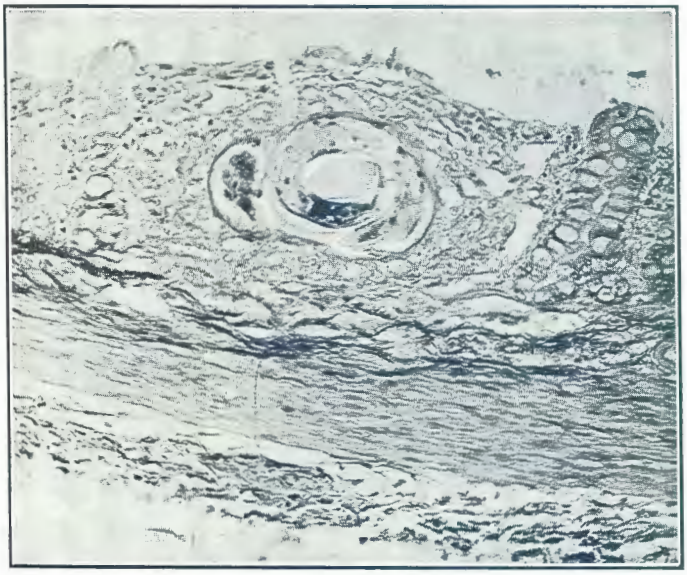


Fig. 3 (150×).—Encysted larva. Large intestine.

fine thread like filament, which at this stage can be differentiated from the outer skin of the parasite by van Giesen's staining. The outer skin of the parasite takes on a yellowish colour whereas the cyst wall stains a light pink colour. The glandular epithelium of the large intestine is in places completely replaced by the cyst and by a zone of tissue which has formed around the cyst. This zone of tissue consists of scattered eosinophiles, fair numbers of round cells, but mainly of cells having large vesicular or spindle shaped nuclei, which are regarded as fibroblasts, amongst which a certain number of epithelioid cells may also be present. This probably represents a type of granulation tissue, which already at this early stage seeks to encapsulate the parasite. This cellular zone around the cyst varies in depth, as it consists of only a few rows of cells in the case of some cysts, whilst in the case of others an appreciable zone of tissue is formed around the cyst.

Numerous serial sections were examined from various portions of the gut and in not a single one was any evidence found that larvae had penetrated the muscularis mucosae.

5 *Days after Infection.*

Lamb (*no number*). *Specimen* 6577.—Many encysted larvae are present against the muscularis mucosae. In Fig. 1, Plate VII two larvae are shown encysted side by side. If the tissues are responsible for the cyst wall, it seems strange that they did not include these two larvae with their associated cyst contents within one cyst wall, there is, however, a possibility that the two larvae became encysted at different times. The cyst generally resembles that which is seen four days after infection. In serial sections from another portion of the large intestine a portion of a larva is seen more or less in longitudinal section, Fig. 2 Plate VII. The head end is against the muscularis mucosae and causes a bulging of it towards the submucosa. Judging from the mouth capsule etc. the structure of this larva conforms to the structure of the fourth stage larva described by Veglia (1923). According to Veglia, Oesophagostomum larvae are, during this time, completing the third ecdysis but they had not yet migrated back to the lumen of the intestine. This larva must have emerged from the cyst and is now migrating as the fourth stage larva. This can be conveniently described as secondary parasitic migration, in contradistinction to what has already been referred to as primary parasitic migration.

6 *Days after Infection.*

Lamb No. 16074 (*Own case*). *Specimen No.* 6578.—Numerous encysted larvae are still present against the muscularis mucosae (Plate VIII). The cysts themselves vary a good deal in appearance. Some have a well-defined delicate threadlike membranous wall (Fig. 4) with very few cells ordinarily associated with granulation tissue and capsule formation, whereas others have in addition a zone of tissue consisting of several rows of cells (Fig. 3). The cells of the central rows, i.e. immediately around the parasite have large vesicular nuclei, some of which are almost elliptical in shape, others more or less round. The cells of the peripheral rows have nuclei

PLATE VII.—Specimen 6577. 5 days after infection.

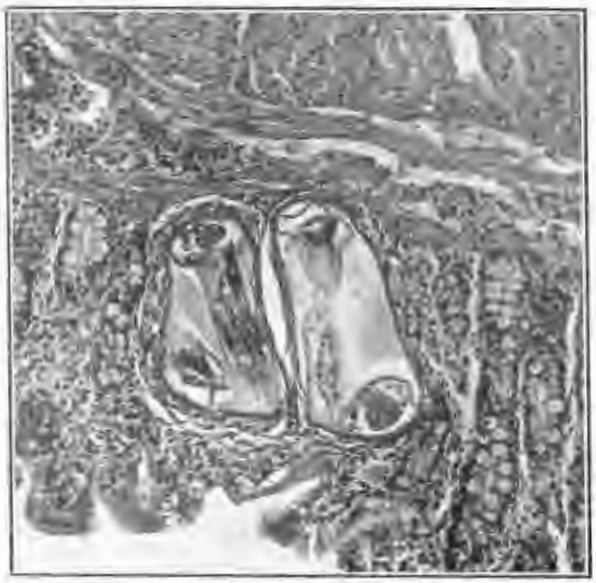


Fig. 1 (150×).—Encysted larvae. Large intestine.

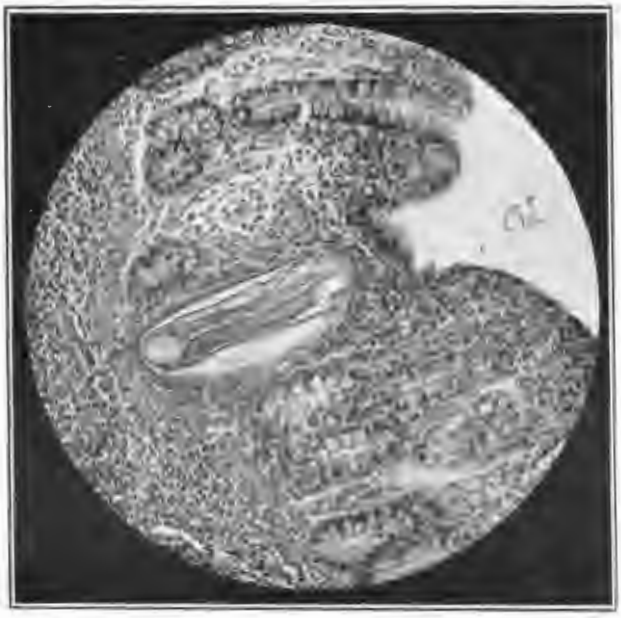


Fig. 2 (150×).—Migrating 4th Stage larva.

which are spindle shaped. These cells are interpreted as being mainly fibroblasts and the tissue is regarded as granulation tissue, which is building a capsule around the parasite. This difference in the structure of the cysts may be due to: (1) a difference in age. It is almost certain that even in cases where infection takes place at the same moment, some larvae will complete their development before others. (2) For some unknown reason, the tissue reactions to individual cysts may vary. It seems unlikely that there would be an inherent difference in the cysts themselves, but possibly the kind of tissue where they become encysted, may influence the type of reaction which is produced.

In places there are empty spaces from which the larvae have migrated. In addition to migrating larvae of the fourth larva or second parasitic stage, the nodules which are produced by some of them are now for the first time seen. (Plates VIII and IX). The nodules are present mainly in the submucous tissues, but some are present in the submucous as well as the mucous tissues. In the earlier sections of the series, one sees merely a more or less circumscribed accumulation of eosinophiles, but in later sections of the series portions of the larvae are easily recognised. In Fig. 1 Plate IX the nodule is seen to occupy the entire mucous membrane and penetrates the muscularis mucosae to extend into the submucous tissues. Here the larva is seen cut more or less longitudinally and details in its structure are easily recognised. The head end lies in the submucosa and is directed away from the mucous membrane. The entire parasite is closely surrounded by infiltrating cells amongst

PLATE VIII.—Specimen 6578. 6 days after infection.

Fig. 1 (75×).—Encysted larvae and young nodule in submucosa.

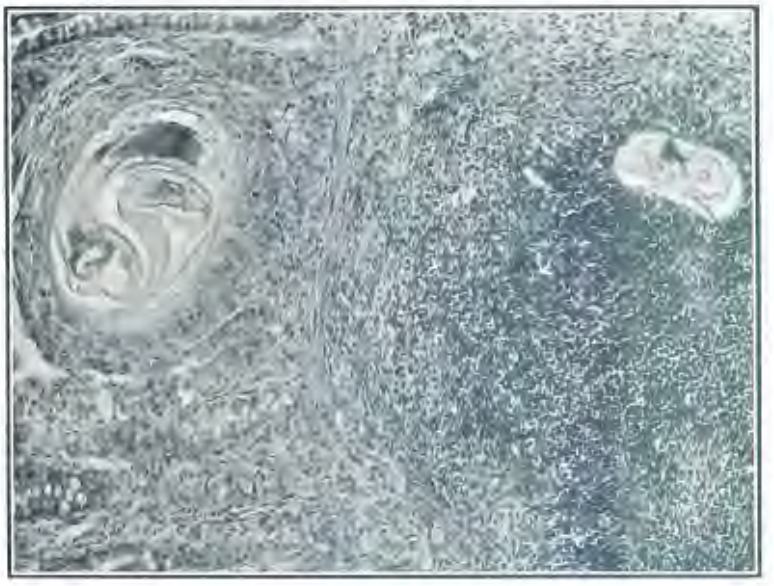


Fig. 2 (150×).—As in Fig. 1.

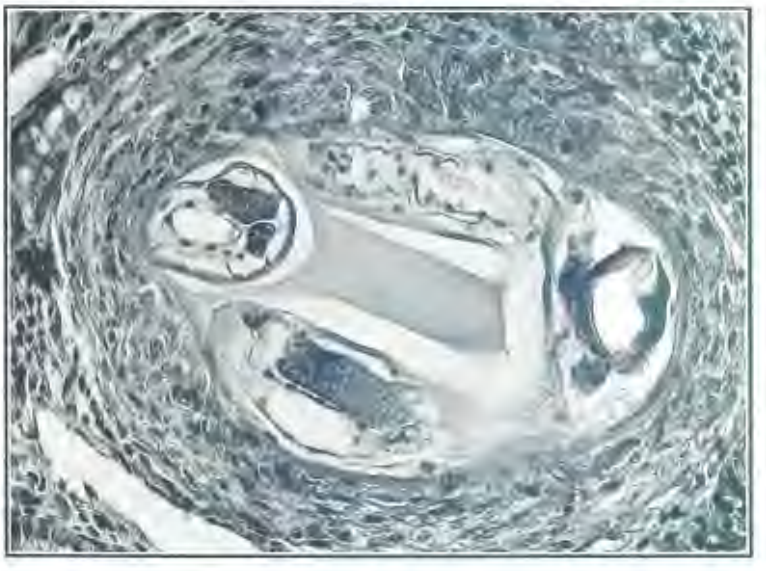


Fig. 3 (300×).—As in Fig. 2. Well developed capsule.

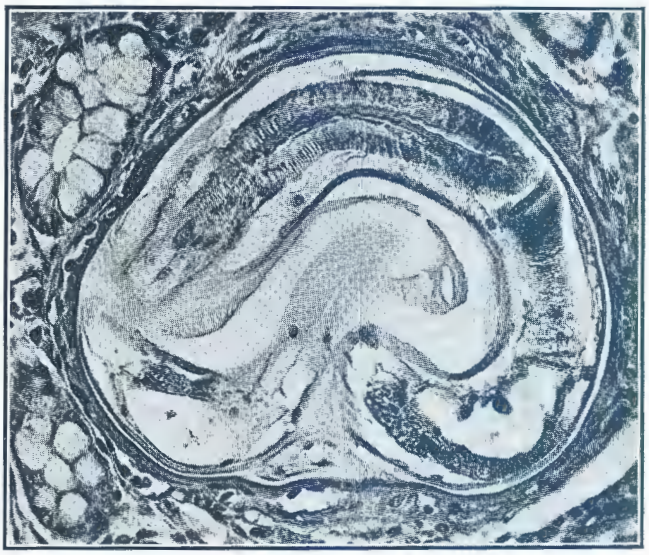


Fig. 4 (300×).—No capsule formation.

PLATE IX.—Specimen 6578. 6 days after infection.

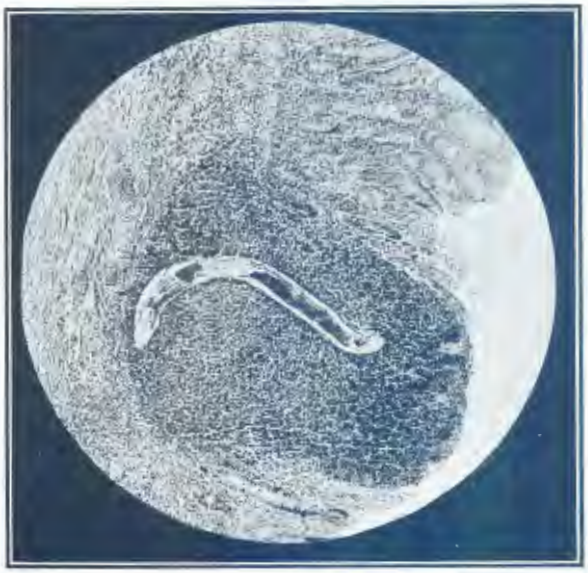


Fig. 1 (75×).—Worm nodule in mucosa and submucosa.

Fig. 2 (150×).—As Fig. 1.

which many eosinophiles are present. In other sections where a larva is cut mainly through its cranial portion, a group of polymorpho-nuclear cells is seen lying at the entrance to the oesophagus. Only the nuclei of these cells can be identified. They are regarded as eosinophiles which the larva had ingested, as there is no reason to suppose that these cells could have been accidentally deposited here in such a regular group during the process of cutting. The early nodules as seen in these sections consist of the parasite with masses of infiltrating cells, the great majority of which are eosinophiles. In addition to such circumscribed accumulations of eosinophiles around the migrating larvae, there is also a diffuse infiltration of eosinophiles throughout the mucous membrane. This must be looked upon as an expression of the defensive mechanism of the intestine, which is being mobilized against the parasites.

7 *Days after Infection.*

Lamb No. 19. *Specimen* 6579.—In quite a number of places there are bloody tracts in the mucous membrane. These may extend to the surface of the mucous membrane, where the parasite has passed out into the lumen of the gut. Migrating and encysted larvae are present in the mucous and submucous tissues of the large intestine. In the case of the ileum larvae are seen wandering in the interstitial tissues of the follicles in Peyers patches, Plate X, Fig. 1.

PLATE X.—Specimen 6579. 7 days after infection.

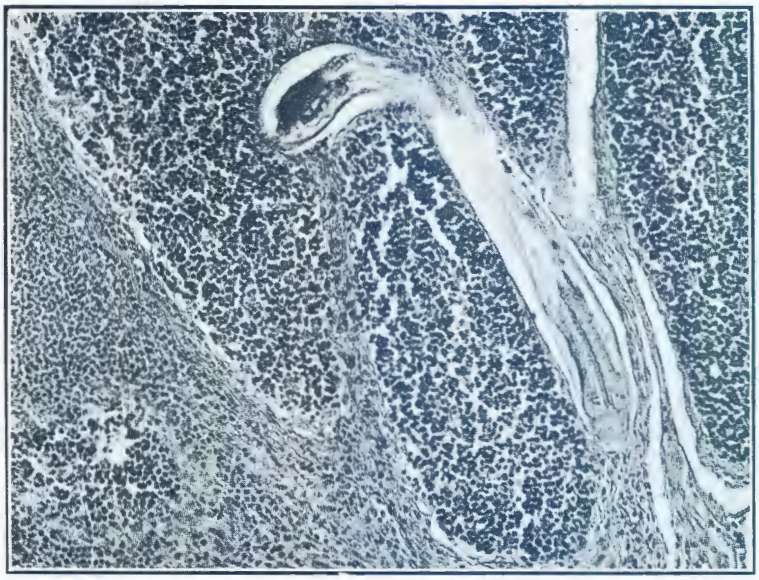


Fig. 1 (150×).—Migrating larva in follicle of Peyers Patch.

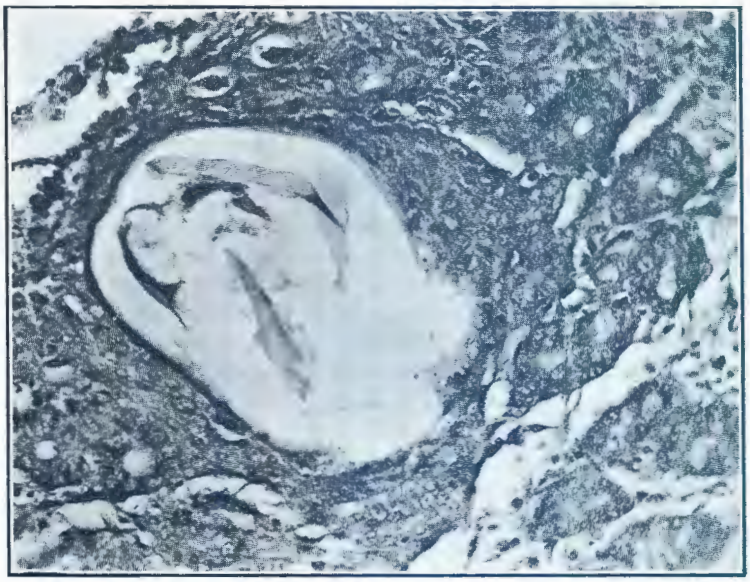


Fig. 2 (225×).—Empty cyst.

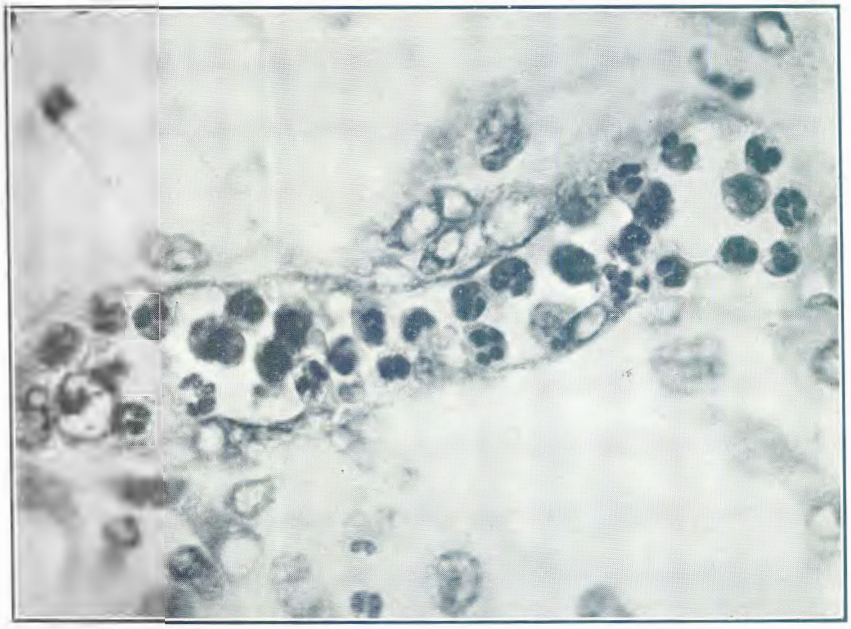


Fig. 3 (1000×).—Blood vessel in submucosa containing numerous eosinophiles.

Fig. 4 (650×).—Showing cast striated skin.

The migrating larvae especially in the submucous tissues are closely surrounded by infiltrating eosinophiles, forming typical young worm nodules as in the previous case six days after infection. Eosinophiles are also diffusely distributed in the mucous membrane. Although the available evidence is inconclusive there would seem to be a possibility that adult worms and to a less extent fourth stage larvae, in the lumen of the intestine, may induce a diffuse eosinophilia in the mucous membrane. Further, eosinophiles are seen to be the dominating cells in certain vessels in the submucosa (Fig. 3, Plate X). These are undoubtedly blood vessels as they contain red cells in addition to the eosinophiles. They are not capillaries, as they are far too wide and can easily accommodate 3 or 4 eosinophiles side by side. Neither are they arterioles as their walls are much too thin. Furthermore, if they were arterioles one must assume that there is active haemopoiesis in the myeloid tissues, from which the eosinophiles were released into the circulating blood. Judging from the preponderance of eosinophiles in these vessels, the vast majority of the leucocytes in the blood ought in that case to be eosinophiles, a condition not yet encountered to such a marked degree in the fairly extensive haematological observations made in cases of oesophagostomiasis. One is therefore forced to the conclusion that these vessels are actually venules, which are draining an area where active secondary migrating larvae are present or from which such larvae are disappearing. When the eosinophiles are passed into the general circulation their numbers will relatively be very much decreased, but they may still be sufficient to produce a transitory eosinophilia. Later, however, they will disappear from the circulation. If this interpretation is correct, an eosinophilia in oesophagostomiasis is not so much an indication of an active tissue verminosis, but rather, that the tissue verminosis has been completed. In such cases one visualizes migrating larvae which penetrate the muscularis mucosae, wander about in the submucous and other tissues, where an intense eosinophilic reaction is elicited. Should they die and find their way back into the lumen of the gut, the eosinophiles may no longer be required for the purposes of defence and are released into the general circulation, from which they will later disappear. Hadwen (1925) had already at that time expressed the view that this may occur in cases of parasitism.

In some sections empty cysts are seen (Fig. 2 and 4. Plate X). The walls of the cysts are intact except at the one end where the parasite has broken through. The suggestion previously put viz. that the walls which were supposed to consist of dead tissue, will disintegrate to release the parasite, is not supported by the structure of this empty cyst, which does not differ from that of the inhabited cyst. When the parasite has reached the end of the first parasitic stage it casts its skin, the striated structure of which is easily recognised (Plate X, Fig. 4). The parasite then breaks through at one end; leaving, as already stated, the remaining portion of the cyst wall intact. The cyst wall, as a definite structural entity, is very clearly seen in the empty cyst reproduced in Plate XI, Fig. 1.

8 *Days after Infection.*

Lamb No. 3585. *Specimen* 6580.—Encysted larvae are only very rarely seen against the muscularis mucosae, but migrating

larvae and nodules produced by them in the walls of the intestines are frequently seen. The nodules are present mainly in the submucous tissues, but in places portions of the mucous membrane itself are etxensively involved, with the complete destruction of a considerable number of villi. In other cases the muscular layers are extensively replaced by eosinophiles. Some of the infiltrating cells have undergone necrosis but in places one can still recognise the polymorphic nature of nuclei of what are probably the remains mainly of broken down eosinophiles. Round cells are also present. This probably represents the early stages of caseation (Figs. 2 and 3, Plate XI), although it should be mentioned that in some of these centres showing commencing necrosis, quite a number of bacteria are present. They are bacilli, all apparently of the same type (Plate XI, Figs. 2 and 3). They are probably not putrefactive bacteria, as there are portions of the same section in which they are not present. (Decomposition can be definitely excluded as the lamb was destroyed and specimens collected in formalin almost immediately after death). It seems as if the migrating larvae have produced such severe changes in the mucous membrane that bacterial infection from the lumen of the bowel took place and one may then be actually dealing with a true inflammatory process in addition to the lesions produced by the worms themselves. The fact that neutrophiles are undoubtedly also present further supports this. However, to anticipate what will be shown later in this paper, it does not seem that bacterial infection is a regular complication of oesophagostomiasis, but seems especially liable to occur when migrating larvae produce extensive lesions in the mucous membrane. Occasionally empty cysts are seen under the muscularis mucosae (Plate XI, Fig. 1).

PLATE XI.—Specimen 6580. 8 days after infection

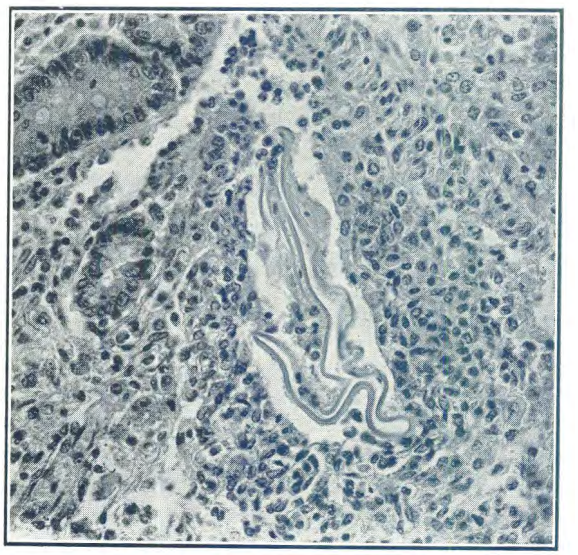


Fig. 1 (250×).—Empty cyst showing structural entity of cyst wall.

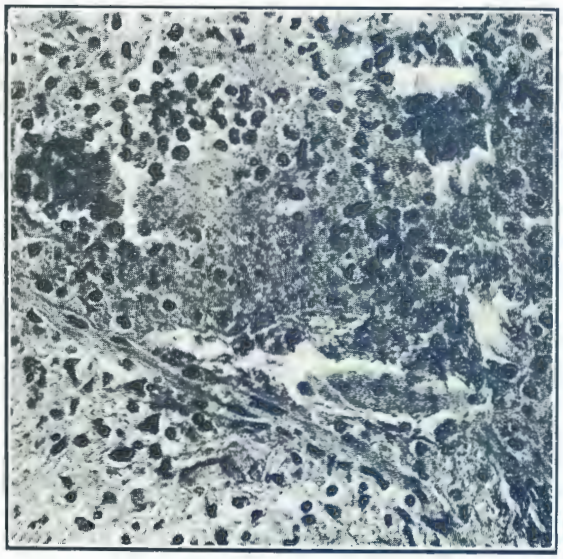


Fig. 2 (375×).—Commencing necrosis with bacteria.

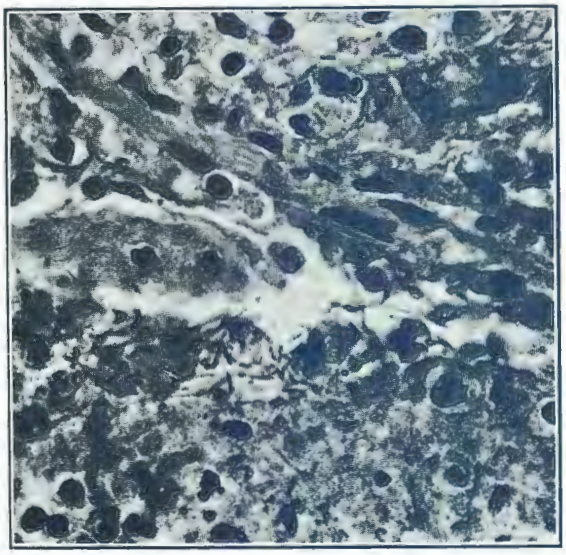


Fig. 3 (750×).—As Fig. 2.

12 *Days after Infection.*

(Two lambs.)

Lamb No. 1. *Specimen* 6581. *Large Intestine.*—Nodules of varying size are present in the mucous membrane, the submucous tissues, the longitudinal and circular muscular layers, and in some cases extending right up to the serosa, which seems to be on the point of being perforated. In these situations the normal tissue has been completely destroyed (Plate XII) and replaced by the nodules. In some places a nodule may be seen to extend from the muscular layers, through the submucosa, right to the surface of the mucous membrane, forming an ulcer which opens into the lumen of the gut.

The nodule at this stage has the following structure. In a few cases the cells (eosinophiles) which mainly form the nodules, are still seen more or less intact, but most of the nodules consist of a large central structureless mass, which stains more or less uniformly pink with eosin. In nodules from specimens which are cut and stained, soon after they are collected, the central structureless mass stains an intense pink colour with eosin, differing in this respect very markedly from the glanders nodule, the Preisz-Nocard abscess and the caseating tubercle. In places nuclear debris can still be seen and this is always in greater abundance towards the periphery of the nodule, where a well defined more deeply staining zone in which the nuclei of the cells are seen in varying stages of necrosis, is usually present. Here intact eosinophiles may be seen and where the living and dead tissues meet the cells of the tissue normal to the part are pycnotic.

PLATE XII.—Specimen 6581. 12 days after infection.

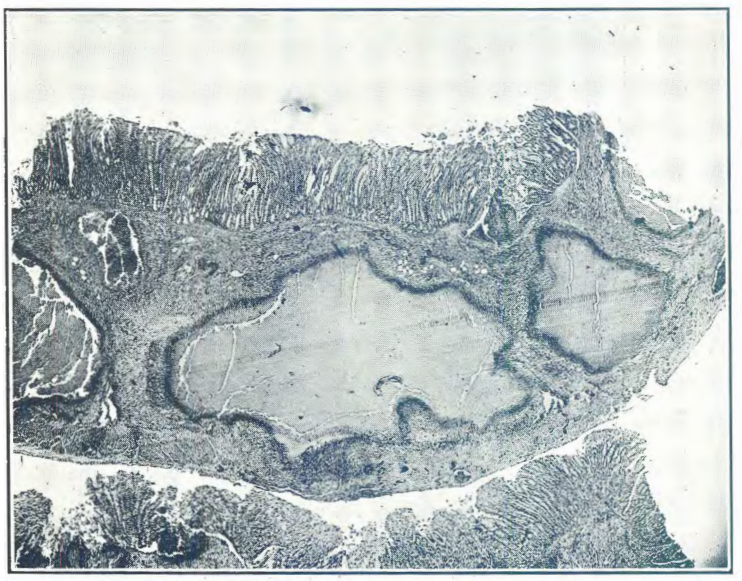


Fig. 1 (13×).—Deep caseous nodules, parasite in one of them. Ulceration of mucous membrane.

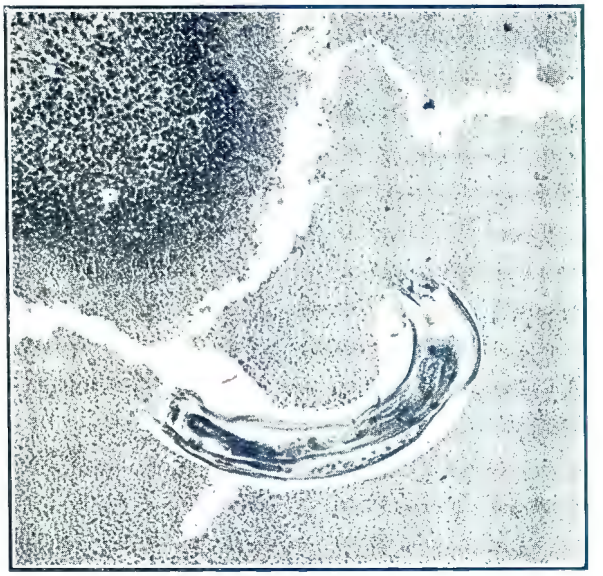


Fig. 2 (150×).—As in Fig. 1.

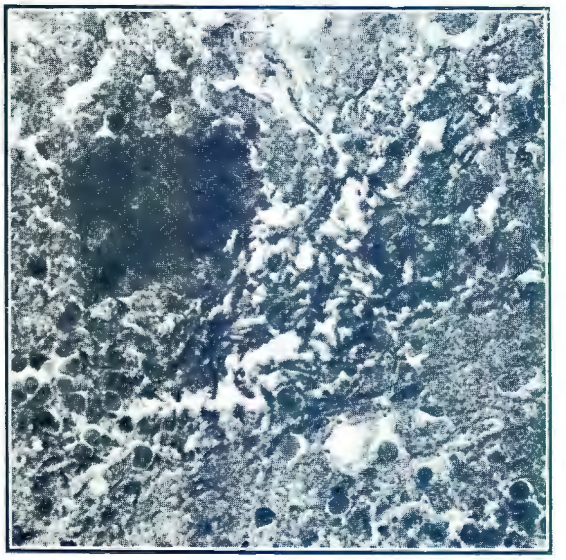


Fig. 3 (750×).—Another nodule, commencing necrosis, numerous bacteria.

In addition to the remains of broken down leucocytes, mainly eosinophiles, but in some cases probably also neutrophiles, one may find in the central caseating mass the following: —

(1) *Bacteria.*—These are present in most of the nodules. They may be scattered throughout the central structureless mass or may occur in groups towards the periphery of the nodule. These bacilli seem to be exactly like those previously described. They appear to be Gram positive rods, and here and there small bodies, resembling spores are present. These bacteria are present not only in the depths of ulcerating lesions, but also in nodules deeply situated in the submucosa, including the muscular layers of the intestinal wall. These bacteria probably gained entrance through the severe lesions produced by the gross parasitic infestation. It is extremely unlikely that the parasites carried them into the walls of the intestine, when they first entered the mucous membrane. If that were so, one would expect to find evidence of inflammation (regenerative or degenerative) in the walls of the intestine, when the parasitic invasion first takes place and when the cysts are formed. This is, however, not the case, and in the cysts themselves one never sees any bacteria or any inflammatory reaction. This is confirmatory evidence in support of the views of Carne and Clunies Ross (1932) who failed to find any Preisz-Nocard organisms in the nodular worm lesions of sheep, which were dosed *per os* with nodular worm larvae as well as with cultures of the Preisz-Nocard organism. Further it is of interest to note that these authors in examining, histologically and culturally, 50 well-developed lesions of oesophagostomiasis of the bowel wall of sheep, were able to identify gram-negative bacilli of the colon-typhoid type in two instances. If infection by paratyphoid bacilli was more common in sheep, one would have to consider the possibility of food poisoning in such cases of severe oesophagostomiasis. Fortunately, however, this does not seem to be the case. Mason (unpublished work) in making a very careful study (at this institute) of the intestinal bacterial flora of 23 sheep, was not able to isolate any paratyphoid organisms.

(2) *Irregular, darkly staining (with haemalium-eosin) particles.* These are regarded as the early stages of calcification, which is characteristic of the old nodules.

(3) *Parasites.*—The oesophagostomum larvae may or may not be present in the structureless central portions of the nodule. Sometimes they are found towards the periphery of the nodule. Several nodules in one field may be produced by different larvae or they may be produced by one migrating larva. It seems that larvae, having emerged from their cysts, can migrate through the mucous membrane of the intestine, without leaving much evidence of their presence. Sometimes an eosinophile infiltration of a greater or lesser degree may be produced, but this probably disappears very soon after the larvae have again returned to the lumen of the intestine. If, however, the migrating larvae penetrate the muscularis mucosae, then an intense reaction is elicited from the tissues concerned, in the form of an active eosinophilic infiltration. It is possible that such wandering larvae may again pass through the muscularis mucosae and the mucous membrane into the lumen of the gut finally to complete their life history. However, the

probability is that they will wander aimlessly about in the depths of the intestinal walls, and according to Carne and Clunies Ross (1932) and others may even be found in the mesenteric glands and such remote organs as the liver, having as it were lost their sense of direction. Most of them will be destroyed and it seems extremely likely that the macroscopic nodules so characteristic of nodular worm infection, indicate as stated by Mönnig (1934), some form of resistance on the part of the host to infestation and represent larvae which are being or have been destroyed, in their wanderings. On post-mortem examination, one sometimes finds very numerous adult worms in the lumen of the bowel when only a few nodules are present. This tends to indicate that in what can be described as the normal development of the parasite, viz. primary migration into the mucous membrane, encystment against the muscularis mucosae and secondary migration back into the lumen of the gut, without penetrating the muscularis mucosae—very little disturbance of a permanent nature is produced in the walls of the intestine. In the abnormal development of the parasite, instead of migrating back through the mucous membrane into the lumen of the gut after it emerges from the cyst, it penetrates the muscularis mucosae and elicits an intense tissue reaction as a result of which the nodules form. That something of this kind may take place is supported to some extent by the observation, which is not infrequently made on post-mortem examination, that numerous nodules are present, when no adult parasites may be found in the lumen of the intestine itself. However, in this connection one should not loose sight of the possibility, indeed even the likelihood, that in some of these cases, the adult worms may have been got rid of by medicinal treatment, as a result of diarrhoea, or may even have died of old age. It is more than likely, that there must be some definite factor (physical, biological, chemical) which induces the parasite to refuse the path of least resistance, from the muscularis mucosae, through the mucous membrane, to complete its development in the lumen of the gut and to prefer to penetrate the barrier, in the form of the muscularis mucosae, only to loose itself in the depth of the intestinal wall.

Towards the periphery of the nodule there is evidence of fibroblastic activity, and in some cases a good deal of fibrous tissue is present. In addition to the nodules themselves, the migrating larvae in this case inflicted considerable mechanical injuries to the intestinal walls, having produced large blood tracts in the mucous membrane the submucous and other tissues right up to the serosa.

(12 *Days after Infection—continued.*)

Lamb No. (2). *Specimen* 6582.—Large intestine. In portions of the intestine pronounced lesions are seen macro- and microscopically. The lesions may involve the entire thickness of the intestinal wall and in places there is actual perforation. In such cases there are multiple, discrete and circumscribed lesions which vary in size up to 2·5 mm. in diameter, on the serosa. (Plate XIII). Centrally there is a well marked pit or depression which is the actual hole where the bowel wall has been perforated. The borders of the lesions are prominent and raised above the surrounding normal tissue surface. The whole lesion has a rather characteristic elliptical shape. In places there is a fibrinous deposit on the serosa (fibrinous peritonitis).

PLATE XIII.—Specimen 6582. 12 days after infection.

Fig. 1.—Large intestine with perforation due to Oesophagostomum larvae.

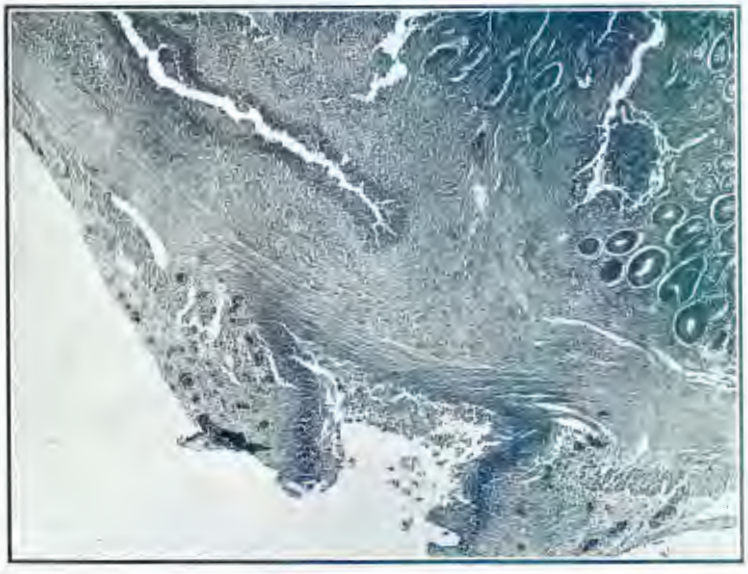


Fig. 2 (38×).—As Fig. 1.

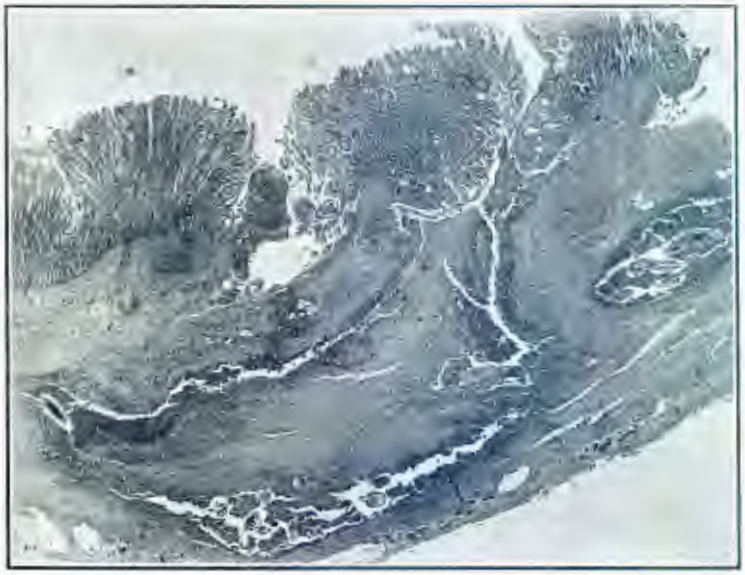


Fig. 3 (20×).—Nodule in wall of intestine, showing wandering larvae and ulceration.

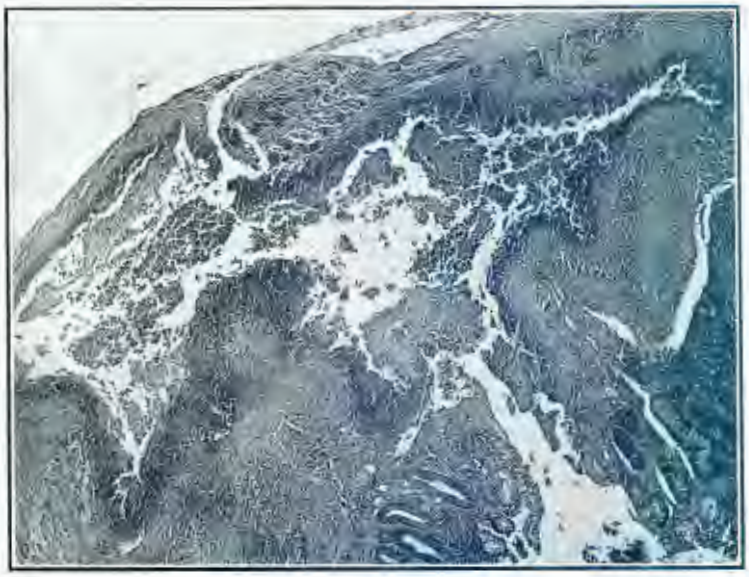


Fig. 4 (38×).—Tracks of wandering larvae and ulceration.

Microscopically the lesions are similar to those described for lamb No. (1), except that in these sections perforation of the intestinal wall is clearly seen (Plate XIII). The tracts of the wandering larvae in the submucosa, represented mainly by masses of eosinophiles, some of which are partially or completely disintegrated, appear in some sections to be quite independent of the tracts of the larvae which have produced the perforation. However, if one carefully follows out a complete series of sections, it is clearly seen that the tracts referred to communicate with one another and in this way it was possible to study the wanderings of the larvae from the mucous membrane through the submucosa and muscular layers, until complete perforation through the serosa has occurred. In such cases one must expect to find larvae in the peritoneal cavity. This observation was actually made by Veglia (1923), confirmation of which will be reported subsequently in this paper. The effect of such perforation would depend largely on the presence of bacteria which could have been introduced from the lumen of the intestine. In this case bacteria were seen to be present in the lesions, where perforation had occurred and it is more than likely that the fibrinous perintonitis already referred to was caused by these organisms. In one case, which will be referred to in greater detail later, when discussing the pathogenesis of the disease, the *bacillus pyocyaneus* was isolated in practically pure culture from the peritoneal cavity. In this case death was due to peritonitis as a result of perforation of the intestinal wall subsequent to infection with oesophagostomum larvae. At the moment a considered opinion as to the extent that peritonitis and other bacterial complications may be important factors in natural cases of oesophagostomiasis cannot be given. However, to judge from the fact that bacteria were repeatedly shown to be present in lesions of the disease during the course of this study, bacterial complications in oesophagostomiasis of sheep would seem to be rather more important, than is suggested by the admittedly very guarded statement of Taylor (1935) that " it seems highly probable that the injuries caused to the bowel wall by parasitic worms in general do not play any important part in bringing about bacterial infection from the lumen of the intestine ".

15 *Days after Infection.*

Lamb No. 64. *Specimen No.* 6583.—Nodules in which parasites may or may not be present, are seen in the small and the large intestine. They are situated in the mucosa, submucosa and in some cases large nodules measuring $1 \times \cdot 5$ cms. are situated beyond the inner circular muscular layer, completely replace the longitudinal muscular layer and cause a bulging of the serosa. In the mucous membrane there are actually ulcers present and in the caseous mass which completely replaces the glandular epithelium, parasites are seen, probably just on the point of passing into the lumen of the intestine. Therefore, it would seem that as long as the wandering larvae remain alive, they may at any time find their way back into the lumen of the intestine. Obviously it is impossible to devise experiments which would supply reliable evidence as to the proportion of larvae which may successfully migrate back into the lumen of the intestine, after having penetrated the muscularis mucosae. My own opinion is that the majority of them are destroyed when this occurs.

Numerous bacteria can be recognised readily on microscopic examination and in addition to eosinophiles, there are also numerous neutrophiles. In these cases one is therefore really dealing with a worm nodule, which is complicated with abscess formation. In order to get further information concerning the constituents of the nodules, smears were made from nodules present in sheep which passed through the post-mortem room at this institute in the ordinary way for routine post-mortem examination. In this way a number of smears were examined from fourteen different sheep. The smears were stained by Pappenheim's May-Grünwald-Giemsa method. Whilst in some cases there was complete destruction of the cellular elements so that they could not be differentiated in any way, in the case of others, the cells were easily recognised. In nearly all cases eosinophilies were present. In quite a number of cases there were, in addition, numerous neutrophiles, not infrequently bacteria were present. These included cocci and various types of bacilli, e.g. short rods, bipolar organisms, and organisms having terminal spores. This is confirmatory evidence that in quite a number of cases, the nodules are infected with bacteria, but in actual practice this does not occur to the extent which is suggested by the statement of Cameron (1933) that " Bacterial contamination is very common and the simple helminthic nodule becomes converted into a pyogenic abscess ". The usual practical experience is that animals showing symptoms of severe oesophagostomiasis make a remarkable recovery if the adult worms are removed by treatment, which cannot have any significant specific effect on any bacteria which may be present in the nodules themselves.

Towards the periphery of the nodule, granulation tissue is being formed, and in addition to fibroblasts there are some cells which resemble very strongly epithelioid cells. In some nodules a certain amount of fibrous tissue has been formed. In a number of places the blood vessels immediately around nodules are markedly distended. This may be due to a certain amount of mechanical interference on the part of the nodule, with the free venous drainage of the intestinal wall.

17 *Days after Infection.*

Lamb No. 3. *Specimen* 6584.—Small and large intestine—no encysted larvae were found. Tracts of wandering larvae are seen throughout the wall of the intestine. The nodules themselves show the presence of very extensive central necrosis. In this caseated mass cellular elements cannot be recognised, but the presence of calcium particles indicate the early stages of calcification. In a number of nodules numerous bacteria (cocci and bacilli are present. At the periphery of the lesions there is a rather darkly staining zone in which the nuclei of the cells are seen in varying stages of disintegration. The whole lesion is surrounded by granulation tissue, which in places has already formed adult fibrous tissue. In this tissue eosinophiles are present in abundance. In many of these nodules the wandering larvae are seen cut either transversely or longitudinally. They may be present in the central structureless

mass, at the periphery of the nodule or sometimes just outside of them. Up to now no definite evidence of dead parasites has been seen, but in this particular animal there are occasional bodies, in which the normal structure is greatly altered, and judging from their outline there is the possibility that they may be portions of the dead bodies of the parasites.

In the ileum, of which complete cross sections were made, well marked changes are seen in the lymph follicles and in the mucous membrane. In the case of the lymph follicles, the lymphocytes are conspicuous by their absence. Indeed it is only here and there that normal lymphoid tissue can be recognised in portions of the follicles. There are not really well defined lesions in the follicles, but the cellular elements seem to have been drained away and the tissue has undergone a kind of rarification as a result of which gaps have literally been left. On the other hand follicles of the control lamb contain dense masses of cellular elements. No definite statement as to the presence or absence of fluid (oedema) in the lymph follicles of the ileum can be made. Unfortunately the usual regional lymphatic glands in well-developed cases of oesophagostomiasis were not available for examination. In the circumstances it is not possible to say if these changes in the follicles of the ileum are definitely due to the effects of the nodular worms, but it is likely that this is the case. The question now arises as to whether the condition is one of lymphoid atrophy or of lymphoid hypoplasia. There is no evidence that the glands are decreased in size and except for the decrease in the number of lymphocytes, there are no recognisable structural changes. It is not known if the glands are producing lymphocytes or other cells at an increased or decreased rate. If there is hyper-activity in the glands, there should be a lymphoid hyperplasia or hypertrophy, but such compensatory processes may be inhibited or controlled by toxins from the parasites. There is a possibility that toxins may interfere with the normal production of lymphocytes and if that is the case, the condition would be one of lymphoid hypoplasia, rather than that of atrophy of the glands, especially in view of the absence of definite evidence that the glands are decreased in size.

In the case of the mucous membrane there is very extensive desquamation of catarrhal cells, which are easily identified in the exudate in the lumen of the intestine in complete transverse sections. This is interpreted as an acute catarrhal enteritis, probably caused by the parasites after they have returned to the lumen of the intestine at the completion of secondary migration. (Plate XIV.)

In one place a wandering larva is seen in a mass of structureless material, which completely replaces the mucous membrane there. (Plate XIV, Fig. 1.) Its head is just free of this material and it appears to be on the point of entering the lumen of the gut. This is further confirmatory evidence that such wandering larvae may find their way back into the lumen of the intestine and that this possibility exists as long as the parasites remain alive.

PLATE XIV.—Specimen 6584. 17 days after infection.

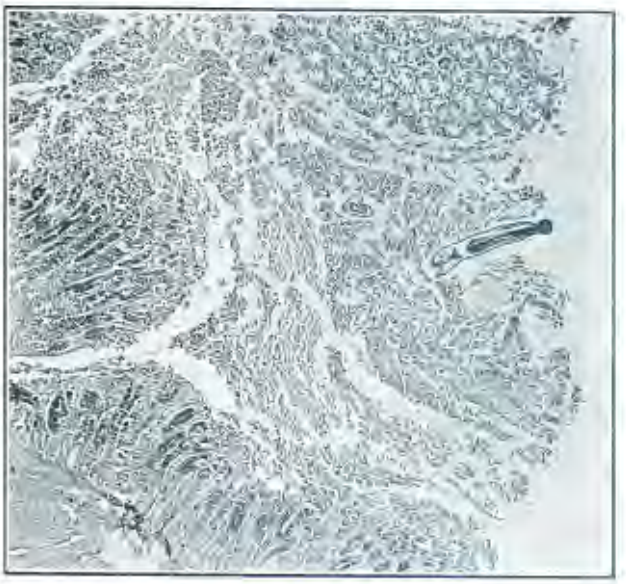


Fig. 1 (35×).—Wandering larva on the point of returning to lumen of intestine.

Fig. 2 (25×).—Small intestine, showing catarrhal enteritis.

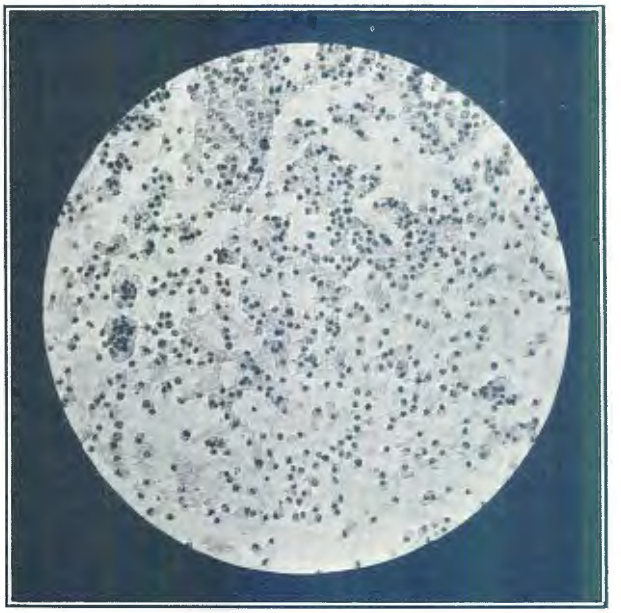


Fig. 3 (220×).—As Fig. 2, showing exudate in lumen of intestine.

Large blood spaces are present, some of which extend from the submucous tissues, through the muscularis mucosae and into the mucous membrane. On careful examination these are seen to be not haemorrhages as they were at first thought to be, but actually distended blood vessels having an endothelial lining. Here again the explanation which suggests itself is a mechanical interference with the free venous drainage of the intestinal wall by worm nodules.

18 *Days after Infection.*

Lamb N. Specimen 6587.—Lesions are present in the small and the large intestine. The nodules are situated mainly in the submucous tissues and their structure is very similar to that of nodules of the previous case (17 days after infection). In some nodules, bacteria and/or parasites are present, whilst in others one or the other or both of these may be absent. The bacteria are chiefly bacillary forms in chains, many with spores which appear as refratile bodies all along the chain. In Plate XV, Fig. 1, a cross section of the ileum is shown, in which there is very marked thickening of the serosa. In this thickened portion are numerous bacteria(Fig. 2). These were probably responsible for peritonitis here. The exudate is now being organised and a good deal of fibrous tissue has already been formed. In the same plate, figures 3 and 4, a nodule is shown in which the parasite appears in longitudinal section. Within its mouth capsule there is a mass of cells which have polymorphic nuclei. The cytoplasm of these cells stains pink, and although no eosinophile granules can be recognised it is believed that they are ingested eosinophiles. It is not clear if the parasite is primarily making use of

PLATE XV.—Specimen 6587. 18 days after infection.

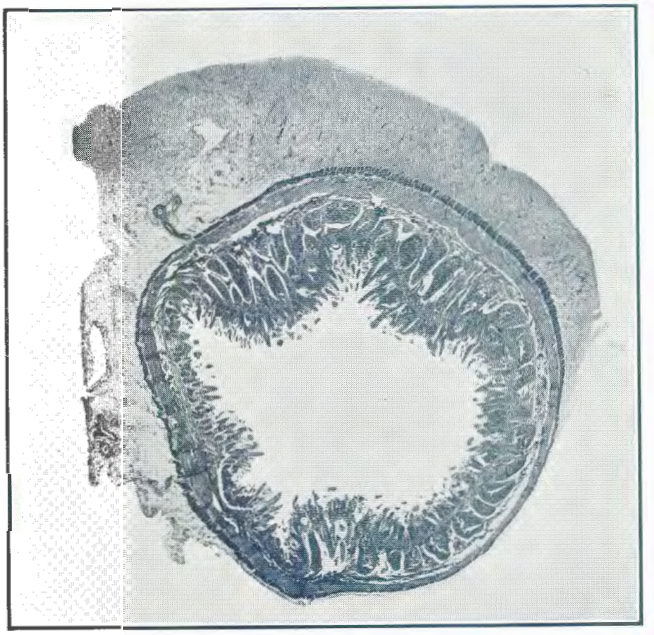


Fig. 1 (8×).—Section Ileum, showing thickened serosa due to peritonitis.

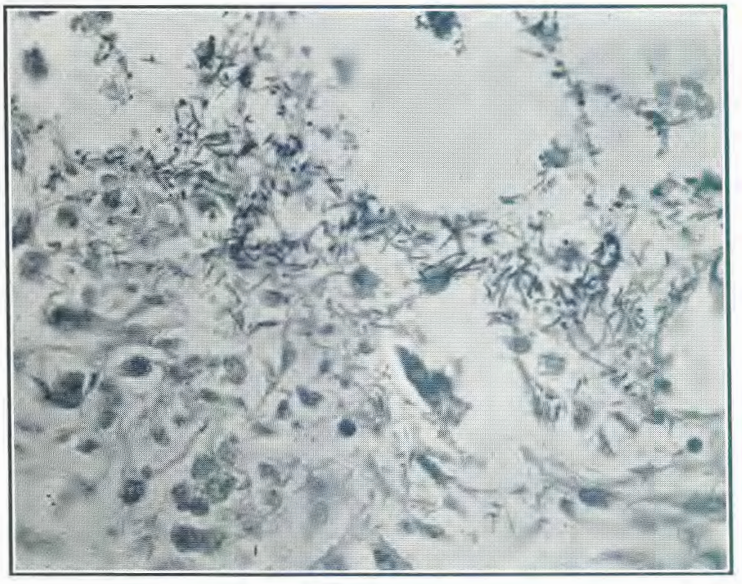


Fig. 2 (750X).—As Fig. 1, showing bacteria at extreme margin of the thickest portion of the thickened serosa.

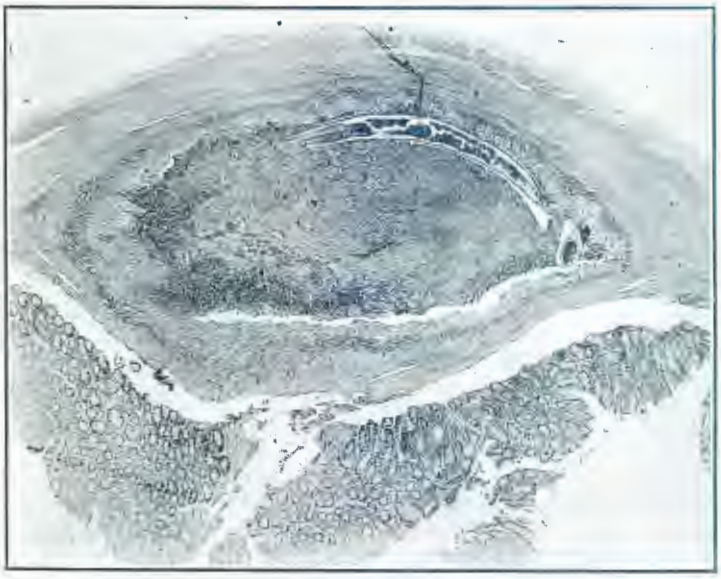


Fig. 3 (25×) and Fig. 4 (220X).—Showing parasite and structure of nodule in the submucosa.

these cells for food purposes, or if it merely devours them in order to protect itself against their attacks. It does not seem likely that the parasite could decrease materially the actual numbers of these cells in this way. To what extent the possibility exists that the ingested eosinophiles have a special biological significance, leading to an immunity of the parasite against their attacks, is at the moment entirely speculative. Should this be the case, the life of these wandering larvae would be considerably prolonged and the damage they inflict would consequently be greater. In several places there are darkly bluish staining (haemalum-eosin) hyaline masses, which lie closely applied to the parasite. An occasional eosinophile is present in the substance of these masses. This is obviously an acid substance which is taking the basic stain. It may possibly be secreted by the parasite itself. Its significance is not understood.

Towards the periphery of the central structureless mass intact eosinophiles and in some nodules probably also neutrophiles can be identified. This is surrounded by a small zone consisting of fairly large cells, having a faintly staining cytoplasm, which sometimes appears to be markedly vacuolated. Some of these cells have more than one nucleus. They are regarded as epithelioid cells and the parasite seems to have made a circular tract all along the epithelioid zone. In the epithelioid zone one sees now for the first time occasional well developed foreign body giant cells. Around this zone there is an accumulation of two kinds of cells mainly. These are eosinophiles and round cells. The round cells are much smaller than the epithelioid cells, amongst which some of them lie. Their nuclei stain rather darkly. These two kinds of cells can almost be said to form another zone here around the epithelioid cells. This zone merges into a zone of granulation tissue, where fibroblasts are readily identified and in places fibrous tissue has already been formed, the whole constituting a capsule. The parasite has now probably become imprisoned within this capsule, and although it may be possible, it seems unlikely, that it would break through this barrier.

Some nodules cause complete destruction of not only the circular but also the longitudinal muscular layers in the wall of the intestine. One can well imagine that in some cases nodules may cause a complete break in the plain muscle of the intestine at a particular place and although as pointed out by Theiler (1921) that this may not be the only cause of intussusception in sheep, it probably is the cause of complete interruption of peristalsis and consequent invagination (reksiekte) in some cases.

There is evidence of hyperaemia and catarrhal enteritis. Not infrequently eosinophiles are seen diffusely distributed throughout the mucous membrane.

21 *and* 22 *Days after Infection.*

Sheep 23 *and Kid* 24. *Specimens* 6589 *and* 6589A.—The lesions (including peritonitis) except that they are rather more advanced are otherwise very much like those of the previous case. The central caseous mass is relatively large (Plate XVI, Fig. 1) and a fair amount of calcification has occured. An epithelioid zone is present,

PLATE XVI.—Specimen 6589. 21 days after infection.

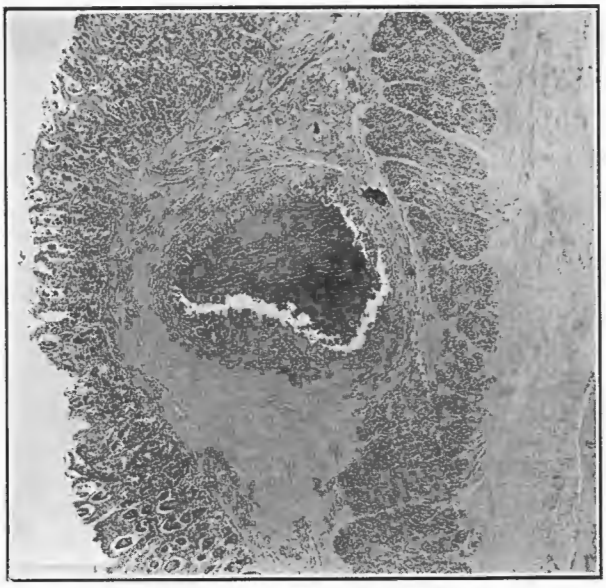


Fig. 1 (25×).—Caseating worm nodule with calcification.

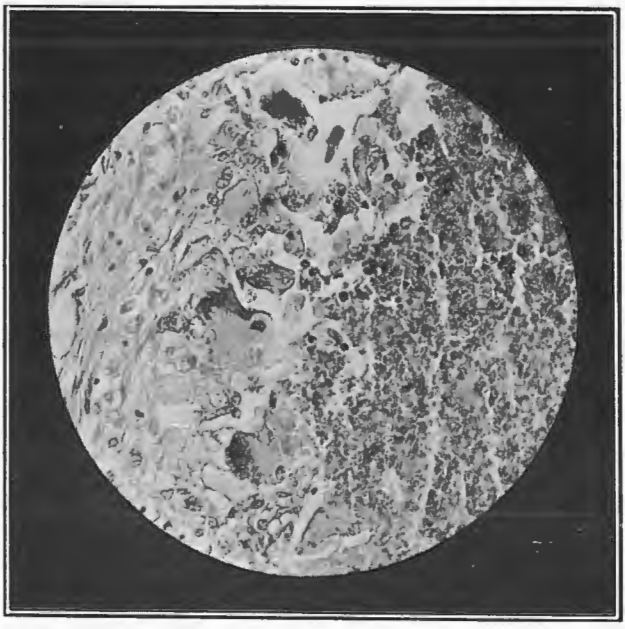


Fig. 2 (220×).—As Fig 1, showing foreign body giant cells.

but this is not very well defined in some nodules and is then to some extent replaced by not infrequent and very typical giant cells. (Plate XVI, Fig. 2.) Many of these have the diffuse distribution of the nuclei, usually associated with foreign body giant cells, but there are some in which the nuclei are present mainly towards the periphery of the cells and resemble then to some extent the Langhans giant cells, usually regarded as typical for tuberculosis. No living parasites could be demonstrated in any of these sections, neither is there any definite evidence of the presence of dead parasites.

28 *Days after Infection.*

Lamb F. Specimen 6590.—In several places migrating larvae are present in the mucous membrane, but no evidence of encysted larvae was found. The older nodules have more or less the same structure as those previously described. Some of the lesions are very extensive with consequent destruction of a good deal of the wall of the intestine. The nodules may actually project beyond the serosa and extend into the peritoneal cavity (Plate XVII, Figs. 1 and 2), or they may cause a bulging of the mucous membrane into the lumen of the intestine. In such cases there may be an actual ulcer and one can frequently recognise a small macroscopic hole in such nodules in the mucous membrane (actually the nodule may also involve the tissues, deeper than the mucous membrane). In the central structureless mass calcified particles are present. Immediately around the central caseous mass are cells which have a radiating or a palisade arrangement. They are epithelioid cells (Plate XVII, Fig. 3) amongst which one sometimes finds well developed giant cells. The nodules are surrounded by a capsule consisting partly of granulation tissue and partly of well formed fibrous tissue.

PLATE XVII.—Specimen 6590. 28 days after infection.

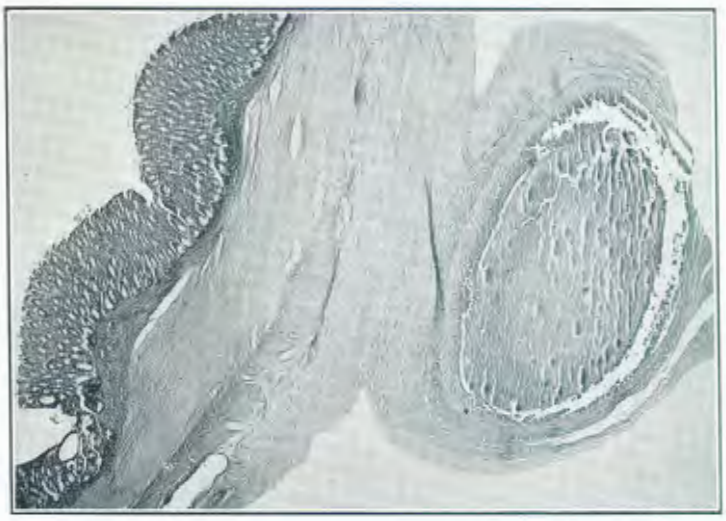


Fig. 1 (18×).—Nodule projecting into peritoneal cavity.

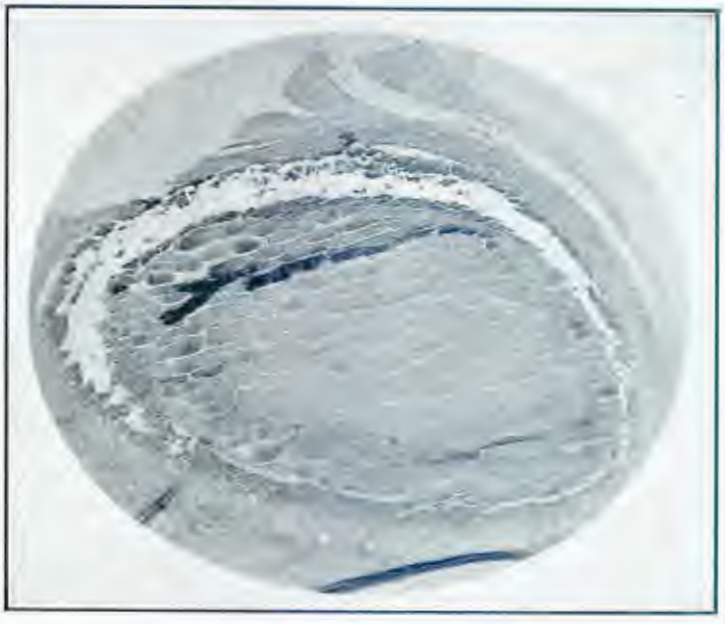


Fig. 2 (30×).—As Fig. 1.

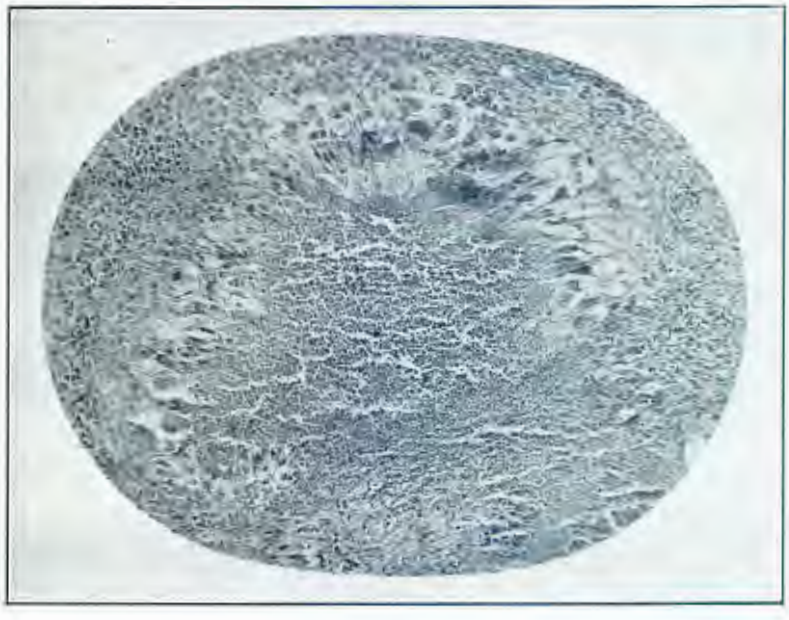


Fig. 3 (120×).—Showing palisade arrangement of epithelioid cells.

30 *Days after Infection.*

Lamb №. 403. *Specimen* 6591.—Lesions are present in the submucous tissues and extend right up to the serosa. Amongst the nuclear debris, polymorphic nuclei can be recognised, but it is not possible to say if they are eosinophile or neutrophile nuclei. Bacilli are present, mostly in the form of chains. There is nothing in the structure of the lesion which suggests definitely its verminous origin; on the other hand it may be an abscess which may be secondary to the verminous infestation.

32 *Days after Infection.*

Lamb 4. *Specimen* 6592.—The usual nodules are present in the walls of the intestine. In some nodules one sees for the first time unmistakable evidence of dead parasites (Plate XVIII, Fig. 1). The disintegrated remains of the parasite lie amongst nuclear debris. Portions of the parasite would seem to be impregnated with calcium. Further calcium particles are found scattered irregularly throughout the nuclear debris. Towards the periphery of the nodule there are not infrequent foreign body giant cells, some of which contain calcified particles. The whole nodule is surrounded by a capsule, which in its inner portions consists of granulation tissue with epithelioid cells and fibroblasts and the peripheral portions consist of fibrous tissue.

PLATE XVIII.—Specimen 6592. 32 days after infection.

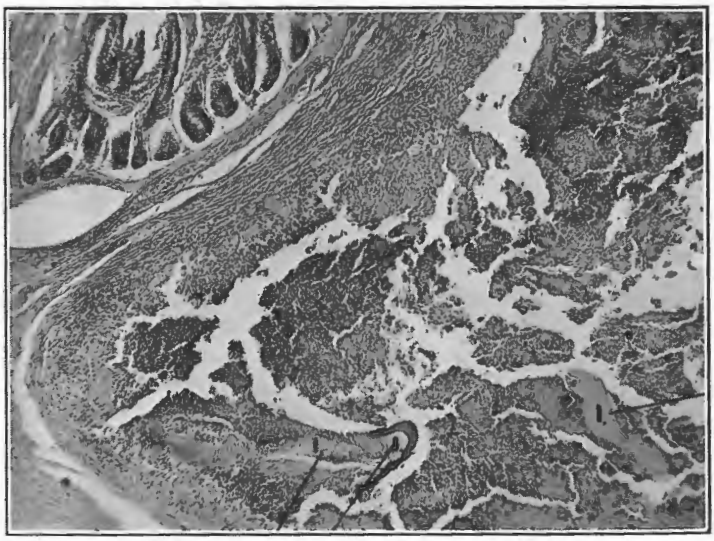


Fig. 1 (65×).—Portions of dead parasite marked by numerals 1.

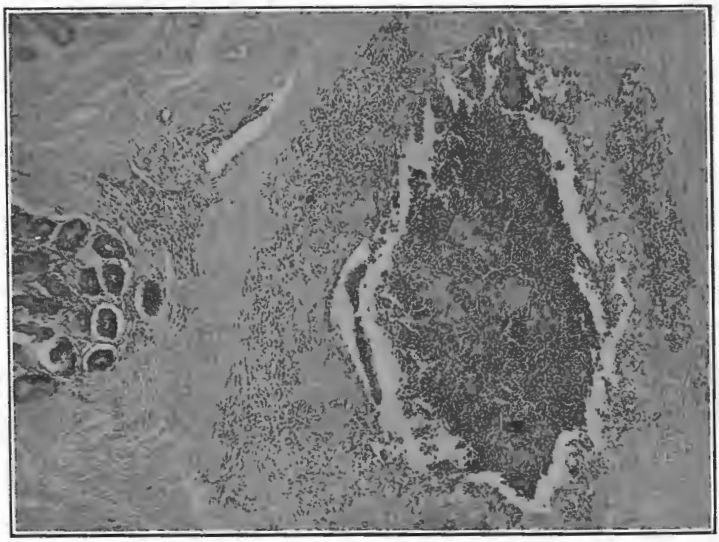


Fig. 2 (65×).—Strand of intestinal epithelium at periphery of nodule.

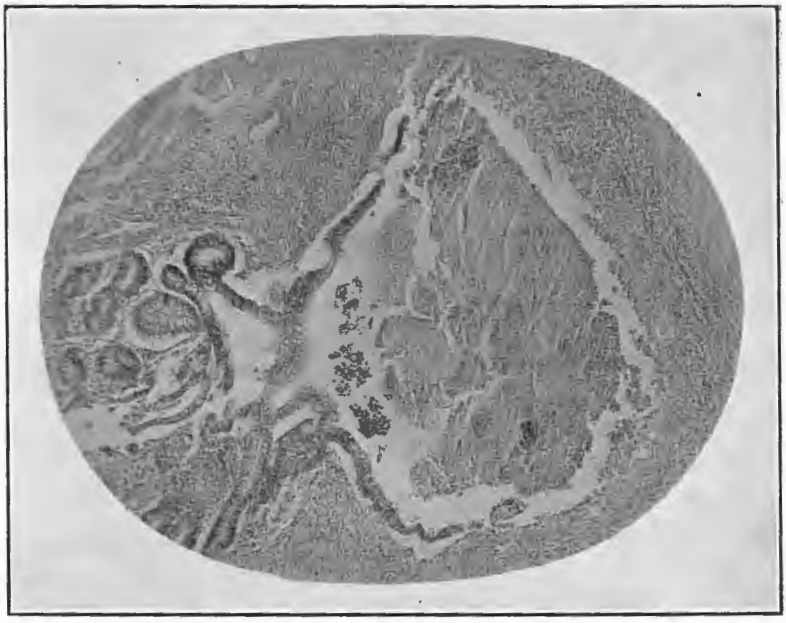


Fig. 3 (65×).—Showing structural relationship between epithelial strands around periphery of nodule and mucous membrane.

The remains of glandular epithelium from the intestine can be seen towards the periphery of some nodules situated in the submucosa (Plate XVIII, Fig. 2). When the serial sections are carefully examined, it can be seen that these apparently isolated strands of epithelial cells, amongst which goblet cells can be recognised, are structurally connected with the mucous membrane (Plate XVIII, Fig. 3). It seems that such cell groups may easily become detached from their normal structural relationship with the mucous membrane and may then probably give rise to anomalies and even tumours.

40 *Days after Infection.*

Sheep 114. *Specimen* 6593.—Numerous nodules, some of large size, are irregularly distributed throughout the wall of the intestine. The wall of a portion of the intestine is markedly thickened and microscopically this thickening is seen to be largely due to an increase of the plain muscular tissue. Although both the inner circular and the outer longitudinal muscular layers are involved, there would seem to be a greater increase of the inner circular muscular layer. At the same time there would seem to be an increase of fibrous tissue, strands of which are irregularly distributed throughout the plain musculature of the intestinal wall. In the absence of a full post-mortem description of this particular animal, one can only speculate as to the cause of this apparent hypertrophy of the plain muscle. The possibility which suggests itself is, that worm nodules in the walls of the intestine caused a partial obstruction to the free passage of intestinal ingesta and that this stimulated increased peristaltic activity of the plain muscle in the intestinal wall, proximal to the obstruction, with consequent hypertrophy of the parts involved.

45 *Days after Infection.*

Lamb No. 32. *Specimen* 6594.—Numerous and extensive worm nodules are distributed irregularly throughout the walls of the intestine. Some nodules project from the serosa into the peritoneal cavity. Where the nodule is attached to the serous surface of the intestine, its outer portion (capsule) consisting of fibrous tissues completely replaces the outer longitudinal muscle fibres of the intestinal wall, but only involves the inner circular muscular layers very slightly. In most cases the nodules are surrounded by well developed capsules, which in the case of some nodules consist of adult fibrous tissue, whilst in the case of others the capsule is partly fibroblastic. The epithelioid cells, when present, again show a marked tendency to palisade arrangement. Many giant cells and eosinophiles are present. The central portion is mostly structureless, calcification is taking place, but is not very extensive. Parasites can be readily identified in some nodules, but it is not known if they are dead or alive. Bacteria could not be identified in any of these nodules.

56 *Days after Infection.*

Kid No. 44. *Specimen* 6595.—Nodules similar to those of the previous case (45 days after infection) are present. Very many eosinophiles are distributed diffusely throughout the mucous membrane even in portions of the intestine where no nodules are present.

It is not clear if the eosinophile reaction is partly or wholly due to the action of adult worms which may be present in the lumen of the intestine or if it is a reaction which must be associated with the nodules present in the deeper portions of the wall of the intestine, even though such nodules may be situated some distance from parts of the mucous membrane where numerous eosinophiles are also present. No definite evidence of the presence of bacteria was found in the sections examined.

71 *Days after Infection* (2 lambs).

(1) *Lamb* 29. *Specimen* 6596.—Nodules are present in the small and large intestines. At the ileo-caecal valve, lesions are seen in both the ileum and the caecum. Here there is very extensive destruction of the wall of the intestine. In places the entire mucous membrane, the submucosa, the circular and the longitudinal muscular layers are completely replaced by a homogeneous necrotic material, which stains pale pink with haemalum eosin and in which, in addition to very many bacteria, there is present also a certain amount of fibrosis. Where the living and dead tissues meet, there is a zone in which the nuclei of the cells are seen in various stages of necrosis. Beyond this, in the living tissues, there is marked increase in fibrous tissue, in which a fair number of neutrophiles and a greater number of larger round cells are present. Eosinophiles are conspicuous by their absence. The lesion, almost certainly initiated by nodular worm larvae, has now the typical structure of an ulcer, in which not parasites, but bacteria play the principal aetiological rôle.

The structure of worm nodules, uncomplicated by bacteria, in other portions of the large intestine, is well shown in Plate XIX, Figs. 1 and 3. The central structureless portion stains an intense pink colour with haemalum-eosin. There does not seem to be any calcification at all. Immediately around the central portion of the nodule are a number of giant cells. Some of them are typical foreign body giant cells, but most have nuclei arranged around the periphery of the cell, in which respect they resemble to some extent Langhans giant cells. In places there are collections of considerable numbers of eosinophiles. Epithelioid cells and fibroblasts are inconspicuous. The whole is surrounded by a well developed fibrous capsule. Eosinophiles although present in the mucous membrane are not numerous.

(2) *Lamb No.* 13101. *Specimen* 6597.—The nodules themselves are similar in structure to those of lamb 29, except that they are perhaps more extensive (Plate XX, Fig 1 and Plate XIX, Figs. 2 and 3) and that a fair amount of calcification has occured (Plate XX, Fig. 3). In some of the lesions, which as far as can be determined from the examination of serial sections, are not in any way associated with ulcer formation, very numerous bacteria are present. In some of the nodules the remains of dead parasites are seen. The detailed structure can no longer be recognised, but only the bare outlines of the parasite can be seen. In others the parasites are in such a good state of preservation that the probability of their still being alive is very great (Plate XX, Fig. 2).

PLATE XIX.—71 days after infection.

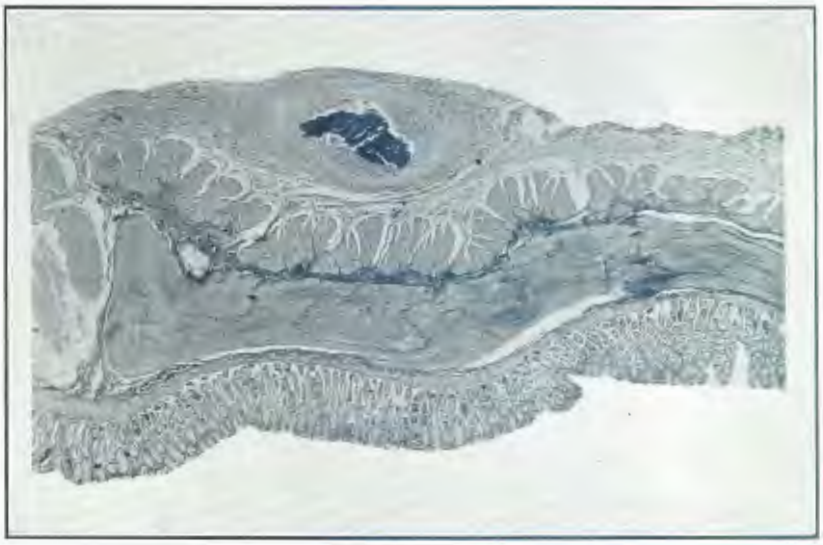


Fig. 1 (18×).—Specimen 6596. Nodule under serosa.

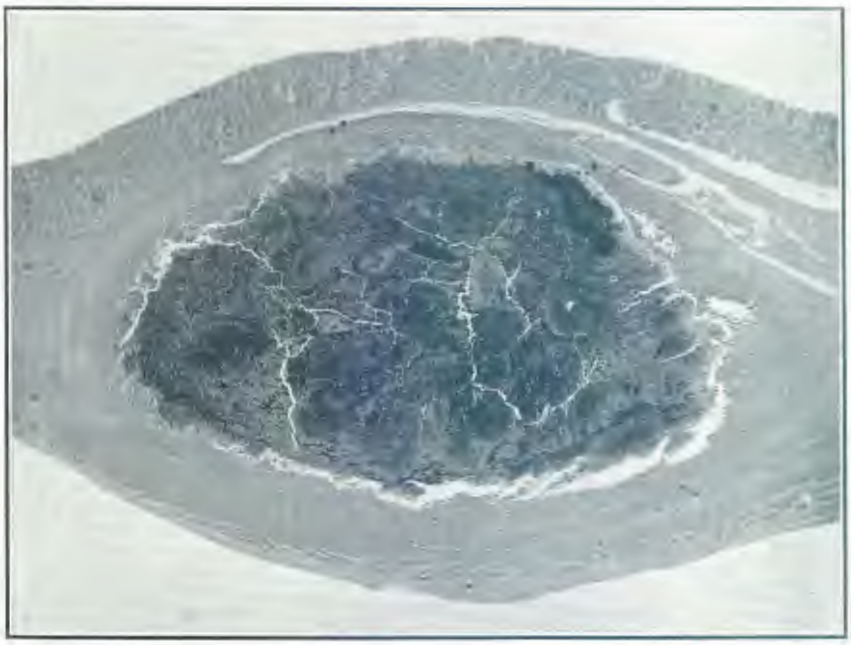


Fig. 2 (18×).—Specimen 6597. Submucous nodule.

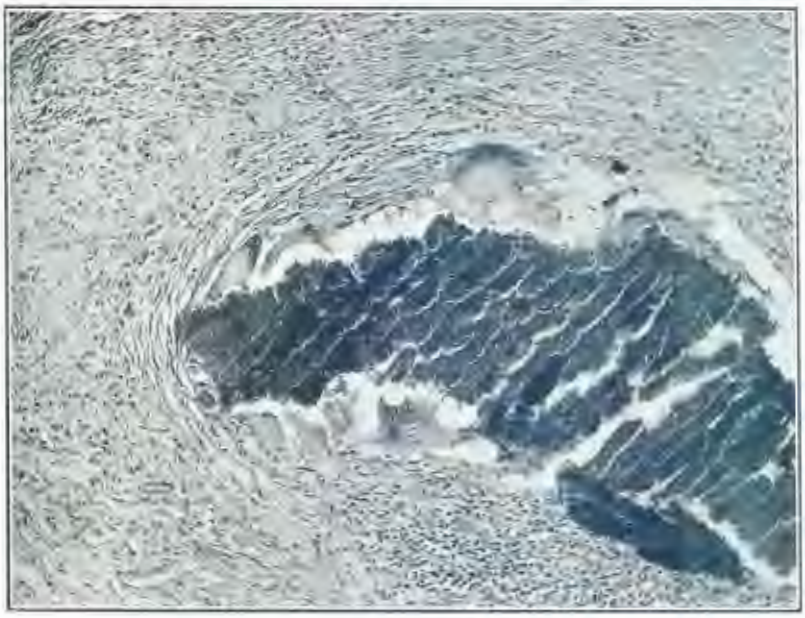


Fig. 3 (120×).—As Fig. 1. Showing giant cells.

Fig. 4 (120×).—As Fig. 2. Showing giant cells.

PLATE XX.—Specimen 6597. 71 days after infection.

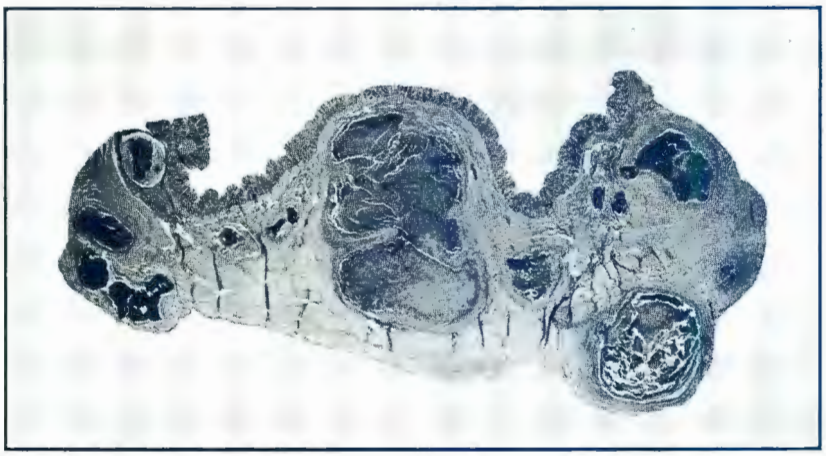


Fig. 1 (6×).—Extensive lesions in the wall of the intestine.

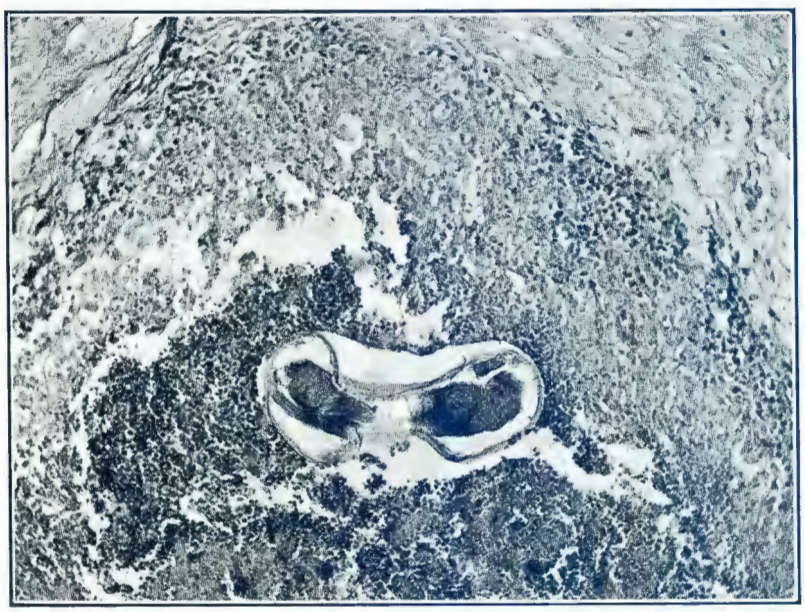


Fig. 2 (120X).—As fig. 1, showing section of the parasite.

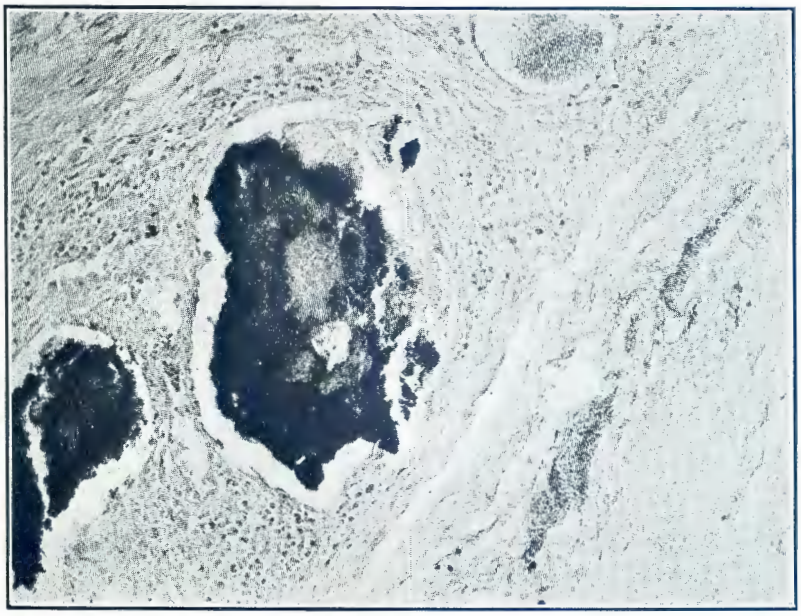


Fig. 3 (120×).—As fig. 1, showing calcification.

106 *Days after Infection.*

Lamb No. 34. *Specimen* 6598.—Well encapsulated nodules are present in the submucous tissues of the small and large intestine. There is no definite evidence of calcification. In some places numerous eosinophiles are diffusely present in the mucous membrane itself. No bacteria can be recognised.

Material from animals known to be infected for exact periods beyond 106 days was not available for examination.

SHORT SUMMARY OF THE PARASITIC LIFE OF THE LARVAL STAGES OF OESOPHAGOSTOMUM COLUMBIANUM IN LAMBS.

(1) 12-24 Hours after Infection.

Twelve hours after infection larvae are demonstrable in the mucous membrane. During this time primary parasitic migration may take place, producing an eosinophilic reaction in the mucosa and sometimes lesions like those of the early stages of a haemorrhagic enteritis.

(2) 24 Hours to 4 Days after Infection.

The larvae encyst against the muscularis mucosae. The muscularis mucosae seems to be merely a convenient mechanical obstruction to the further penetration of the larvae into the wall of the intestine.

The cyst wall consists of a delicate membrane which in a microscopical section has a threadlike appearance, and the nature of which could not be determined with certainty. The larva is coiled within the cyst, in which, in addition to the larva, there are red cells and sometimes structureless, pink staining (with eosin) fluid, which probably consists in part, if not entirely, of haemolysed red cells. Any eosinophile attack directed against the encysted larvae, if at all present, is slight. It does not seem as if the encysted larvae carry bacteria which produce pathological disturbances in the cysts themselves. Two kinds of cysts can be distinguished, viz. (1) those in which there is very little tissue reaction, with only slight fibroblastic activity and (2) those in which greater fibroblastic activity is present, resulting in the formation of a well defined capsule. Larvae in such cysts may be imprisoned there as suggested by Veglia (1923), but no evidence of dead larvae was ever found in the mucous membrane.

(2) 5 Days after Infection *et seq.*

Secondary parasitic migration takes place. In one case an empty cyst was found three days after infection indicating that in some cases secondary parasitic migration may commence somewhat earlier than was thought to be the case by Veglia (1923). This migration may occur (*a*) normally and (*b*) abnormally.

(*a*) *Normal secondary parasitic migration.*—The fourth stage larva emerges from its cyst. The muscularis mucosae is a mechanical obstruction to its deeper penetration into the submucosa and the parasite is directed through the mucous membrane towards the lumen of the intestine. During its passage through the mucous membrane a secondary eosinophilic reaction (as against the primary eosinophilic reaction, during the primary parasitic migration prior to encystment) takes place, and eosinophiles may be found diffusely distributed throughout the mucous membrane. Although unmistakable evidence that fourth stage larvae in the lumen of the intestine as well as adult worms can produce a diffuse eosinophilia in the mucous membrane, was not found, there is a possibility that this may occur. In the normal migration of the larvae, nodules do not form in the walls of the intestine. In such cases numerous adult worms may eventually be present in the lumen, when only occasional nodules are found in the walls, of the intestine.

(*b*) *Abnormal secondary parasitic migration.*—For reasons which are not understood, the larvae penetrate the muscularis mucosae. Once they have done this, they seem to loose all sense of direction and wander about in the tissues deeper than the muscularis mucosae. During this time an intense eosinophilic reaction is produced and after a time the nodule forms. The very young nodule consists of eosinophiles and the parasite. Within a few days (8 days after infection) some of the eosinophiles disintegrate. This eosinophilic disintegration is the cause of the formation of the central structureless portion of the nodule. However, in the case of some nodules, bacterial activity may contribute to the formation of the central structureless portion of the nodule. Later on calcification may take place. Towards the periphery of the nodule granulation tissue and intact eosinophiles are present. The granulation tissue consists of fibroblasts and epithelioid cells. Later on giant cells are seen and eventually a well developed fibrous capsule is formed.

In wandering about in the walls of the intestine, the larvae may produce very marked destruction of the normal tissues, leading to physiological disturbances associated with a partial stenosis or even interrupted peristalsis (invagination). However, it seems that very marked tissue destruction may take place in the wall of the intestine deeper than the muscularis mucosae, with very little nutritive disturbances, provided no or only very few adult worms are present in the lumen of the intestine. If, on the other hand, numerous primary and secondary migrating larvae produce gross changes in the mucous membrane, bacterial invasion and bacterial complications can readily take place.

During the wanderings of the parasites one of a number of things may happen. (1) It may by good fortune, after repenetrating the muscularis mucosae, find its way back to the lumen of the intestine to complete its development. (2) The tissues may succeed in imprisoning the parasite, which then dies there. It is almost impossible to prove, but, if as is believed, this is the most usual course of events, then the old nodules in the intestine would represent the graves of the parasitic cemetery in the walls of the intestine. (3) The parasite may perforate the intestine and pass into the peritoneal cavity, or may, according to Hall, Carne and Clunies Ross and Mönnig, already quoted and other authors, even pass to remote organs such as the liver, where they eventually die. If the infestation is such that sufficient destruction of the mucous membrane has occured, with consequent bacterial invasion, a fatal peritonitis may take place, with perforation of the intestinal wall. This may explain the hitherto unrecognised cause of some cases of peritonitis of sheep, not infrequently met with in South Africa.

PATHOGENESIS OF OESOPHAGOSTOMIASIS.

In order to understand the pathogenesis of the disease a brief survey of the outstanding clinical features is necessary. There is diarrhoea, accompanied by a very marked emaciation and cachexia. No morphological changes in the red cells, usually associated with anaemia, are present. There are usually no clinical respiratory or circulatory disturbances. The worms actually cause the death of the animal, but exactly how they do this, is not understood. The immediate cause of death is frequently ascribed to toxaemia and exhaustion. At post-mortem one usually finds: adult worms in the large intestine; nodules may be numerous or infrequent; ulcerative or other forms of enteritis may or may not be present; very marked muscular atrophy; marked serous atrophy of fat with emaciation; atrophy of the spleen; degenerative changes of the liver.

In discussing the pathogenesis of the disease the primary, as well as the secondary, factors concerned, will be referred to, viz.: (1) Toxins. (2) Haematology. (3) Complications: (*a*) Those of a specific nature—bacterial infection—enteritis, peritonitis, etc.; (*b*) those of a mechanical nature: (i) destruction of normal tissue with consequent nutritive and other disturbances; (ii) intussusception (reksiekte).

1. Toxins.

In unpublished work Mönnig and the writer made an attempt to reproduce the symptoms of oesophagostomiasis by repeated injections into susceptible sheep of extracts made from worms collected from sheep which died at Onderstepoort. The results were entirely negative. There is no direct experimental evidence that toxins are mainly or even partly responsible for the symptoms of this disease. However, their presence has been inferred mainly on circumstantial evidence. One meets with fatal cases of the disease in which relatively few nodules are present and in which no evidence of complications such as enteritis is present, in spite of many adult worms in the large intestine. In such cases, the emaciation, general atrophy and advanced degenerative changes of a fatty nature in the liver, seem to be due to toxic principles, derived from the worms themselves. However, there is also the possibility that toxic materials may be derived from the damaged mucous membrane. Whipple quoted by Hewlett (1923) isolated duodenal loops in dogs, with rapidly fatal results in 36-72 hours time. Similar loops from the large intestine were only slightly toxic. If these results in dogs can be applied to sheep, there would seem to be a possibility that toxic substances may actually be formed from the mucous membrane of the large intestine as a result of physiological or other disturbances due to the action of the nodular worms. In severe infestations when the symptoms are most marked, such toxic substances can be formed along the entire length of the large intestine, (as against experimental loops of Whipple) where the worms are present; and even though only a small amount of toxin is formed, its cumulative effect over a period of weeks or months may be quite marked.

2. Haematology.

As far as is known a systematic examination of the blood of sheep suffering from a pure infestation of nodular worms, has not been recorded. Fourie (1931) referred to the difficulties experienced in producing a progressive and fatal disease with amongst others, pure faeces cultures of *Oesophagostomum columbianum*. Nevertheless, with the assistance of Veglia a few such cases were produced and these are the cases which will mainly be referred to in so far as the anaemia is concerned.

The technique employed is exactly that referred to by Fourie (1931). However, in more recent work, it was sometimes found that considerable difficulty was experienced in counting the red cells, as a result of macroscopic agglutination which took place when blood from some sheep infested with oesophagostomiasis and ancylostomiasis, was diluted in Hayem's solution in the ordinary way for counting. Such agglutination was almost completely eliminated, in the few cases which were available for study by heating the Hayem's solution slightly before the blood was added to it. Agglutination will not take place subsequently if the diluted blood is allowed to cool down to room temperature. However, agglutination which has taken place in cold Hayem's solution will not be broken down

appreciably by subsequent slight heating. The detailed results from one sheep, the blood of which showed macroscopic agglutination in cold Hayem's solution, are reproduced below.

A. Dilution in Cold Hayem's				B. Dilution in Warm Hayem's.				C. (B) But Allowed to Cool Down.			
54	60	62	60	59	45	45	45	53	42	47	54
72	60	71	59	41	53	59	38	41	47	54	52
73	68	61	44	47	53	59	36	45	48	52	52
64	69	49	51	52	54	51	49	55	47	46	50
263	257	243	214	199	205	214	168	194	184	199	208
	9·77				7·86				7·85		

A sufficient number of cases of this type of agglutination was unfortunately not available for repeated observation, but to judge from the above case it would seem that although the total counts in (B) and (C) correspond almost exacly, the distribution is much more uniform in the warm Hayem's diluting fluid which was subsequently allowed to cool down before counting (C).*

Haematology of Sheep 14512 *and* 15970 (*Pure Infection Oesophagostomiasis*) and 11899 (*Mixed Infection* Oesophagostomum columb. *and* Haemonchus contortus).

Twelve worm free sheep were infected with faeces cultures of nodular worm larvae. Of these only three (14512, 15970, and 11899) developed a progressive disease from which they died. On post-mortem examination death was found to be due to a pure infestation of *Oesophagostomum columbianum* in sheep 14512 and 15970 and a mixed infestation of *Oesophagostomum columbianum* and *Haemonchus contortus* in sheep 11899. The haematological observations concerning these three sheep are recorded in Table 1, Graph 1 (sheep No. 14512), Table 2, Graph 2 (sheep No. 15970) and Table 3, Graph 3 (sheep No. 11899).

Haematology of Sheep 14512.

The exact age of this animal is not known, but it was under six months. Infection was commenced on the 28th July 1926, at the rate of 200 larvae every second day until a total of 1,600 larvae had been given.

Faeces cultures were positive on the 7th, 9th, 29th, and 30th September 1926. On the 2nd October 1926, the animal was in poor condition and had diarrhoea. On the 11th October 1926, the condition of the animal was very poor and it was weak. The animal died on the 15th November 1926.

* The statistical analysis of the above figures made by Mr. A. P. Malan (Statistician) confirmed this.

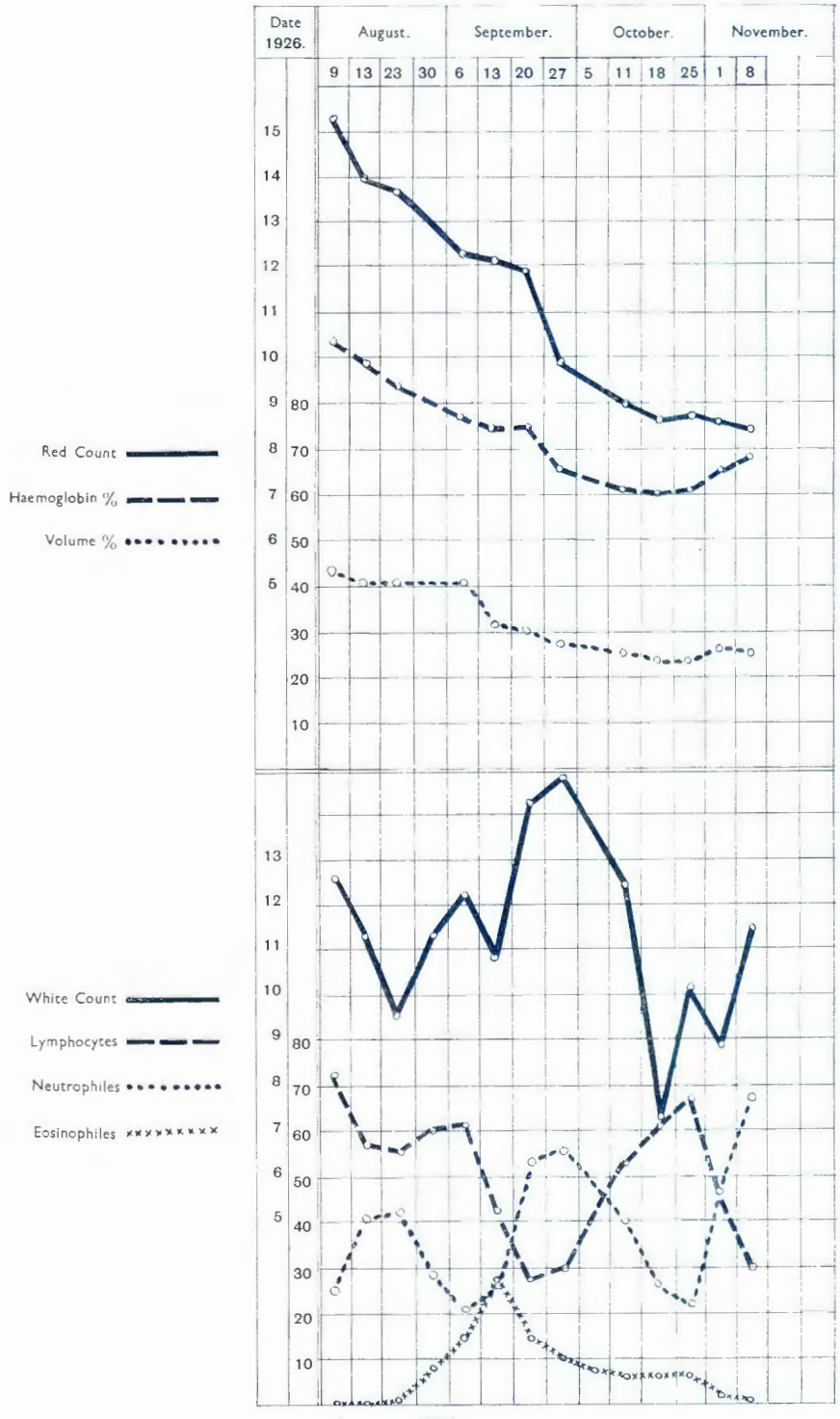
TABLE I.

Sheep No. 14512.

Infection commenced 28.7.26 *at the rate of* 200 *Oesophagostomum larvae every second day until* 1,600 *had been given. Infection completed* 11.8.26.

Date.	Source.	R.C.	R.P.	Hglb.	W.C.	L.	M.	N.	E.		Remarks.
9.8.26.....	Jugular.....	15·3	44	93	12,700	72	2	26	—	—	
13.8.26.....	,, ...	14·0	41	89	11,300	58	1	41	—	—	
23.8.26.....	,, ...	13·7	41	84	9,600	57	—	42	1	—	
30.8.26.....	,, ...	12·3	—	—	11,300	60	2	29	8	1	
6.9.26.....	,, ...	12·3	40	78	12,200	61	3	21	14	1	
13.9.26.....	,, ...	12·1	32	75	10,800	43	3	26	27	1	
20.9.26.....	,, ...	11·9	30	75	14,300	28	2	53	14	3	Loosing condition.
27.9.26.....	,, ...	9·9	28	66	14,900	30	—	56	10	4	
5.10.26.....	,, ...	8·5	—	—	14,300	33	3	57	7	—	Condition poor (diarrhoea).
11.10.26....	,, ...	9·0	26	61	12,400	53	—	40	6	1	
18.10.26....	,, ...	8·7	24	60	7,200	61	1	27	6	5	
25.10.26....	,, ...	8·8	24	61	10,100	68	3	22	6	1	
1.11.26.....	,, ...	8·6	27	66	8,900	47	1	47	2	3	
8.11.26.....	,, ...	8·4	26	72	11,500	30	1	68	1	—	

GRAPH I.—SHEEP 14512.



Changes in the Red Cells.—There is a continuous decrease in the number of red cells almost up to the time of death. It is not clear to what extent this decrease in the number of red cells may be due in part to a normal decrease which may take place in the blood of young animals as they grow older. Fraser (1929/30) refers to variations in the number of red cells of the sheep of different age groups. Unless one can make systematic haematological observations on the blood of a sufficient number of lambs from birth and until they are adult, no reliable conclusions as to the changes which may occur in the number of red cells in sheep as they grow older can be drawn, particularly in view of the variations which are known to occur in the number of red cells of the same sheep as pointed out by Wirth (1931) and Fourie (1931).

The animal still had more than eight million cells per c.c. of blood when it died. This can be a more or less normal count for some sheep. No morphological changes usually associated with an anaemia were present in the red cells. Obviously, therefore, there is no primary haemopoietic or other disturbance in which the number of red cells can be regarded as deficient (clinical anaemia) in spite of the oligocythaemia which is admittedly present. Any changes in the decrease of the haemoglobin and the percentage volume of red cells, seem to be entirely dependent on the decrease in the number of red cells already referred to.

The oligocythaemia may be due to a diminshed efficiency in the normal function of the haemopoetic organs, which may also be involved in the general atrophy characteristic of the disease. If this is the case the toxins may interfere with the normal regenerative changes which are constantly taking place in the haemopoetic system, with the result that the replacement of worn out cells does not occur normally and a gradual decrease in the number of red cells is then inevitable. There is no direct experimental evidence in support of this view. Furthermore, there is no appreciable morphological differences between the cells of this animal in the earlier stages of infection (9.8.1926) and at the time of death. If there is any atrophy of the bone marrow, it is conceivable that the bones themselves would be subject to similar changes in this disease and although a special examination of the bones was not included in this study one would expect that any defect of the bones such as brittleness would be revealed by a predisposition to fractures. This is not a clinical feature of the disease. Nevertheless, it is possible that atrophy of the haemopoietic system, resulting in oligocythaemia, but not to the extent of clinical anaemia, may similarly affect the bones themselves to a moderate degree only, so that in actual practice a predisposition to fractures is not present.

Changes in the Leucocytes.—The figures presented in Table I and Graph 1 loose a good deal in value on account of the fact that preinfection counts were not made and in view of the likelihood that a number of larvae may already have commenced and some even completed their secondary parasitic migration, when the first counts were made 12 days after infection was commenced.

There would appear to be a definite leucocytosis on the 20th and the 27th of September (more or less two months after infection). This is caused mainly by an increase in the number of neutrophiles.

During the next two weeks there would almost seem to be a collapse in the total number of leucocytes, affecting mainly the neutrophiles, an observation which tends to support the view in favour of a possible atrophy of the blood forming tissues previously postulated, particularly as this occured at a time when a similar kind of collapse in the number of red cells took place. During the later stage of the disease the neutrophile counts are definitely high, but the total number of leucocytes do not reach the same high levels as on the 20th and 27th of September. Not knowing the preinfection normal counts, it is useless to calculate the absolute numbers of neutrophiles and lymphocytes etc. This is unfortunate, as there may merely have been a relative neutrophilia, which possibly could have been accounted for by a lymphocytic leucopaenia as a result of a possible greater atrophy of the lymphoid tissues. Probably the neutrophilia indicates that bacterial complications are taking place and if there is a certain amount of atrophy of the blood-forming tissues, the neutrophile counts will not reach the same high levels as would be the case with a normally reacting bone marrow.

Eosinophiles.—There is a very steep ascent in the eosinophile curve which reaches its peak (27 per cent.) six weeks after infection was commenced, and then subsides gradually until the death of the animal. The peak of the eosinophile counts occured at a time when the total leucocytic counts were relatively low. If these total counts can be regarded as more or less normal, the possibility which suggests itself is that previous to, or, during the time that the eosinophile counts were increasing, there was no significant reaction in the myeloid tissues and that the eosinophiles were produced elsewhere possibly in the lymphoid tissues of the intestine. This is not incompatible with the view previously expressed that an eosinophilia may indicate that the active tissue verminosis has been completed, when these cells are temporarily released into the circulation. The subsidence of the eosinophiles may be due to either (1) a diminished or (2) an increased effect of the parasitic factor.

(1) *The Diminished Effect of the Parasitic Factor.*—The last dose of infective larvae was administered just about a month before the peak of the eosinophile counts occured. During this time the larvae should all have completed their normal secondary parasitic migration, so that from this source there should be no stimulus for the production of eosinophiles. Also it is possible that during this time there may be a decrease in the effects produced by abnormal secondary parasitic migration, but no definite statement to this effect can be made as it seems that until the parasite dies or finds its way back into the lumen of the gut, the abnormal parasitic migration may continue indefinitely. It is not known when the parasites will die and the factors such as food, age, general hygiene of the sheep etc., which may influence the life of the parasite, during parasitic migration are not understood.

(2) *The Increased Effect of the Parasitic Factor.*—This may cause atrophy of the tissues which produce the eosinophiles. During the two weeks subsequent to the time that the peak in the eosinophile counts was reached, there is a very definite increase in the total

leucocytes, accounted for exclusively by an increase in the number of neutrophiles. This speaks against a myeloid atrophy during this time. Similarly the increase in the differential lymphocytic counts which occured during the latter part of October may be regarded as evidence against atrophy of the lymphoid tissues. But here again the differential counts may be a very unreliable index of the true state of affairs as actually there may have been a neutrophile leucopaenia during this time, accounting for the relative increase in the number of lymphocytes.

The haematological changes can be summarised as: Oligocythaemia but no clinical anaemia; eosinophilia which subsides gradually during the later stages of the disease; neutrophilia probably indicating that bacterial complications are taking place, but there is a possibility that, especially during the two weeks before death, the neutrophilia may be merely relative and possibly due to a lymphocytic leucopaenia.

Haematology of Sheep 15970.

This animal was a control in a wireworm experiment but later (8th February 1927) it was infected with nodular worm larvae at the rate of 5,000 per day until 35,000 had been given. The animal died 13 days after infection and because of the acute course of the disease the haematological observations do not reveal any significant changes, except for a leucocytosis six days after infection and about a week before the death of the animal.

This case has been included here mainly on account of its special pathological interest. It is a two-tooth ewe in fair condition. There are many erosions and even ulcers especially in the mucous membrane of the small intestine. In many places there is perforation of the intestine, particularly the ileum. The perforations as seen from the serosa are discrete and more or less elliptical defects, with raised borders, presenting a pathological picture almost identical to the case previously described (Plate XIII). Where perforation has occurred the omentum is adherent to the intestine. There is a fibrinous peritonitis from which the *Pseudomonas pyocyanea* was isolated in practically pure culture. The caecum shows an acute haemorrhagic enteritis. There are numerous nodules in the small intestine, but only a few in the large intestine. The liver shows well marked fatty degeneration. Unfortunately there is no record of the number of parasites present in the lumen of the intestine, and an examination was not made for the fourth stage larvae in the peritoneal cavity. Here then, is a case in which a young sheep was subjected to a gross infection with nodular worm larvae and in which in numerous cases, there was abnormal secondary parasitic migration, leading to perforation of the wall of the intestine. As a result of the extensive destruction of the mucous membrane and other parts of the intestine by primary and secondary migrating larvae, bacterial infection took place and later caused the peritonitis which was the immediate cause of death.

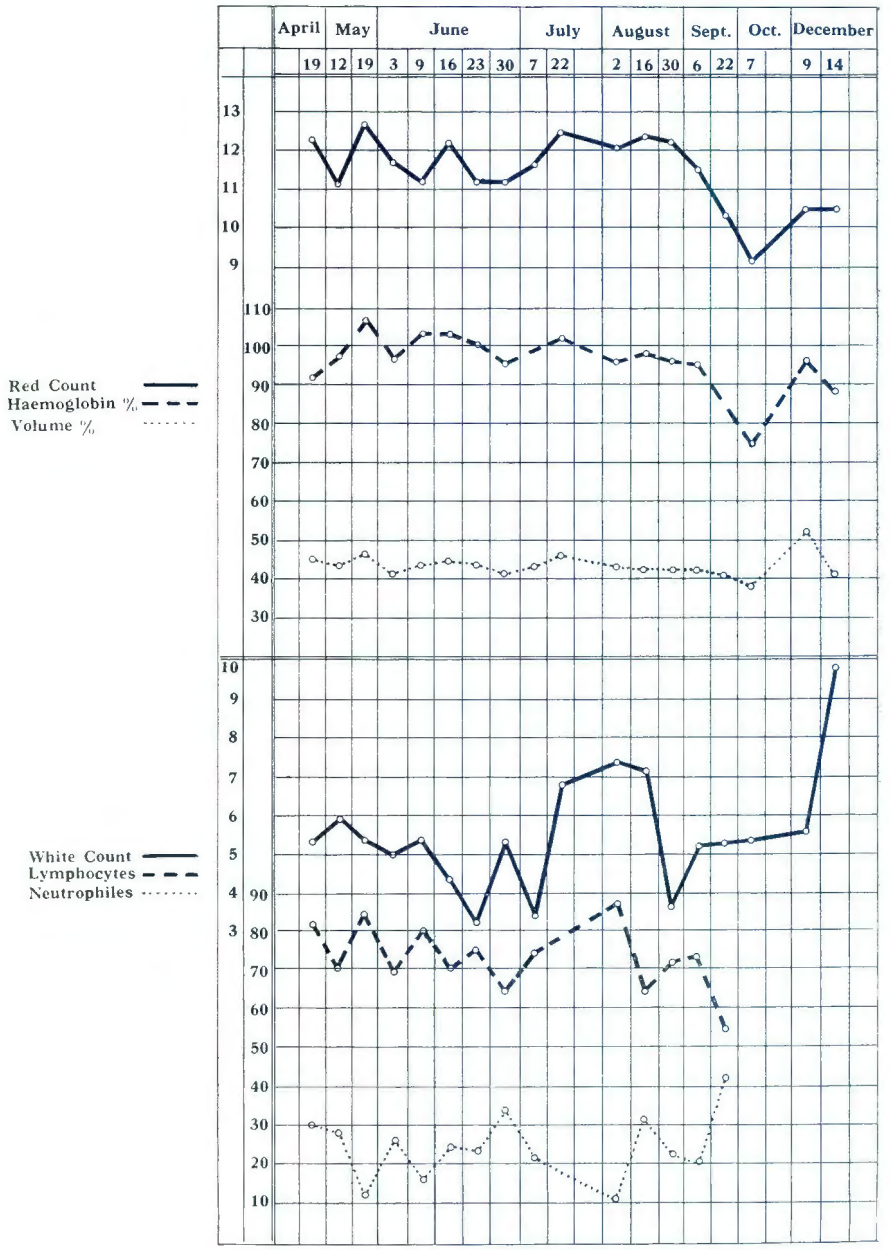
Table II.

Sheep No. 15970.

Infection with Oesophagostomum columbianum *larvae was commenced* 8.12.27 *at the rate of* 5,000 *larvae per day until* 35,000 *larvae had been given.*

Date.	Source.	R.C.	R.P.	Hglb.	W.C.	L.	M.	N.	E.	B.	Remarks.
19.4.27.....	Jugular.....	12·3	46	91	5,300	81	4	13	2	0	
12.5.27.....	,, ...	11·2	44	98	5,900	70	1	28	0	1	
19.5.27.....	,, ...	12·8	47	107	5,300	85	1	12	2	0	
3.6.27.....	,, ...	11·7	41	96	5,000	69	0	27	3	1	
9.6.27.....	,, ...	11·2	44	102	5,300	80	0	16	4	0	
16.6.27.....	,, ...	12·2	44	102	4,400	70	1	24	4	1	
23.6.27.....	,, ...	11·2	44	100	3,200	75	0	23	1	1	
30.6.27.....	,, ...	11·2	42	95	5,300	64	2	33	0	1	
7.7.27.....	,, ...	11·7	43	—	3,400	74	0	21	1	4	
22.7.27.....	,, ...	12·5	47	101	6,800	Smear unsatisfactory.					
2.8.27.....	,, ...	12·0	43	96	7,400	86	1	11	2	0	200 cells counted.
16.8.27.....	,, ...	12·4	42	98	7,100	64	3	31	1	1	
30·8·27.....	,, ...	12·2	42	96	3,600	71	5	22	1	1	
6.9.27.....	,, ...	11·6	42	95	5,200	73	3	20	3	1	
22.9.27.....	,, ...	10·2	47	—	5,300	54	4	42	0	0	
7.10.27.....	,, ...	9·2	38	75	5,400	—	—	—	—	—	
9.12.27.....	,, ...	10·5	42	97	5,600	—	—	—	—	—	
41.12.27....	,, ...	10·5	41	88	9,700	—	—	—	—	—	

GRAPH II.—SHEEP 15970.



Haematology of Sheep 11899.

This case has been included here in order to show the type of blood picture which can be expected with a mixed infection of nodular and wireworms. Infection with nodular worm larvae was commenced on the 16th November 1925, at the rate of 200 or 5,000 larvae every second or third day, until a total of 51,400 larvae had been given. In the course of time, the animal became also grossly infected with wireworms accidentally.

The animal died on the 11th February 1926 and showed on post-mortem examination: Very marked anaemia (hydraemia, paleness of all mucous membranes); cachexia, with serous atrophy of fat; general atrophy of organs and the musculature; degenerative changes of the myocardium, liver and kidney; worm nodules, severe infestation with nodular and wireworms.

On referring to Table 3 and Plate XXI, it will be seen, that the outstanding morphological changes in the red cells are those of poikilocytosis and anisocytosis, and that cells showing punctate basophilia were definitely not present to the extent they occur in most cases of haemonchosis, according to Fourie (1931). Fourie further believes that punctate basophilia in haemonchosis indicates regenerative changes and is a sign of active haemopoiesis. The fact that these cells are present to a slight degree only in this case of mixed infection, may possibly indicate that the nodular worms have caused atrophy of the haemopoietic tissues, as a result of which active regenerative changes which are usually caused by a well developed wireworm infestation, with its resultant anaemia, are now largely dominated by degenerative changes, the atrophied bone marrow being no longer able to respond efficiently to the stimulus for active regeneration.

Haematology of Sheep 18000, 17995, *and* 18344.

In view of the possibility that eosinophiles in a sheep infected with nodular worms may be produced outside the myeloid tissues, total and differential white cell counts were made from blood collected from the jugular, aorta, caudal vena cava, mesenteric veins and, in some cases also, from the portal vein, from two sheep (17995 and 18344) infected with nodular worms, as well as from a control uninfected sheep (18000).

The sheep were infected on 26th September 1927 and blood was collected under anaesthesia ten days later.

In the control sheep eosinophiles were found to the extent of 1 per cent. in the jugular vein, but none were found in the other vessels, when 200 cells were counted.

In sheep 18344 the eosinophile counts were: jugular, 1 per cent; mesenteric, 6 per cent; and aorta, 3 per cent. In sheep 17995 the smears were such that differential counts could not be made, except in the case of the mesenteric vein, where no eosinophiles were found. Although the percentage of eosinophiles is fairly high especially in the mesenteric vein of sheep 18344, no reliable conclusions can be drawn from these results. Such an experiment can supply reliable results only, if total and differential counts can be made at varying periods after infection.

TABLE III.

Sheep No. 11899.

Infection with Oesophagostomum columbianum *larvae commenced* 16.11.25 *at the rate of* 200 *or* 5,000 *larvae every second or third day until* 51,400 *larvae had been given. Infection completed* 23.12.25. *Animal died* 11.2.26.

Date.	Source.	R.C.	R.P.	Hglb.	Viscosity.	W.C.	L.	M.	N.	E.	B.	Remarks.
3.11.25	Jugular	12·4	39	—	—	5,500	62	5	31	2	—	
5.11.25	,,	12·6	34	83	—	4,100	61	1	37	1	—	
9.11.25	,,	12·5	34	80	—	4,800	60	2	37	1	—	
11.11.25	,,	9·8	30	76	—	4,500	53	4	43	—	—	
30.11.25	,,	11·0	35	84	—	6,000	50	3	45	2	—	
25.11.25	,,	11·5	33	86	—	3,700	44	1	55	—	—	
2.12.25	,,	11·1	35	87	—	2,700	58	3	34	3	2	
10.12.25	,,	12·3	35	89	—	3,400	62	1	35	2	—	
17.12.25	,,	10·6	34	84	—	6,100	39	3	54	3	1	
23.12.25	,,	11·7	34	83	—	5,700	32	2	66	—	—	Cells normal.
29.12.25	,,	9·2	28	73	3·1 29	3,600	45	—	50	5	—	Cells normal.
7.1.26	,,	7·3	20	51	2·4 30	3,900	69	3	28	—	—	Anisocytosis.
13.1.26	,,	5·2	16	39	2·1 30	4,200	44	7	43	6	—	cells normal.
15.1.26	,,	5·1	15	38	2·0 29	3,800	42	3	53	1	1	
18.1.26	,,	4·0	12	32	2·0 25	4,100	—	—	—	—	—	Poikilocytosis—Red cells crenated.
20.1.26	,,	4·4	13	32	2·2 26	4,400	32	2	63	1	2	Poikilocytosis punctate basophilia not infrequent.
23.1.26	,,	4·5	13	32	2·1 27	4,600	51	1	46	1	1	Poikilocytosis marked, punctate basophilia infrequent.
27.1.26	,,	3·6	12	28	1·9 28	4,900	49	2	48	1	—	Poikilocytosis marked, occasional punctate basophilia.
29.1.26	,,	4·0	9	25	1·7 28	5,900	48	0	50	2	—	Poikilocytosis, marked anisocytosis (micro- and macro-cytes).
3.2.26	,,	3·9	9	24	1·4 30	3,800	56	1	41	1	1	Poikilocytosis and anisocytosis less marked. No punctate basophilia seen.
5.2.26	,,	3·2	6	21	1·3 31	4,000	40	2	58	—	—	Poikilocytosis very marked. Punctate basophilia not infrequent.
9.2.26	,,	3·3	7	21	1·3	5,300	53	1	46	—	—	Poikilocytosis very marked. Punctate basophilia very rare.

GRAPH III.—SHEEP 11899.

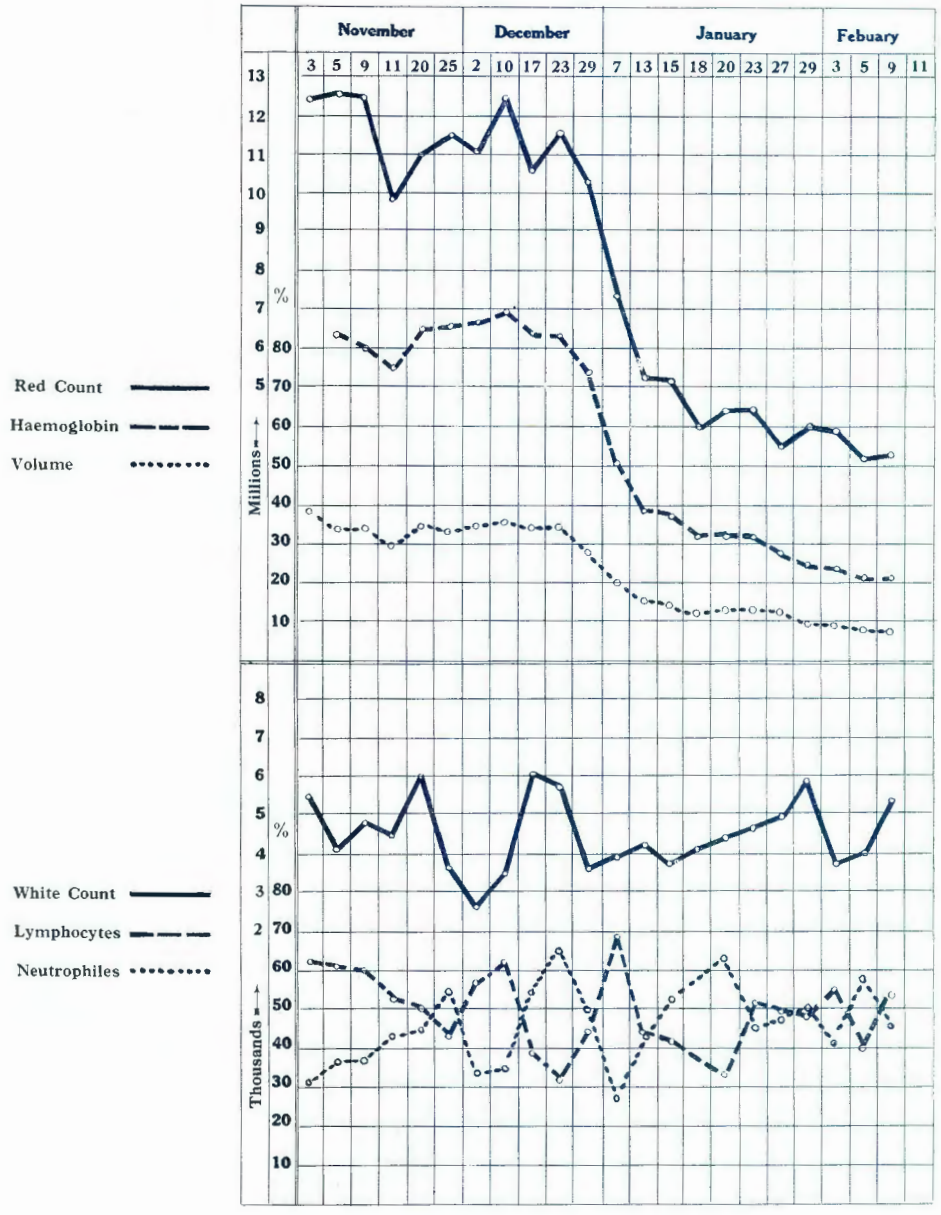


PLATE XXI.—Sheep 11899. Mixed infection Haemonchus and Oesophagostomiasis.

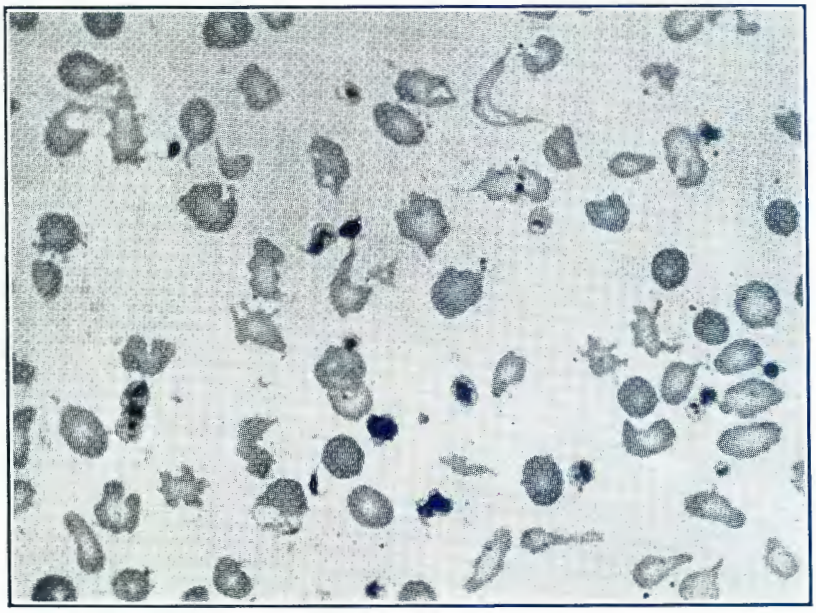


Fig. 1. (1250×).—27.1.26. Poikilocytosis.

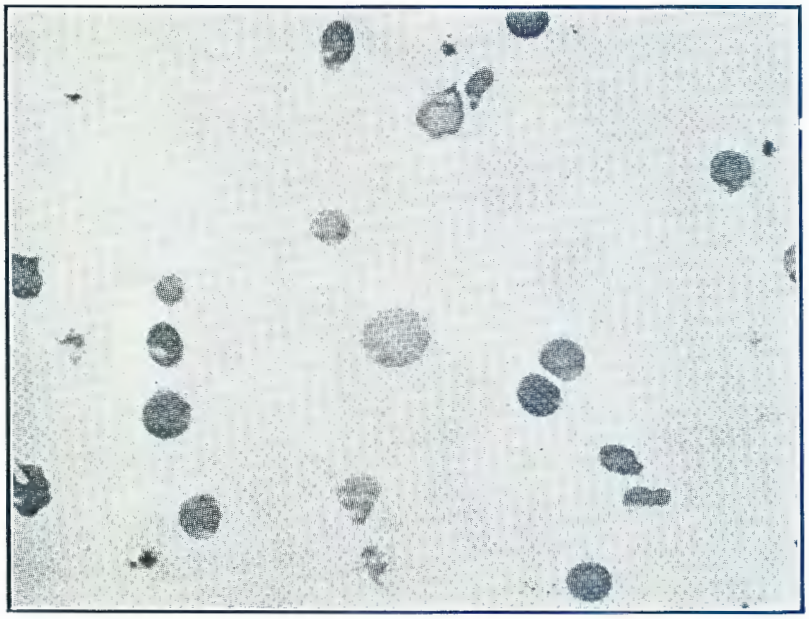


Fig. 2 (1250×).—29.1.26. Anisocytosis.

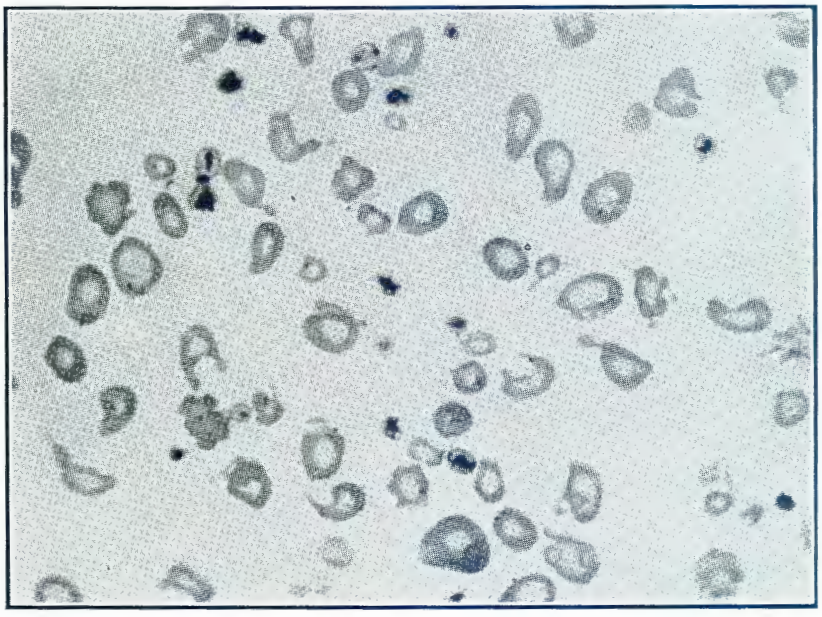


Fig. 3 (1250×).—5.2.26. Poikilocytosis and anisocytosis.

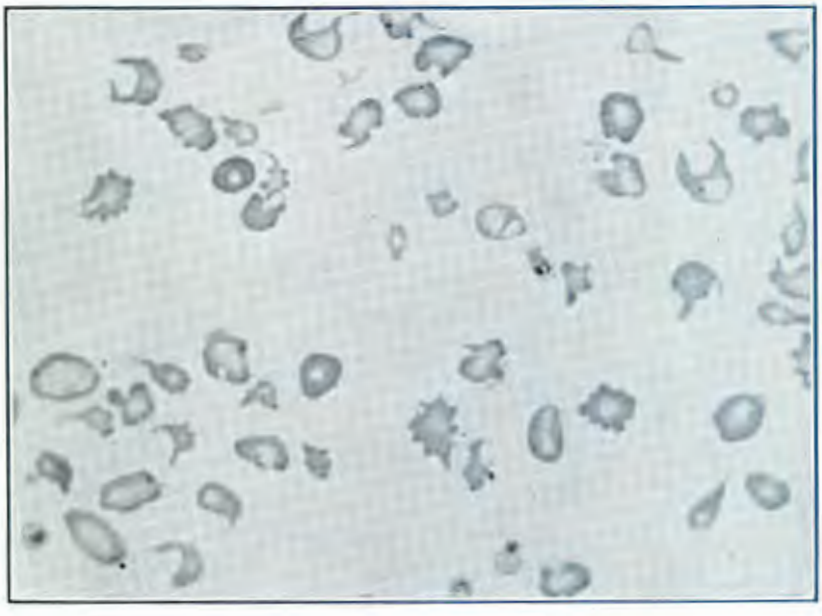


Fig. 4 (1250×).—Poikilocytosis and anisocytosis.

(3) Complications.

(*a*) *Bacterial Infection.*

As already stated there is no evidence that worms carry pathogenic bacteria with them during primary parasitic migration. The primary migrating larvae may, however, produce such gross anatomical changes in the mucous membrane, especially in severe infestations, that bacterial invasion of the mucous membrane can take place from the lumen of the intestine and produce various forms of enteritis (enteritis superficialis). When the animals are exposed to continuous infection on the pasture, primary and secondary parasitic migration will probably be present in many animals at the same time. In such cases bacterial invasion of the submucosa and other tissues of the intestinal wall deeper than the muscularis mucosae can also occur and produce deep inflammatory processes in the intestinal wall (enteritis profunda) and peritonitis in cases where the serosa becomes perforated. If enteritis is present the condition may be a contributory factor in the production of the diarrhoea characteristic of the disease. However, diarrhoea is a symptom in cases which, on post-mortem examination, were found to be free from bacterial complications. Therefore in such cases it seems that the parasites in the large intestine produce the irritation leading to increased motility of the intestine with consequent diarrhoea. Exactly how they do this (toxins, methods of parasitic feeding, etc.) is not known.

It is very difficult to assess the importance or otherwise of the rôle played by bacteria in oesophagostomiasis. There is no doubt whatsoever that the parasites can and do produce the death of the animal, in cases where bacterial complications are not present, but to judge from the number of cases in which bacteria were shown to be present in the deeper portions of the intestinal wall, during the course of this study, there must be quite a number of cases in which bacteria are a considerable contributory factor in the production of symptoms and of mortality.

(*b*) *Complications of a Mechanical Nature.*

(i) Hypertrophy of the plain muscle in the intestinal wall was described. It is likely that this may have been due to a partial stenosis, as a result of the presence of worm nodules and it is conceivable that in some cases a complete stenosis may be produced. One would think that the actual destruction of the tissues of the intestinal wall may lead to nutritive disturbances in those cases where very numerous nodules are present. This would not seem to be the case. One repeatedly finds, at post-mortem, very numerous nodules in animals which are in excellent condition. In such cases any bacterial invasion which may have occured has presumably been overcome and any adult worms which may have been present have been got rid of.

(ii) *Intussusception* (*Reksiekte*).—This is a condition which is sometimes met with in South African sheep. Whilst there is no evidence that this condition is always caused by oesophagostomum nodules, there is, in some cases of oesophagostomiasis, a very definite anatomical basis for the development of the condition. In these

cases the larvae cause complete destruction of the circular and longitudinal muscle fibres at a particular place during abnormal secondary parasitic migration. As a result of this, peristalsis may become completely interrupted here and invagination may take place.

SUMMARY OF THE PATHOGENESIS OF OESOPHAGOSTOMIASIS.

(1) Although toxins for the experimental reproduction of the disease have not actually been obtained from the nodular worms, there is very strong circumstantial evidence that during the course of the disease poisonous substances are formed and that these can produce the symptoms, lesions and death in the absence of bacterial and other complications.

(2) Although insufficient cases were available for haematological study, there seems to be a possibility that the toxic action of the parasites may also produce a certain amount of atrophy of the haemopoietic tissues leading to oligocythaemia, but a deficiency of the red cells to the extent of a clinical anaemia was not observed.

In some cases there is an eosinophilia. Whether this is due to their increased production in the myeloid tissues and their subsequent mobilization, or, whether they are produced locally in the walls of the intestine and are released temporarily into the circulation at the conclusion of the active tissue verminosis, cannot be stated with certainity.

(3) In some cases bacterial complications producing various forms of superficial and/or deep enteritis, as well as peritonitis, are contributory factors in the causation of symptoms and mortality in the disease.

(4) In some of the lesions there is a definite anatomical basis for the development of partial stenosis and intussusception. Apart from such accidents, the nodules themselves, even though they may be responsible for very extensive tissue destruction, do not seem to produce nutritive or other disturbances, in the absence of parasites in the lumen of the intestine and in the absence of bacterial complications.

REFERENCES.

CAMERON, T. W. M. (1933). Comparative Pathology of Helminths. *The Veterinary Record*, Vol. 13, No. 15, pp. 325-331.

CARNE, H. R., AND CLUNIES ROSS, I. (1932). The Association of the Bacillus of Preisz-Nocard with Lesions caused by *Oesophagostomum columbianum* in Sheep. *The Journal of Comp. Path. and Therap.*, Vol. 45, Pt. 2, pp. 150-157.

FENG, L. C. (1931). Studies on Tissues Lesions produced by Helminths *Archiv für Schivs- und Tropen-Hygiene. Path. u. Ther. exot. Krank*, Bd. 35, Nr. 1, pp. 1-10.

FOURIE, P. J. J. (1931). The Haematology and Pathology of Haemonchosis in Sheep. *17th Report of the Director of Veterinary Services and Animal Industry, Onderstepoort*, Pt. II, pp. 495-572.

FRASER, A. C. (1929-30). A Study of the Blood of Cattle and Sheep in Health and Disease. *First Report of the Director, University of Cambridge Institute of Animal Pathology*, pp. 114-163.

GOODEY, T. (1922). Observations on the Ensheathed Larvae of some Parasitic Nematodes. *Annals of Applied Biology*, Vol. 9, No. 1, pp. 33-48.

HADWEN, S. (1925). Ascariasis in Horses. *The Jnl. of Parasitology*, Vol. 12, No. 1, pp. 1-10.

HALL, M. C. (1920). Parasites and Parasitic Diseases of Sheep. *Farmers Bulletin* 1150, United States Dept. of Agric., pp. 42-45.

HEWLETT, A. W. (1923). " Intestinal Obstruction " Pathological Physiology of Internal Diseases, pp. 201-202.

JOEST, ERNST (1926). Handbuch der Speziellen Pathologischen Anatomie der Haustiere, pp. 802-804.

KEASBEY, E. (1923). On a New Form of Leucocyte (Schollenleukozyt, Weill) as found in the Gastric Mucosa of the Sheep. *Fol. Haemotolog.*, Vlo. 29, pp. 155-171.

MAXIMOW (1928). The Lymphocytes and Plasma Cells. *Special cytology*, Vol. 1. Edited by Cowdry, p. 348.

MÖNNIG, H. O. (1934). Veterinary Helminthology and Entomology. London. Bailliere, Tindall and Cox.

SPINDLER, L. A. (1933). Development of the Nodular Worm. *Oesophagostomum longicaudum* in the Pig. *Journl. of Agric. Res.*, Vol. 46, No. 6, pp. 531-542.

TAYLOR, E. L. (1935). Do Nematodes assist Bacterial Invasion of the Host by Wounding the Wall of the Intestinal Tract. *Parasitology*, Vol. 27, No. 2, pp. 145—151.

THEILER, A. (1921). The Nodular Worm and the Lesions caused by it. *Journal of the Department of Agriculture*, Vol. 2, pp. 44-51.

THOMAS, H. W. (1910). The Pathological Report of a Case of Oesophagostomiasis in Man. *Annals of Tropical Medicine and Parasitology*, Vol. 4, 1910-1911, pp. 57-88.

VEGLIA, F. (1923). Preliminary Notes on the Life-history of *Oesophagostomum columbianum*. *9th and 10th Reports of Dir. of Vetu. Educ. and Res.*, pp. 811-821.

WEINBERG, M. (1909). Oesophagostomose des Anthropoides et des ginges Inferieurs. *Archives de Parasitologie*, Tome 13, pp. 161-203.

WETZEL, R. (1934). Zur Ernährung und pathogenen Wirkung der Oesophagostomen. *Deutschen Tierärztlichen Wochenschrift*, Nr. 37, pp. 602-603.

WIRTH, D. (1931). Grundlagen einer klinischen Häematologie der Haustiere (Urban und Schwarzenberg, Berlin und Wien).