B. PATHOLOGY

Pathology and Bacteriology of Early Lesions in Leprosy

D. S. Ridley[1]

It is accepted by most people that the leprosy bacillus makes its entry into the body through the skin, though the evidence for this view is based on epidemiology (2, 3, 8, 20, 22) and so far lacks pathologic support. How the organism establishes itself thereafter is a matter of some confusion. It was already known by the turn of the century that in early macules the bacilli were to be found in the small nerve branches of the skin, and that ascending degeneration of the nerve followed (4, 5, 6). It was also widely held that the early lepromatous foci developed in the small blood vessels of the skin, from which dissemination occurred via the blood and lymphatic circulation (13). Khanolkar (12) put forward the view that all leprosy is neural in its inception, and others (25) that the bacilli find their way to Schwann cells, their target, via the blood stream. Recently it has been suggested that muscles serve as a target site comparable in importance to nerves (9). Although the pattern of established infections is thought to be governed by cell-mediated immunity (23), it is not known at what stage of the infection it becomes effective (21). A further study of the pathology of early lesions seemed to be indicated.

MATERIAL AND METHODS

This study is based on skin biopsies from approximately 100 patients who were suspected clinically of having early leprosy. We are concerned, however, only with 51 in whom the diagnosis was confirmed by histology as either (a) indeterminate (unclassifiable) leprosy, or (b) early classifiable leprosy in which the granuloma occupied no more than 1/20 of the dermis. Clinically the lesions were macules, hypopigmented patches, tuberculoid patches or areas of anesthesia. In the majority of cases only one solitary lesion was present; these solitary lesions are often regarded as being the primary site of infection, though proof is lacking (2, 15, 20). It is likely that these biopsies represent a later phase in the infection than those of Khanolkar (12), who examined contacts of patients, and a somewhat earlier phase than the one dealt with by Büngeler (1), many of whose patients had multiple lesions. All the accepted patients were thought to be untreated, apart possibly from native remedies. The group included patients from various parts of the world, but the majority were from Papua and New Guinea.

Only one biopsy was received from each patient and only one section was examined for bacilli if it was a good sized specimen; two or three were examined in the case of small punch biopsies.

RESULTS AND DISCUSSION

Classification. The patients can be placed conveniently in four groups, according to their degree of success in restricting the infection to a single site and in restraining the multiplication of bacilli. The original grouping, however, was made on a purely histologic basis; it was found only later that this correlated with evidence of resistance.

In group I the essential feature was invasion of the epidermis by lymphocytes (Fig. 1), or by epithelioid cell granuloma which eroded the basal layer and was to some extent intraepidermal. No acid-fast bacilli (AFB) were found in any of the eight cases in this group. The lesions were confined to a single site, though there was a tendency to develop satellite lesions or tubercles. This group was judged, therefore, to have maximal resistance.

In group II there was a small epithelioid cell granuloma originating within a nerve bundle, usually in the deep dermis. In most

[1]D. S. Ridley, M.D., F.R.C. Path. Hospital for Tropical Diseases, London, N. W. 1.

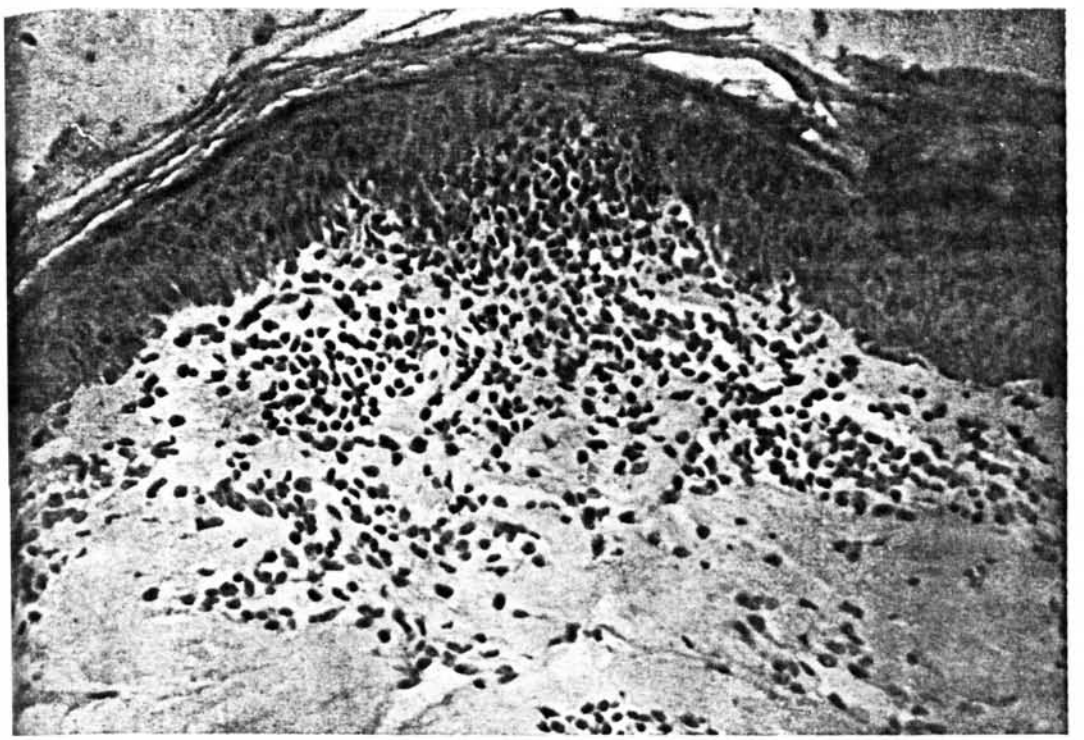

FIG. 1. Cellular infiltration of the epidermis. Group I. No AFB found.

cases the perineural sheath was intact. AFB were found in eight out of 11 cases in this group, and in all except one the patient had only a solitary lesion. There was some overlap between groups I and II. Any patient in whom infiltration of the epidermis was observed was put in group I, but two patients in group I also showed a granuloma in a nerve, and two other cases (one in each group) could have been placed almost equally well in either group.

Group III, the largest of the four groups, consisted of the indeterminate cases with a nonspecific infiltrate in the dermis, which have been studied in great detail by Büngeler (1). As Büngeler states, the reasons for considering these lesions as being due to leprosy are (1) the finding of AFB in a proportion of cases, and (2) the manner of evolution of the lesions with the subsequent development of tuberculoid or lepromatous characteristics. The histologic features of indeterminate leprosy are: (1) infiltration of lymphocytes and histiocytes around hair follicles and other skin structures, in addition to perivascular infiltration; (2) proliferation of spindle-shaped cells in the superficial dermis, which look like fibrocytes, but in established lesions prove to be phagocytic; neither of these features is conclusive evidence of leprosy, but for the purpose of this study they were regarded as confirming a clinical diagnosis; (3) more definite is a proliferation of Schwann cells; and (4) conclusively, the finding of intracellular bacilli in association with the histologic features referred to above (17). Twenty-three patients were grouped as indeterminate (i.e., group III) and bacilli were detected in nine, a lower proportion than in group II. On the other hand, a higher proportion of patients in the indeterminate group, one-third, had multiple lesions and in them the infection was safely assumed to have reached the stage of dissemination. Since the lesions in this group are less mature histologically than those of groups I and II, and no larger in size, the fact of their early dissemination is taken to indicate a lower level of resistance than in the other

two groups. The indeterminate patients are not, however, a homogeneous group. It was noted that the lesions in which Schwann cell proliferation was prominent tended to be solitary lesions, and probably had a tuberculoid element in their make-up whereas many of the lesions in which infiltration was predominantly perivascular were, not surprisingly, disseminated. Many of these may, perhaps, have had a lepromatous tendency. It has been observed, however, that three-quarters of patients with early indeterminate lesions are self-healing ([14]), and the same would probably apply to the patients in groups I and II.

In the lesions of group IV there was a small granuloma with the characteristics of early borderline or lepromatous leprosy. There were nine cases and in all except one the granuloma was situated in the superficial zone of the dermis around small blood vessels. Bacilli were found in the granuloma in every case. Although the clinical records are incomplete, it appears that the lesions were multiple except in two cases that verged on group II, and it is reasonable to assume that dissemination occurred at an early stage via the blood stream. Resistance, therefore, was minimal.

In terms of the five group spectrum that is used in the classification of established leprosy infections ([18]), the four groups of the early lesions can be interpreted approximately as follows:

Group I: 1st and 2nd stages of TT

Group II: 2nd stage of BT

Group III: 1st stage of BT, BB, BL and LL

Group IV: 2nd stage of BB, BL, and LL; also some cases close to BT.

If self-healing does not take place, group III evolves to Group IV. Group II may also evolve to group IV.

Sites of Bacilli. In group I there were no bacilli and in group IV bacilli were multiplying freely in the granuloma, though they were found also in other situations. In groups II and III (with granuloma in nerve, or indeterminate histology) bacilli were not multiplying freely, and their location was of some interest. The two groups can be considered together, making a combined total of 17 bacteriologically positive cases (Table 1).

Bacilli were detected more or less equally in small nerve bundles, in arrector pili

TABLE 1. *Locations of bacilli in Groups II and III (17 bacteriologically positive patients).*

	SEZ	Muscle	Nerve	Capillary
Number of cases + ve.	9	8	10	3
Sole location	4	1	5	0
Total number of bacilli	28	21	27	6
Mean number of bacilli per section:				
Group II	3.0	1.0	2.0	0.8
Group III	0.5	1.5	1.0	0.6
Morphologic index	32	60	88	83

muscles and in the subepidermal zone (SEZ). Less frequently they were found in capillary endothelium; they were not found in any other sites in these two groups. There was no significant difference in the distribution of bacilli between the two groups except that they were observed in capillaries only in group II. Bacilli in this group were also twice as numerous per patient as in group III, confirming perhaps that lesions of group II were in some ways more advanced than those of group III even though they were not disseminated. The morphologic index was highest in bacilli in nerve, and lowest in those in the SEZ. Club forms of bacilli were scanty especially in nerves, though in early relapsing lesions in skin they are numerous ([19, 26]).

It is well known that in this type of lesion bacilli are to be found in nerves, and surprising that they should be found to occur in almost equal numbers in the SEZ and arrector pili muscles. My attention was first drawn to their presence in the SEZ by Dr.

J. P. Wiersema (personal communication). As he said, they often appear to be lying in collagen, and this may sometimes be the case. Others may well be situated in the fixed tissue histiocytes whose branching cytoplasmic processes are not visible in haematoxylin-eosin sections (Fig. 2). They were found immediately beneath the basal layer of the epidermis (Fig. 3). There was never any cellular reaction around the bacilli in the SEZ.

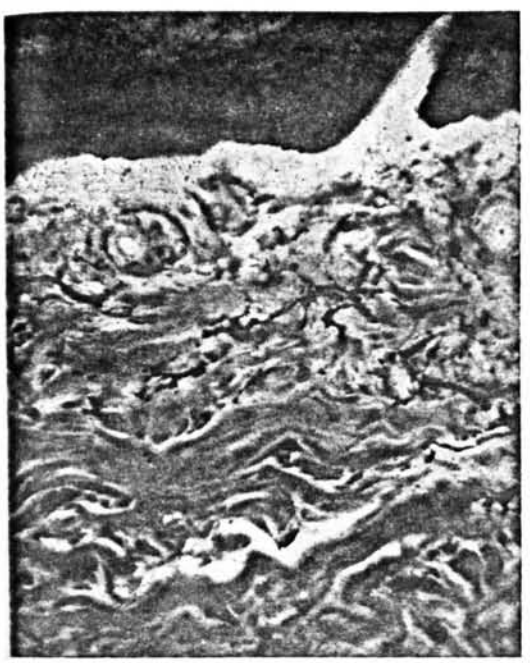

FIG. 2. Fixed tissue histiocytes in the superficial epidermis of normal skin (Weil-Davenport stain).

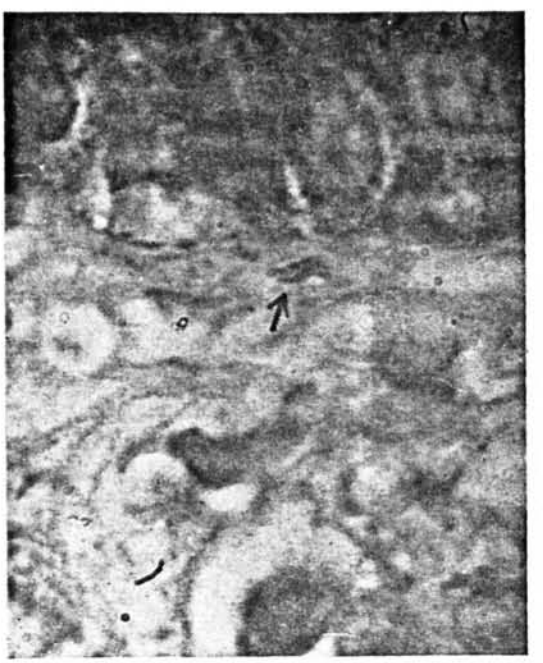

FIG. 3. A small group of AFB just beneath the basal layer of the epidermis. Group II.

In muscle bundles bacilli were often not oriented to the direction of the muscle fibers. Cellular reaction in muscle was fairly infrequent, and whenever infiltration of lymphocytes and histiocytes was seen bacilli were absent at that site. In nerves, by contrast, solid-staining bacilli appeared to survive in the presence of quite severe cellular infiltration by chronic inflammatory cells invading through the perineurium; bacilli, however, were never found in the presence of epithelioid cells in a nerve bundle. A granulomatous reaction was much more frequent in nerves than in muscle: in the whole series of 51 biopsies there were 14 granulomas in nerve against three in muscle.

Two points to be emphasized: (1) Nerves offer a safer refuge for bacilli than muscle or the SEZ, if the evidence of the morphologic index is accepted. (2) An epithelioid cell reaction was commonly observed in nerves while in other parts of the section AFB were seen to be lying unmolested in the SEZ or a muscle (Fig. 4). I will come back to these points later.

Pathogenesis of Lesions: Bacterial Invasion. Infiltration of the epidermis by inflammatory cells or granuloma is an exceptional occurrence in the field of dermatology. It must be interpreted in conjunction with the observations (1) that it is found only in leprosy patients with the highest resistance—group I of the early lesions or polar tuberculoid in established lesions, in each of which it is not normally possible to detect bacilli; (2) that in this group the nerves of the dermis are histologically ap-

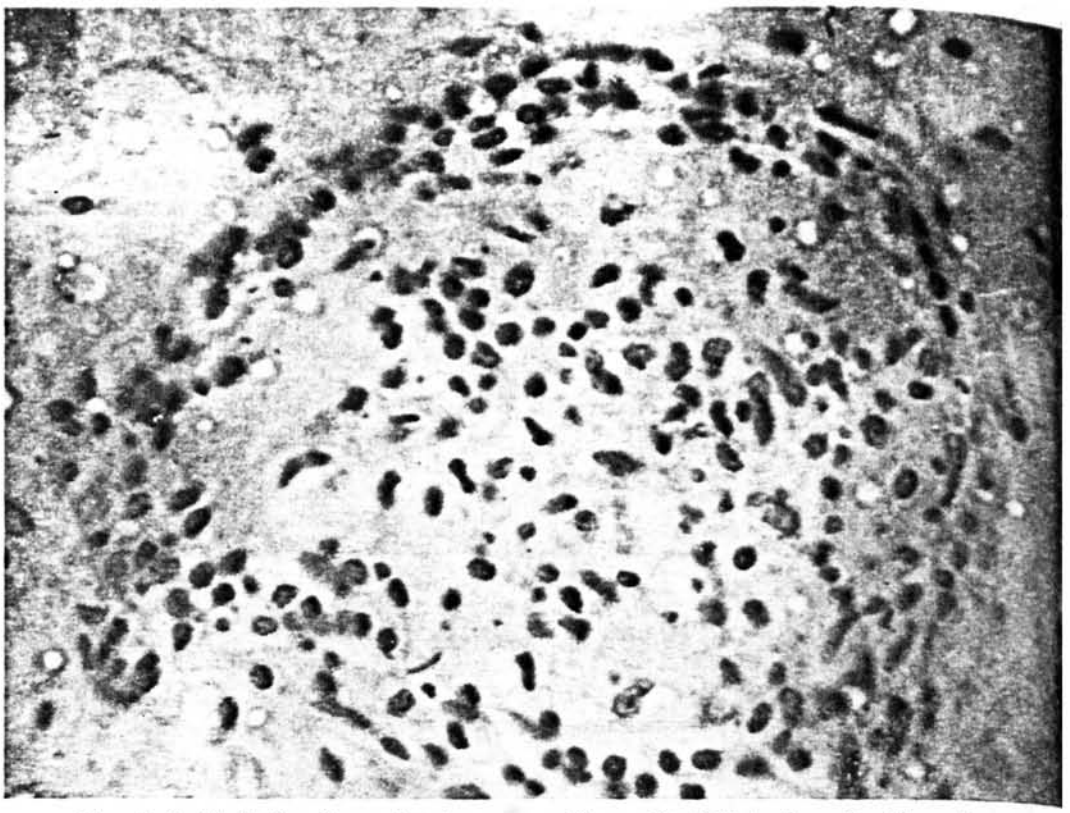

FIG. 4. Epithelioid cell reaction in a nerve. Group II. AFB in the subepidermal zone caused no tissue reaction.

proximately normal, at least in the early stages; and finally (3) that these lesions are nearly always solitary and therefore presumably the site of primary infection. All of this points to the likelihood that bacilli enter via the skin and in patients with maximal resistance they are mopped up by the inflammatory reaction before they have penetrated the epidermis (Fig. 1); or, if that fails, they are contained in a granuloma at the level of the basal layer.

In group II immunity is less effective. Bacilli penetrate the epidermis and are found in the SEZ immediately deep to the basal layer. Further support for this hypothesis is given by an examination of the lesions of group III, some of which are solitary, some multiple. Thus far I have been able to detect bacilli in the SEZ only in the solitary lesions, i.e., at the sites of primary infection in which bacilli have penetrated the epidermis. Bacilli have not been found in the SEZ in the multiple lesions, although at a later stage (group IV) they are seen in the capillaries of the papillary zone.

Bacilli sometimes look as if they have made limited multiplication in the SEZ, and produced small clumps of about six organisms. But in general the MI of these bacilli is low and they appear not to establish themselves unless they succeed in gaining access to a muscle bundle, or preferably a nerve. How they arrive in one of these situations is not clear.

If immunity is low, however, dissemination via the blood stream may occur at an early stage with the production of multiple lesions showing nonspecific perivascular infiltration. Bacilli are not often found in lesions of this type. When ultimately they make their appearance it is in a small granuloma, at which stage the lesion belongs to group IV. But even at this stage

the bacilli show a predilection for nerves in some cases.

To confirm that leprosy bacilli enter the body via the skin it would be necessary to demonstrate them in the epidermis or at a site of injury or an insect bite. This might be difficult. I have failed to find AFB in the epidermis in any of the cases in groups II and III. They were present in several of the cases in group IV, but the objection here is that bacilli might have been displaced from the granuloma in cutting the section. One of the patients with a solitary lesion, however, showed fairly numerous bacilli in the epidermis. Further sections of this lesion were cut, therefore, in a downward direction so that displacement of bacilli would be away from the epidermis, and the knife was carefully cleaned with xylol in between each section. After this procedure up to ten bacilli per section were found in the epidermis, mainly in squamous cells. It is known that epidermal cells can phagocytose particulate matter with which they come in contact ([16]). Whether these bacilli were making their way inward or outward through the epidermis, they suggest that under some circumstances *M. leprae* may be able to penetrate human epidermis. But the evidence is still not conclusive.

Pathogenesis of Lesions: Immunity. Kato and Gozsy ([10, 11]) thought that in the pregranulomatous stage of murine leprosy mast cells play an active role in defence, governing the phagocytic activity of capillary endothelium. Their number reached its maximum at 40 days, and their subsequent disappearance coincided with the dissemination of the infection. To some extent this was confirmed in the 49 human lesions.

Mast cells accumulated around the small inflammatory foci and the affected muscles or nerves of groups I and III and to a lesser extent in group II. In group IV the number of mast cells appeared to vary inversely with the number of bacilli in a focus. In large granulomas of established leprosy lesions they were absent, but they were found to reappear in old regressing lesions when the bacilli became scanty after prolonged treatment. They are seen also in reactions with scanty bacilli. The reason for the sharp fall in the number of mast cells around a granuloma above a certain size is no doubt that they become degranulated and probably disrupted in response to some stimulus, but whether this is the cause or effect of the progression of the infection is hard to say.

A point of interest is that at Karamui, in New Guinea, it is the local practice to treat white blotches of the skin by linear scarification with a sago palm thorn and rubbing ash or plant juices into the wound (D. A. Russell, personal communication). The people believe that a few cases of leprosy have been cured in this way ([7]). A few of these scarified lesions have been biopsied. As would be expected the injury causes an increase of mast cells which is beyond anything seen in leprosy. There was some evidence that in one or two lesions the leprosy might have been modified and displaced from the superficial dermis where mast cells are most numerous to the subcutaneous border where they are scanty. But on balance it seems unlikely that the mast cell response often has a decisive influence on the course of a leprosy infection. If ever it is decisive it could only be in the very early stage.

Apart from mast cells, the predominant inflammatory cell types in all groups were lymphocytes and phagocytic cells of the histiocytic series. These cells are such a common feature of all early inflammatory reactions that it would be unwise to attach much immunologic significance to them. The infiltration of the epidermis by lymphocytes in group I and the dense perineurial cuffing of these cells in groups II and III does, however, suggest that cell-mediated immunity may be operative by the time lesions have become clinically apparent. In group I it appears to be operative at a very early stage.

It is difficult to give a sensible immunologic interpretation of the cellular reaction in early lesions. Whatever the nature of the immunity may be, its mechanism is clear: leprosy bacilli are destroyed in epithelioid cells (whose development is thought to depend on lymphocytes). The safest refuge for the bacillus, to judge from the MI, is a nerve bundle, and yet this is the very

situation in which an epithelioid cell response is most likely to develop. As already stated, bacilli are quite commonly found to have been destroyed in nerve by an epithelioid cell reaction, while elsewhere, in SEZ or muscle, they are found to be unmolested by cellular reaction. At a later stage, when the bacillus is no longer dependent on neural protection, the most likely site in which to find active bacterial multiplication is the superficial zone of the dermis or even the SEZ, where previously activity was most sluggish. The most likely explanation is that bacilli are active in different habitats according to the level of immunity, which may well vary in different phases of the infection. The predilection of leprosy bacilli for nerves is often attributed to protection against immunological forces, but nerves are clearly not protective against the lymphocyte-epithelioid cell system. Furthermore, lymphocyte-mediated immunity is operative in other mycobacterial infections and leishmaniasis, in which the organisms find no advantage in a neural habitat. If nerves are protective, therefore, it must be against some other defense system which is operative in the early stages of leprosy infections, which may, or more likely may not, be the mast cells.

In conclusion, it seems likely that in a small proportion of patients who contract leprosy the lymphocyte-mediated system may be effective from a very early stage (group I). In them nerve involvement appears to be unimportant. In a larger group lymphocyte-mediated immunity is moderate, or only becomes effective at a later stage, and the initial defense depends partly on some other factor against which nerves offer protection until bacilli have established themselves (group II and some cases of group III). In the remaining cases one or both of the defense mechanisms are weak and dissemination of the infection occurs at an early stage. Wade and Ledowsky [24]

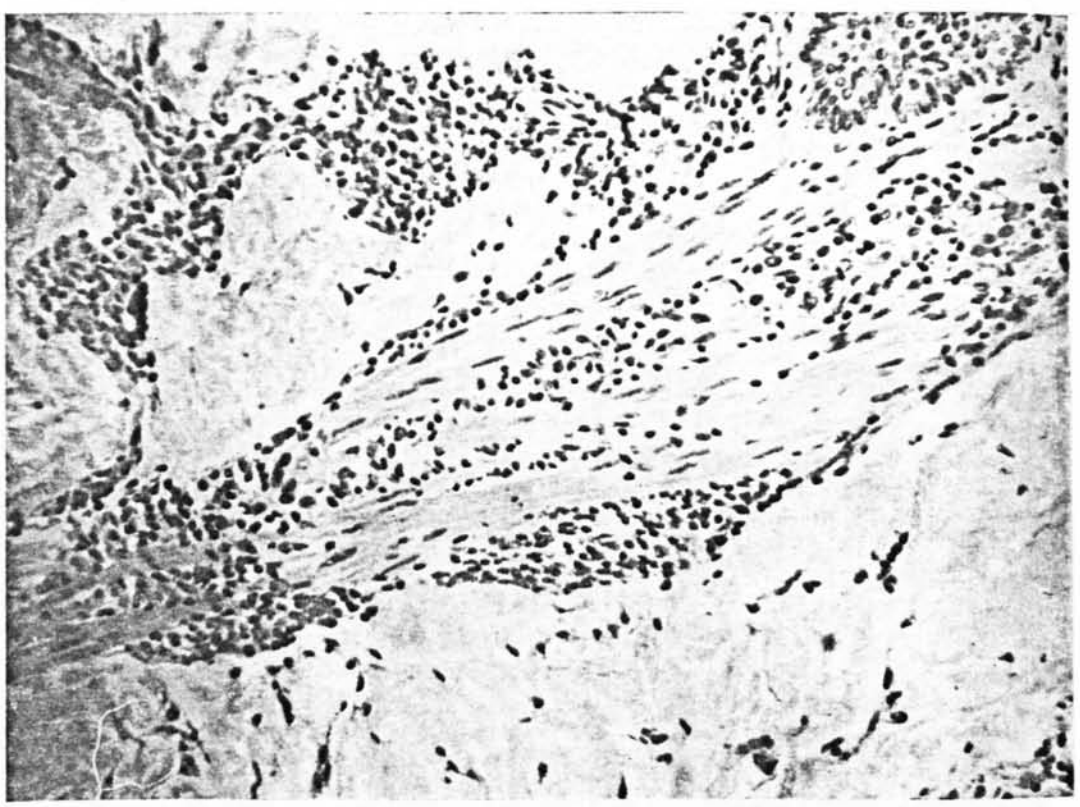

FIG. 5. Arrector pili muscle as a barrier to cellular infiltration. Lymphocytes have infiltrated between the bundles but without penetration. Group II.

found epidemiologic evidence that immunity to leprosy and the immunity that governs the type of leprosy were not the same.

The reason why muscle is an advantageous habitat for the leprosy bacillus is not yet clear. It could be metabolic or it could be immunologic. Muscle appears to be a fairly effective barrier to the infiltration of lymphocytes, possibly more effective than the perineurium (Fig. 5).

Work on this study is still in progress. More recent results confirm the principal findings, but will necessitate the amendment or addition of some points.

SUMMARY

Fifty-one early leprosy lesions were classified histologically as follows: In group I there was cellular infiltration of the epidermis by lymphocytes or epithelioid cells. Group II cases showed a tuberculoid granuloma originating in a nerve bundle. Group III was characterised by a nonspecific cellular infiltrate around (a) a nerve bundle or (b) other skin structures and blood vessels. In group IV the lesion was a perivascular granuloma in the superficial dermis with borderline or lepromatous features. Lesions of groups I and II and IIIa were mostly single (primary). Those of groups IIIb and IV were mostly multiple (disseminated).

Acid-fast bacilli were not found in group I. They were present in some cases in groups II and III, being found more or less equally in the subepidermal zone of the dermis, arrector pili muscle and nerve bundles. Their morphologic index was highest in nerve, lowest in the subepidermal zone. Bacilli were present in all cases in the granuloma in group IV.

It was noted that: (1) In patients with maximal immunity (group I) nerve involvement was slight. (2) Although nerve appeared to be the optimum site for the multiplication of bacilli in the initial phase, destruction of bacilli by an epithelioid cell reaction was frequently seen in nerves while elsewhere bacilli excited no reaction. (3) Muscle was the second best "target site." (4) Mast cells were increased.

The evidence favors the view that leprosy bacilli make their entry via the epidermis. If immunity is maximal they are arrested in the epidermis. If immunity is partial they reach the subepidermal zone and may subsequently find a more secure foothold in nerve or muscle. If immunity is weak early dissemination of the infection occurs via the blood stream.

It is suggested that there may be two systems of immunity: (1) an unidentified system which is operative only in the early stage of the infection, and (2) lymphocyte-mediated. Nerves appear to provide protection for the leprosy bacillus against the former rather than the latter.

Acknowledgments. I am most grateful to Dr. D. A. Russell for sending me the New Guinea biopsy specimens, and for his clinical notes and cooperation. Similarly, I am glad to thank other contributors: Dr. Zarina Fazelbhoy (Pakistan), Dr. W. H. Jopling (London) and Drs. J. M. H. Pearson and M. F. R. Waters (Malaysia). I am indebted to my wife, Mrs. M. J. Ridley, for the histologic processing. I acknowledge with thanks grants for technical expenses from Messrs. Parke Davis in respect of the New Guinea biopsies, and from the Medical Research Council (London) in respect of the other biopsies.

REFERENCES

1. Büngeler, W. Die pathologische Anatomie der Lepra, II. Arch. Path. Anat. (Virchow's) **310** (1943) 493.
2. Chaussinand, R. La Lèpre. Paris, 1950. p. 51.
3. Cochrane, R. G. A critical appraisal of the present position in leprosy. Internat. Rev. Trop. Med. New York: Academic Press. **1** (1961) 1.
4. Dehio, K. (1897) Reprinted in Leprosy in India **24** (1952) 78.
5. Ermakova, N. Studies on leprosy; the central, sympathetic and peripheral nervous systems. Internat. J. Leprosy **4** (1936) 325.
6. Gerlach, W. Die Beziehungen zwischen Hautflecken und der Nervenerkrankung bei der Lepra anaesthetica. Arch. Path. Anat. (Virchow's) **125** (1891) 126.
7. Glasse, R. M., Leprosy at Karamui. Papua and New Guinea Med. J. **8** (1965) 95.
8. Horton, R. J. and Povey, S. The distribution of first lesions in leprosy. Leprosy Rev. **37** (1966) 113.

9. Job, C. K., Karat, A. B. A., Karat, S. and Mathan, M. Leprous myositis—a histopathological and electron microscopic study. Leprosy Rev. **40** (1969) 9.
10. Kato, L. and Gozsy, B. Studies on the physio-pathology of experimental murine leprosy; reticulo-endothelial capillary and mast cell response. A review. Rev. Canadienne de Biol. **23** (1964) 217.
11. Kato, L. and Gozsy, B. Mast cell response in murine leprosy. Internat. J. Leprosy **33** (1965) 50.
12. Khanolkar, V. R. Studies on the histology of early lesions in leprosy. Indian Council of Medical Research. Special Report Series No. 19, 1951.
13. Klingmüller, V. Die Lepra. Vol. 10/2 of Jadassohn's Handbuch der Haut und Geschlechtskrankheiten. Berlin, Springer, 1930, pp. 525 & 570.
14. Lara, C. B. and Nolasco, J. O. Self-healing or abortion and residual forms of childhood leprosy and their possible significance. Internat. J. Leprosy **24** (1956) 245.
15. Nolasco, J. O. Histologic studies on the primary lesions of leprosy in children of leprous parents; other related studies including one case with necropsy. J. Philippine Med. Assn. **28** (1952) 1.
16. Platt, H. The engulfment of particulate and colloidal materials by epidermal cells. J. Path. Bact. **86** (1963) 113.
17. Ridley, D. S. Histological diagnosis of leprosy, in J. H. Walter's Tropical Medicine Conference, 1967. London, Pitman, p. 143.
18. Ridley, D. S. and Jopling, W. H. The classification of leprosy according to immunity: a five-group system. Internat. J. Leprosy **34** (1966) 255.
19. Ridley, D. S. and Ridley, M. J. The possible significance of club-forms of *Mycobacterium leprae*. Internat. J. Leprosy **36** (1968) 339.
20. Rogers, L. and Muir, E. Leprosy. Bristol, Wright, 1930, p. 158.
21. Skinsnes, O. K. First infection type leprosy. Internat. J. Leprosy **37** (1969) 310.
22. Susman, I. A. A limited investigation into the significance of the site of the first lesion in leprosy. Leprosy Rev. **38** (1967) 37.
23. Turk, J. L. Cell-mediated immunological processes in leprosy. Bull. World Health Org. **41** (1969) 779.
24. Wade, H. W. and Ledowsky, V. The leprosy epidemic at Nauru. A review. Internat. J. Leprosy **29** (1952) 1.
25. Weddell, G. and Palmer, E. The pathogenesis of leprosy: an experimental approach. Leprosy Rev. **34** (1963) 57.
26. Wise, M. J. Club-forms of *Mycobacterium leprae*. Leprosy Rev. **34** (1963) 68.