FURTHER OBSERVATIONS ON A LEPROSY-LIKE DISEASE OF THE RAT.

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THE object of this paper is to record briefly the result of certain observations and experiments on a disease of the rat, which is chiefly interesting from the close resemblance it bears to leprosy in the human subject. Not only are the lesions similar in their macroscopical and microscopical appearances, but the causal bacillus closely resembles the *Bacillus leprae* in its morphological characters, distribution in the tissues, staining reactions, and refusal to adopt a saprophytic habit of life.

A large number of acid-fast organisms have been isolated and studied within the past few years; from smegma, dung, various grasses, milk, butter, and nasal mucus, etc. However, only a few organisms belonging to this group have been observed which bear a causal relation to naturally occurring diseases among animals. Indeed these may be limited to *B. tuberculosis*, the bacillus of Verruga of Peru, *B. leprae*, and certain acid-fast organisms of the streptothrix group giving rise to somewhat ill-defined diseases.

Until this condition in the rat was observed no disease was known in any of the lower animals which pathologically or bacteriologically had any very close resemblance to leprosy in man.

The disease was first observed by Stefansky (1903), while working at plague in Odessa. He found that 5% of the rats (*Mus decumanus*) destroyed suffered from a condition affecting the skin and underlying muscles, and the lymphatic glands. Associated with the lesions, and apparently the causal agent, was a bacillus belonging to the acid-fast group. Stefansky gave an excellent description of the chief features of the disease. Rabinowitsch (1903), who had been working at Odessa during the time that Stefansky made his observations, on returning to Berlin was able to confirm Stefansky's results, for the rats there.

The disease had been observed in England and its nature recognised by the writer (1903) before the appearance of Stefansky's paper, and the experiments commenced in February, 1903, have been continued up to the present time. Since the first case was noted here, six other well marked examples of the disease, as it occurs naturally in the rat, have come under observation. Stefansky, Rabinowitsch, and the writer all stated that the animals which had been inoculated up to the time of their reports showed no signs of having acquired the disease, but since that time the writer has succeeded in obtaining a number of positive inoculation results in the case of rats.

The Disease as it occurs naturally.

Stefansky pointed out that there were two chief types of the disease, the one affecting primarily the skin and musculature, and the other confined to the lymphatic glands. There is, however, no very strict line of demarcation between the two types.

Skin and Muscle Lesions.

Patches of alopecia are the most marked feature. These may be extensive, affecting in some cases almost the whole thorax and abdomen. Above the surface of these bare patches of skin, elevated bosses, or even nodules, the size of a bean are frequently present. The surface of these elevations may be ulcerated, usually at the most prominent point. Ulceration is not, however, an essential feature and appears to result in part at least from mechanical causes (Fig. 1, Plate VI).

On incising an area affected in this manner the skin appears greatly thickened (up to half an inch), due to the presence of a pale yellow infiltration which cuts with a clean, dry, cheese-like surface. In most cases there is little tendency to softening and breaking down of the material. A scraping from its surface, stained by Ziehl-Neelsen's method, shows enormous masses of bacilli, many of them lying within the cells. Microscopical examination of a section through the skin and subjacent tissues reveals appearances which are almost identical with those found in the skin in lepra. The epithelial layers of the skin are but little affected; a few bacilli may be found to have forced their way between, or even into, the epithelial cells. The subepithelial infiltration is found to consist of cells lying in a stroma of connective tissue. The cells in part consist of leucocytes of different forms including lymphocytes, and in part of connective tissue cells; the endothelial cells

taking a prominent part in the formation of this granulation tissue. These cells are round, oval, or polygonal with a large nucleus and abundant protoplasm.

In a section appropriately stained one is struck by the presence of an enormous number of acid-fast bacilli with which nearly the whole of the cells of the tissue are packed (Fig. 5, Plate VII). The same difficulty in deciding whether the groups of bacilli lie in the lymphatic spaces is found as in the case of leprosy, but some of the appearances observed can be most satisfactorily interpreted in this way.

There is no doubt, however, that the majority of the bacilli actually lie within cells, and where these cells are of the large endothelial type an appearance strikingly resembling lepra cells results. The sebaceous glands and hair roots have mostly disappeared; those remaining appear to be undergoing a process of atrophy.

In the subcutaneous connective tissue the most prominent feature is the disappearance of fat. The whole region is infiltrated with cells, for the most part filled with bacilli. Here and there are small rounded or oval areas in which all appearance of cellular structure has disappeared; only *débris*, fragments of cell nuclei, and enormous masses of bacilli being visible. In one case in which the mammary gland was well developed the connective tissue framework and lymphatics were extensively invaded by the bacilli, but none of these could be found within the acini or ducts.

An interesting feature of the disease is the manner in which the striated muscle is invaded by the bacilli. Between the muscle fibres are frequently seen groups of cells of the types already mentioned. As these foci increase in size they cause atrophy of the muscle fibres and at the same time some of the cells burst and the bacilli penetrate the muscle fibre. In the early stage the bacilli are seen lying in the fibre, later this becomes degenerated, loses its striation, and as the invasion progresses only its outline is indicated by a tightly packed mass of bacilli round the nucleus. Even the outline may be lost by fragments of muscle fibre becoming more or less spindle-shaped and thus presenting the appearance of large cells.

Lymphatic Gland Lesions.

The lesions next in importance to those of the skin occur in the lymphatic glands. The glandular enlargement may be widespread; the axillary, cervical, and inguinal glands may all be affected, or only one of these groups. The enlargement may be considerable (Fig. 2,

Plate VI). In one case, in which most of the glands were involved, some of these had reached the size of a small hazel-nut. On section of the glands there are neither nodules nor necrotic areas visible to the naked eye.

Microscopical examination. The capsule may be thickened and may contain great masses of bacilli. The chief invasion of the gland itself appears to be along the sinuses, but where the disease is advanced the whole gland may be invaded. Along the sinuses there are large numbers of cells apparently of endothelial origin, rounded, oval, or polygonal, with abundant protoplasm and large nucleus. The whole of the protoplasm is packed with bacilli, and a few organisms are also found lying free (Fig. 3, Plate VII).

Giant cells are numerous and occasionally of enormous size, the transverse diameter reaching 70—80 μ , and containing many nuclei which may be situated either in the centre or at the periphery of the cell. They are sometimes vacuolated. The protoplasm of these cells is almost entirely occupied by masses of bacilli. They form beautiful objects either in smear preparations of glands or in sections (Fig. 2, Plate VII). Their mode of origin is difficult to determine just as in the case of lepra. It seems probable that they are greatly enlarged endothelial cells in which multiplication of the nuclei has taken place. The view, however, may be held that they are small lymphatic vessels which have been blocked by bacilli and that the nuclei are those of the endothelium originally lining the channel. The continuity of the cell outline is retained till a great size is reached, but in certain cases the cells seem to have become disintegrated from the overgrowth of bacilli.

Other Lesions.

When the cervical lymphatic glands were greatly enlarged an invasion of the neighbouring structures such as the submaxillary salivary glands was observed. Visceral lesions are rare—only in one case in a naturally infected animal has a small necrotic area containing the bacilli been found in the liver. The bone-marrow was found to be invaded.

An acid-fast bacillus was found in two cases in the nasal secretions, probably identical with that causing the lesions. This is interesting in relation to Stricker's (1899) observation of the frequency of the *Bacillus leprae* in the nasal mucus.

In one rat the loss of several toes was observed, and in others part of the tail had disappeared. As the writer has observed that bacilli

sometimes invade the nerve-trunks these losses may be connected with nerve changes. The lesions may, however, have been the result of trauma and not of disease.

The health of the diseased animals is sometimes seriously affected, and emaciation is a marked feature. Some of the animals were captured in broad daylight wandering outside their holes in a dazed condition.

The disease is evidently of wide distribution since it has been observed in Odessa, Berlin, and England. Six of the seven well-marked examples of the disease which have been examined were obtained within a few miles of Elstree, Herts.; the seventh was found in Norfolk.

The Bacilli.

The bacilli are straight or bent rods with round, or more rarely pointed ends, about 0.5μ broad and for the most part from 3 to 5μ in length, though sometimes longer forms are observed. They may have a beaded appearance, the beads being few and large, and causing a bulging of the rod. The bacilli are strongly acid- and alcohol-fast. They stain readily with carbol-fuchsin, and resist the action of 25% sulphuric acid or 3% hydrochloric acid in alcohol, or 2% anilin-hydrochloride—followed by alcohol. They stain by Gram's and Claudius' methods.

An interesting point about the staining of the bacillus which it has in common with that of leprosy is that if mounted in even slightly acid Canada balsam it gradually loses its bright red colour.

Cultivation experiments.

All attempts at cultivation of the bacillus in its acid-fast form have entirely failed. A large number of tubes containing all the ordinary laboratory media have been inoculated and kept under aerobic and anaerobic conditions but no growth took place. Special media have also been tried, such as a medium made from rat's flesh, and a medium with the addition of rat's blood-serum, but these have also failed to give a growth of the bacillus. Many pieces of tissues swarming with bacilli which have been left for over a year on appropriate media still show the bacilli staining well but no growth has taken place. As Rabinowitsch has pointed out, this makes it improbable that the bacillus is one of the known earth or dung bacilli, because these are easily cultivated and appear to be innocuous to rats. The writer has succeeded in obtaining from two of the affected rats cultivations of a diphtheroid bacillus.

This observation is of interest because a diphtheroid bacillus has been isolated by Babes and a number of observers from cases of lepra.

In his later publications Babes (1899) states that he has isolated this organism from twelve cases. Spronck (1898) isolated the same organism and found that it was agglutinated by the blood-serum of leprous patients, though not more so in certain cases than by the serum of non-leprous persons. As is remarked by Babes, a positive agglutination test would not prove the identity of this diphtheroid bacillus with the lepra bacillus, since if the bacillus is frequently associated with the acid-fast bacillus in cases of lepra it would naturally give rise to an agglutinating serum in these patients. The identity of the two bacilli would be demonstrated in a satisfactory manner only (1) if the diphtheroid bacillus could be so cultivated as to become acid-fast, (2) if it were demonstrated to have specific properties in relation to lepra such as the production of a specific tuberculin-like reaction, or (3) if it was found capable of producing lepra. Babes has carried out experiments in certain of these directions without, however, obtaining conclusive results.

Levy (1897 and 1899), Czaplewski (1898), Barannikow (1899), Teich (1899), and Kedrowski (1901) have all obtained cultures of bacilli resembling those of Babes. Kedrowski (1904) believes that he has obtained lesions resembling those of lepra by injecting his bacillus into rabbits.

The diphtheroid bacillus, isolated by the writer from rats, resembles the organism isolated by these workers, but its significance is open to the same criticism since, so far, it has been found impossible to convert it into the acid-fast one. There was no evidence of the acquisition of acid-fast properties by the diphtheroid bacillus after a sojourn of three months in the peritoneal cavity of the rat.

The only observation that makes it possible that the two organisms are in any way related will be found under the head of agglutination. A probable explanation of the presence of diphtheroid bacilli in lepra, and it may be also in the rat disease, is the fact that diphtheroid bacilli appear frequently to have their habitat in sebaceous glands, and in a weakened state of the skin they may be able to penetrate it and grow in the tissue.

Brief description of the main characters of the diphtheroid bacillus which was isolated from two of the diseased rats.

Morphology. In young cultures (24 hours old, on agar, or blood serum) it has the form of a small diplo-bacillus, or is sometimes very

like a Hofmann's bacillus. On the second or third day it becomes longer and has all the appearances of a typical diphtheria bacillus with segmentation, club-shaped ends, etc., and later it may form short, much segmented and branching filaments. In a two days' old culture, the club-shaped forms become very large, much segmented, and recall those of the *B. xerosis*.

Cultures. On solidified blood serum the growth is indistinguishable from that of the Klebs-Loeffler bacillus. On neutral agar and glycerine agar the growth is slower, the colonies are more delicate and transparent than those of the ordinary diphtheria bacillus. On neutral and alkaline broth it forms no pellicle, but grows in the form of clumps along the test-tube wall, or as a flocculent deposit at the bottom of the test-tube. In glucose broth the growth is granular but not so abundant as that of the diphtheria bacillus. It forms acid in 24 hours, but not so strongly as the diphtheria bacillus. It forms no visible growth on potato.

Pathogenicity. The diphtheroid bacillus has a feeble pathogenic action on young rats. Three out of four young rats that received 2 c.c. of a broth culture a week old died within a week. Old rats proved resistant.

Agglutination tests.

No great importance can be attached to these agglutination tests in view of the absence of specificity which some observers have demonstrated in regard to the agglutination of the acid-fast group of bacilli. The tests are of a preliminary character and are included in this paper merely as an indication of a line of research which it was thought might throw some light on the relationship of the organisms.

Preparation of bacterial suspension. A piece of tissue, very rich in the acid-fast bacillus of the rat, was removed from the methylated spirit in which it had been preserved, allowed to dry, and ground up in a mortar with glass powder in normal salt solution. After standing for some time it was found that nearly all the fine pieces of tissue had sedimented, and that the supernatant opalescent fluid consisted almost entirely of acid-fast bacilli. The fluid was brought to a degree of opacity suitable for carrying out naked eye tests in Wright's agglutination tubes which were used on account of a very small quantity of serum being available. The bacterial suspension remained almost unaltered for 24 hours.

*Leprosy-like Disease of Rat*TABLE I. *Result in three hours.*

TEST 1.

Dilution	Normal Serum	Leprous Serum
1 : 2	Nil	Large sediment
1 : 4	"	" "
1 : 8	"	" "
1 : 16	"	" "
1 : 32	"	Distinct sediment
1 : 64	"	Trace

TABLE II. *Result in sixteen hours.*

TEST 2.

Fresh Dilutions	Normal Serum	Leprous Serum
1 : 2	Nil	Large sediment
1 : 10	"	" "
1 : 20	"	" "
1 : 40	"	Distinct sediment
1 : 50	"	Trace
1 : 60	"	Nil

TABLE III. *Agglutination of diphtheroid bacillus emulsion prepared from agar culture of three days' growth by means of shaking with glass beads.*

Serum	1 : 2	1 : 10	1 : 20	1 : 30	1 : 40
Normal (human)	0	0	0	0	0
Human (tubercular case)	0	0	0	0	0
Lepra serum	+	+	+	+	0
Normal rat	0	0	0	0	0
Rat (inoculated with acid-fast bacilli)	+	+	0	0	0
Rat 2 (ditto)	+	+	0	0	0

In these tests :—

(1) the serum from a case of human leprosy agglutinated the acid-fast bacillus from the rat; normal human serum had no agglutinating power;

(2) the serum of rats inoculated with the acid-fast bacilli agglutinated the diphtheroid bacillus; normal rat's serum did not agglutinate the bacillus;

(3) normal human serum and the serum from a tuberculous patient failed to agglutinate the diphtheroid bacillus, whereas the serum from a case of leprosy had distinct agglutinating properties.

Note. The serum from only one normal, one tuberculous, and one leprosy patient, was used in these tests. I wish here to express my thanks to Dr Lie of Bergen for his kindness in supplying some blood from a case of leprosy.

Inoculation Experiments.

I. Rats.

Thirty black and white rats have been inoculated with material consisting of emulsions made from the infiltrated skin and enlarged lymphatic glands obtained from the naturally, or experimentally, infected animals. A considerable number of these died of intercurrent disease without obvious signs of having been infected with the acid-fast bacillus, though in many of them, even in the absence of naked-eye changes, the organism could be demonstrated to be present in the peritoneum after two or three months. Nine animals, however, showed marked lesions, evidently produced by a multiplication and invasion of the acid-fast bacillus, and in some of these the lesions were of a very striking character.

In three cases the infection was carried on successfully from the naturally infected animal to an experimental animal and from the experimentally inoculated rat to a third rat: the lesions in the two generations of experimentally infected animals were similar.

Course of the Disease in the Inoculated Rats.

The disease runs a very slow course in the inoculated animals. In one case a rat died about one year after inoculation with well-marked evidence of a progressive invasion of the bacillus. In others well-marked lesions developed and death occurred within six months of inoculation. There was no definite evidence of increase of virulence in the third generation.

Pathology.

Subcutaneous Inoculation was in several instances followed by the production of a local lesion but this was not a constant result. The lesion consisted of a nodule containing a semi-caseous-like substance resembling the material which was present in the skin infiltration of the naturally infected animals. The acid-fast bacilli were very numerous in these local lesions. The lymphatic glands in such cases, even at a distance from the lesion, were sometimes invaded by the bacilli and showed the changes already described. For example, in one case where the local lesion was situated in the groin the axillary lymphatic glands were infected.

Intraperitoneal Inoculation. The most marked lesions resulted from intraperitoneal injections. In these the epiploön presented a

striking appearance. It was frequently infiltrated with grey opaque looking masses of material which on microscopical examination proved to be masses of cells containing bacilli. The peritoneum was in some cases thickened, and pale yellow patches of infiltration occurred on both the visceral and parietal surfaces.

Liver. As a rule, even when the infection was well-marked, the liver showed nothing abnormal or the capsule only was involved. In one case, however, the organ was beset with small, pale, yellow, sharply circumscribed areas to such an extent that about a third of the liver substance seemed to be destroyed. A second case showed the condition in a less advanced stage. The areas under the microscope were seen to be sharply circumscribed and surrounded by flattened liver cells. The process was very extensive close to the peritoneum and seemed to extend along the Glisson's capsule. When stained by Ziehl-Neelsen's method the appearance presented under a low power is shown in Plate VII, Fig. 1, which is typical of what is found throughout the liver. The bulk of each nodule appears to be composed of masses of acid-fast bacilli. At the periphery these are mostly arranged in round masses as if lying in cells or cell remains. Some of these round masses are very large and so tightly packed with bacilli that they appear almost homogeneous. In the centre of the nodule the cellular appearance may be less marked, many of the bacilli appearing to be free. Round some of the nodules the capillaries are greatly dilated, and in the endothelium of these there are groups of acid-fast bacilli.

Spleen. The capsule was thickened and contained bacilli in several cases, but only in one was the organ itself markedly involved. This occurred in the case in which the liver was most affected.

Kidneys, Bladder, and Genital Organs. In several instances the capsules of these organs were thickened and infiltrated with bacilli but so far as could be found no extension of the process to the organ itself had occurred. In the case of the testicle and epididymis groups of bacilli were found lying in the connective tissue framework of the organ.

Thorax. In three of the inoculated animals a very striking appearance was found on opening the thorax. A tumour-like mass occupied the anterior mediastinum, and the posterior surface of the sternum was covered with an infiltrating material which in one case reached a thickness of a quarter of an inch. The pale yellow tumour-like mass occupied a considerable part of the thorax and was moulded over the pericardium and apices of the lungs. On microscopical

examination only small areas of lymphatic gland tissue were visible, the bulk of the mass consisting of the acid-fast bacilli. This tendency, especially in rodents, for the anterior mediastinal glands to become infected from the peritoneum has been frequently observed.

Lungs. Lesions of these organs were not common, but in two cases a few small nodules, the size of millet seed, were observed. These appeared to originate from the lymphatic vessels lying beside the small bronchi and vessels.

The *Pericardium* and *Epicardium* were secondarily invaded in these cases with retrosternal tumours.

Myocardium. A small nodule containing the bacilli was in one case found in the myocardium.

Blood. On several occasions bacilli were seen lying within the blood-vessels.

The Central Nervous System. In two cases the bacilli were looked for in the brain and spinal cord but were not found.

Nerve-trunks. Bacilli were found to have invaded these both in the experimental animals and in those naturally infected.

II. Inoculation of Animals other than Rats.

Guinea-pigs. Thirteen animals were inoculated; of these only one developed a small local subcutaneous lesion which contained the acid-fast bacilli. None of the others showed any effect.

Rabbits. Six animals inoculated; showed no effect.

Mice. Ten animals inoculated. Six of these died, but without showing any lesions which could be attributed to the acid-fast bacillus.

Monkey (Macacus rhesus). After 18 months showed neither local nor general signs of infection.

These results show that we are not dealing with Tubercle bacilli, especially as in some of the guinea-pigs and rabbits very large doses were given.

Remarks and Conclusions.

One of the chief features common to this disease in the rat and to lepra in the human subject, namely the presence of the bacilli in such large numbers within the cells, has been regarded by Babes as the evidence of a form of symbiosis between the cells and the bacilli. The bacilli invade the cells and live within them without interfering with their vital functions.

It seems probable, however, that we are both here and in leprosy dealing with the phagocytosis of a relentlessly invading germ of great resistance, which, notwithstanding the fact that it finds itself in a suitable medium, has little power of producing toxic substances. In the case of tubercle, after a few bacilli have been taken into a cell, so much toxin is secreted by the bacilli that necrosis and death of the cell occurs. Here, on the other hand, the bacilli when taken into the cells are able to continue their growth and having only feeble toxigenic properties they have relatively little deleterious action on the cells, which maintain their vitality and for long continue to struggle against the invaders. The slowness of the struggle may possibly account for the enormous size of the giant cells.

It would be out of place here to go into the literature of the attempts and failures to inoculate animals with the *Lepra* bacillus; one may, however, refer to the work of Iwanow (1902), who, working in Metchnikoff's laboratory, succeeded in obtaining results in guinea-pigs which he regarded as indicating that a certain multiplication of bacilli may have taken place. The appearances which he observed in the epiploön of the guinea-pig resemble those observed in the slighter form of invasion in the case of the rat disease. It would be of interest to know whether the rat has been used for inoculation experiments with leprous material. So far as could be learned from a brief review of the subject this does not appear to have been the case [Wolters (1893); Finger (1901), and Hansen (1902)].

The features of resemblance between these two diseases are so strong that they point to a close relation between the associated bacilli.

The consideration that man and the rat are fellow victims of almost equal susceptibility to other disease germs such as the plague bacillus, might even suggest the possibility that in lepra and in this rat disease we are dealing with the same micro-organism affecting two species.

There is no intention here, however, in the present state of our knowledge to uphold such a hypothesis, which is obviously rendered improbable from epidemiological considerations alone.

As has been indicated in discussing the interpretation of the appearance in the infected animals, many of the pathological features of resemblance probably depend on the slow invasion of, and the defence offered by, the animal's tissues against a highly resistant organism which is not markedly toxigenic.

Fig. 1.

Fig. 2.

FIG.1.

FIG.2.

FIG.3.

FIG.4.

FIG.5.

CONCLUSIONS.

1. This disease of the rat has a remarkable resemblance, both in its pathological, anatomical, and in its bacteriological features to *Lepra* in the human subject.

2. All attempts at cultivation of the acid-fast bacillus have failed.

3. A diphtheroid bacillus has been isolated and cultivated from two cases of the disease, but so far attempts to convert this into an acid-fast form have been unsuccessful.

4. The experiments on agglutination having been of a preliminary character do not permit as yet of any definite conclusions. Nevertheless the bacillus was agglutinated by the serum of a leprous patient.

5. The disease could be conveyed to other rats (but not to rabbits, guinea-pigs, mice or a monkey) by subcutaneous and by intraperitoneal injection of emulsions of the bacilli from infected tissues.

6. A large number of the rats inoculated succumb to intercurrent diseases but a certain number develop well-marked characteristic lesions which can be again reproduced by reinoculation.

7. The disease in these artificially infected animals runs a very slow course, which may extend to about one year.

EXPLANATION OF PLATES VI AND VII.

Plate VI. Fig. 1. Photograph of rat (*Mus decumanus*) showing the appearances presented in a case of natural infection with the acid-fast bacillus. Note the patches of alopecia, two of which show small ulcers.

Fig. 2. Photograph showing a dissection of the enlarged cervical and axillary lymphatic glands in a case of natural infection of the rat with the acid-fast bacillus.

Plate VII. Fig. 1. Drawing of the microscopical appearances presented by a section of the liver of a white rat which had been inoculated intraperitoneally six months before death. The red colour of the affected areas in the liver is due to the presence of masses of the acid-fast bacillus. Ziehl-Neelsen's solution, 3% hydrochloric acid-alcohol, aqueous methylene blue. $\times 100$.

Fig. 2. Drawing of a giant cell containing bacilli. From a smear preparation of the axillary gland of a white rat which died eleven months after subcutaneous inoculation. Ziehl-Neelsen's solution, 25% sulphuric acid, aqueous methylene blue. Under oil immersion lens. $\times 825$.

Fig. 3. Drawing of the microscopical appearances of a section of an infected lymphatic gland. Shows the cells of endothelial origin packed with acid-fast bacilli. Ziehl-Neelsen's solution, 3% hydrochloric acid-alcohol, aqueous methylene blue. $\times 825$.

Fig. 4. Same cell as seen in Fig. 2. $\times 100$.

Fig. 5. Drawing of the microscopical appearances of a section through the affected skin of a rat suffering from the naturally acquired disease due to the acid-fast bacillus. Most of the red colour below the epithelium is due to the presence of large numbers of bacilli. Stained with Ziehl-Neelsen's solution, 25% sulphuric acid, aqueous methylene blue. $\times 100$.

The drawings are by Mr M. H. Lapidge.

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