Lepr. Rev. (1975) **46**, 51-67

Perineurial Changes in Untreated Leprosy

J. M. H. PEARSON* AND A. G. M. WEDDELL†

Leprosy Research Unit, National Leprosy Control Centre, Sungei Buloh, Malaysia
and
Department of Human Anatomy, South Parks Road, Oxford, England

Perineurial changes were studied by light microscopy in biopsies of skin and nerve from 64 patients covering the full range of the leprosy spectrum. In all types of leprosy a multilayered appearance of the perineurium could be observed: in lepromatous cases the layers tended to consist of swollen cells which contained more leprosy bacilli than the Schwann cells; in borderline and tuberculoid biopsies they took on a thinner, more fibrotic appearance, and bacilli were scanty or absent. Epithelioid changes were seldom observed in this site. The possible effects of perineurial damage in the endoneurium, and of endoneurial damage on the perineurium, are discussed.

Introduction

The "scientific era" of leprology may be considered as starting in 1847, when Danielssen and Boeck published their treatise on leprosy. They divided patients into two groups, "nodular" and "anaesthetic," according to their dominant clinical features. Clearly therefore nerve damage was already well recognized as commonly present in the disease.

Virchow (1864) examined nerves from leprosy patients, and considered that there was an interstitial neuritis, in which an important role was played by "brownish cells" in the endoneurium. He also however commented on the presence of a perineuritis i.e. proliferation of the outer layers of the nerve. This appearance has been consistently noted by workers since that time, and it has been considered to play a part in causing nerve dysfunction by exerting a strangling action on the nerve bundles.

With the advent of electron microscopy it became possible to study the ultrastructure of perineurium, and to show that it is not a simple membrane but a compact multilayered structure, composed of up to ten laminae, each covered by a basal lamina. It is anatomically well suited to act as a semi-permeable barrier to isolate the interior of nerve from some of the contents of the fluid outside the nerve, and has, indeed, been shown to have that function (Waggener, Bunn and Beggs, 1965). The sequence of events following experimental perineurial injury

* Present address: MRC Leprosy Project, P.O. Box 1005, Addis Ababa, Ethiopia.
† Requests for reprints should be addressed to AGMW.
Received for publication 10 October, 1974.

has been analysed by Morris, Hudson and Weddell (1972); this study also indicated its importance as a barrier.

There have been a number of studies of nerves in leprosy in recent years, both by light and electron microscopy (Iyer and Desikan, 1968; Job, 1971; Dastur, Ramamohan and Shah, 1973): but the perineurial changes receive only incidental mention. Pearson (1972) discusses the perineurium, but only in relation to lepromatous leprosy. The purpose of this paper is to describe the changes seen by light microscopy in the perineurium in untreated leprosy of all types. Possible mechanisms causing these changes, and the interrelationship of perineurium and endoneurium are also discussed.

Patients and Methods

Sixty four patients were studied, covering the whole range of leprosy, from tuberculoid to lepromatous. Men, women and children over 12 years were included, their nationalities being Malay, Chinese, Indian, or Gurkha. Patients with both early and advanced lesions were selected.

All patients appeared to be suffering from active untreated disease, and all denied receiving previous treatment. No patient was suffering from other significant disease, and in no case was dapsone found in the urine. Classification was according to the system of Ridley and Jopling (1966) and there was good agreement between clinical and histological diagnoses.

All patients were subjected to biopsy of an active skin lesion and of an enlarged nerve. The nerve usually chosen was the superficial radial at the level of the styloid process of the radius (Pearson and Weddell, 1971), but in some cases enlarged subcutaneous nerves in the immediate vicinity of tuberculoid skin lesions were biopsied. Two separate skin lesions were biopsied in two cases. Not all the nerve biopsies however, were processed for light microscopy—figures are given in Table 1.

TABLE 1

Classification of patients and number of biopsies examined

Classification		Number of patients	Number of skin biopsies	Number of nerve biopsies
Tuberculoid	TT	3	3	2
	BT	24	24	14
Borderline	BT/BB	3	3	1
	BB	6	6	4
	BB/BL	2	2	1
Lepromatous	BL	8	8	4
	LI	16	17	8
	LL	2	3	1

The skin biopsies were divided into two, one part being examined for independent histological classification by Dr D. S. Ridley, the other fixed by immersion in 10% buffered formaldehyde and examined in the Department of Human Anatomy, Oxford. Nerve biopsies were intended primarily for electron microscopy, and in most cases were fixed by immersion in 3% glutaraldehyde for

2 h; larger specimens were sometimes subdivided for light as well as electron microscopy. Some nerve biopsies, however, to be used primarily for light microscopy, were fixed in 10% buffered formaldehyde.

The specimens for light microscopy were subjected to routine processing, embedded in wax, and cut into 6 μm sections. They were always stained with haematoxylin and eosin, and haematoxylin and cold carbol fuchsin. Additional stains used in some cases included methods for elastic fibres, PAS stains, and silver techniques for axons. In a few cases silver stains of 25 μm sections were combined with staining for acid-fast bacilli. Additionally in a few cases thin (1 μm) sections of araldite embedded material stained with toluidine blue were available.

Findings and Comments

The appearance of normal perineurium is shown in Fig. 1.

TUBERCULOID LEPROSY

Twenty seven patients were included in this category. Eight biopsies (all BT) were taken from lesions which appeared clinically to be rather early in their evolution; and a further two (both BT) were biopsies taken across the edge of the lesion and extending into apparently normal skin outside it. In such biopsies the earliest stage of the evolution of the disease could be seen outside the active edge, and its development followed across the edge and into the lesion.

The remaining biopsies were from the active areas of mature lesions; three were classified TT and the remainder BT.

(1) *Early Lesions (10 cases, all BT).* The dermal nerves in these cases were usually normal in size or only slightly enlarged. Most of them however had a rather fibrotic appearance, and a few showed marked swelling and hypercellularity of the endoneurium, though there was never a mature epithelioid granuloma. In general, the more normal a nerve looked, the more likely were bacilli to be found within it.

Even in these early cases the perineurium was usually markedly abnormal. Instead of being a very compact structure, the layers appeared to be separated into thin fibrotic looking strands, and there were a large number of inflammatory cells, chiefly lymphocytes, lying among the strands and apparently invading the perineurium from outside (Fig. 2). It was not uncommon for there to be the appearance of a dense cellular cuff occupying the perineurial zone, with a somewhat fibrotic looking but relatively normal and preserved endoneurium. Only in one instance was the perineurium normal in appearance, and even in this case (where the only abnormality of the nerve itself was the presence of very scanty bacilli lying within Schwann cells) there were a number of lymphocytes clustering round the outside of the nerve. Acid-fast bacilli were never seen in the perineurial zone.

(2) *Mature Lesions (17 cases; TT-3, BT-14).* Dermal nerves of fairly well preserved morphology could be identified in eight cases (TT-1, BT-7), and structures recognizable as formerly neural in another 7 (TT-1, BT-6). Only in 2 biopsies could no nerves be found. The destroyed nerves were grossly swollen, chiefly due to expansion of the endoneurium, in which there were seldom recognizable Schwann cells, the whole zone being replaced by epithelioid cells.

In the more normal looking nerves the perineurium looked much the same as in the early cases; there was a multilayered fibrotic appearance with some cellular

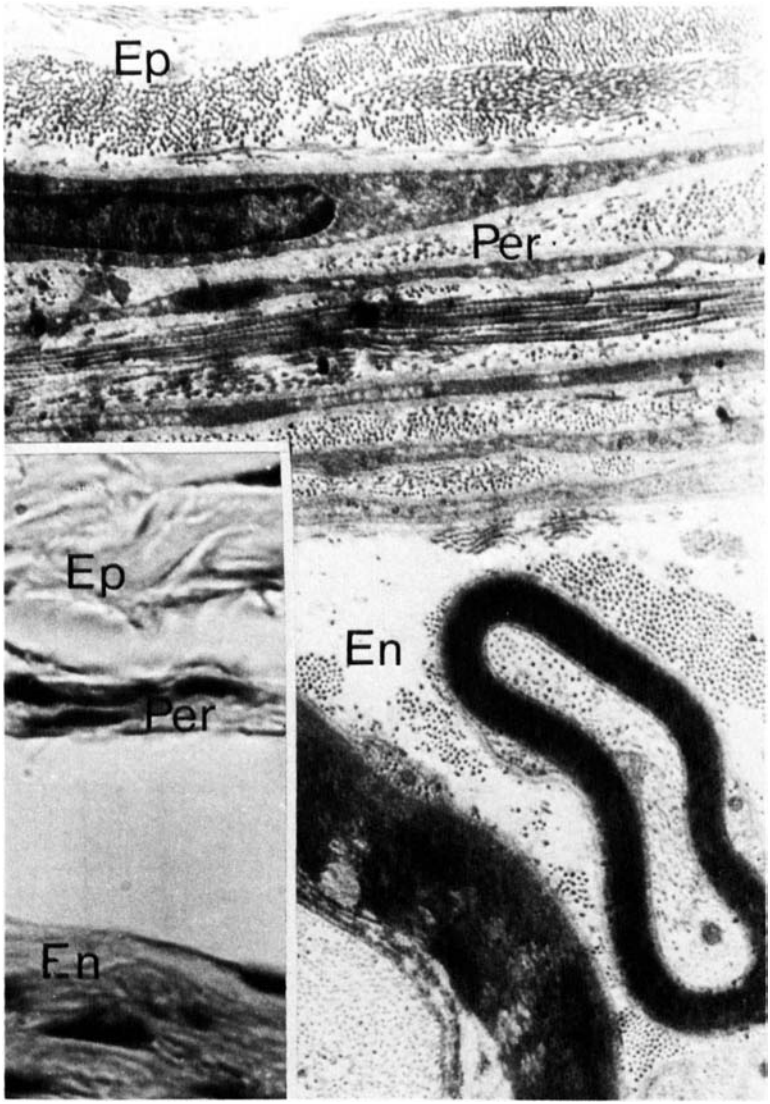

Fig. 1. Electron-micrograph of mouse sciatic nerve (×16,000). Inset: light photomicrograph of fascicle of normal human radial cutaneous nerve (×1500). Note the multilayered structure of the perineurium which is hardly to be seen by light microscopy. The separation of perineurium from the other nerve layers in the light micrograph is a shrinkage artefact. Ep = epineurium; Per = perineurium; En = endoneurium.

infiltration. In addition there sometimes appeared to be fine septa of perineurium extending into the endoneurium, forming multiple compartments in the normally monofascicular nerve bundles.

In the grossly swollen nerves the perineurium was less abnormal than the endoneurium, but it never remained intact, and sometimes appeared to be hanging

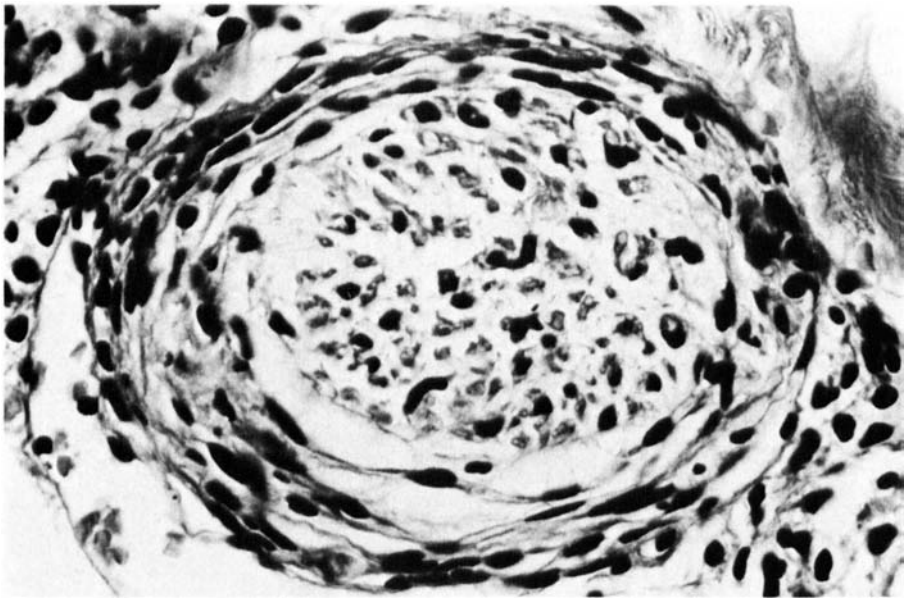

Fig. 2. Early tuberculoid (BT) leprosy. This dermal nerve shows a multilayered perineurial zone with infiltrating lymphocytes. The endoneurium is almost normal. (×800.)

in thickened shreds outside the nerve. More commonly, however, it formed a widened perineurial zone with lymphocytes within the fibrotic perineurial strands (Fig. 3). These lymphocytes tended to form rather dense foci at the point where blood vessels penetrated the perineurium and were about to pass into the endoneurium. At these points it appeared that inflammatory cells could readily emerge from the blood vessels. In two biopsies the perineurium appeared, in some places, to be replaced by epithelioid cells which were usually in continuity with endoneurial granuloma.

(3) *Nerve Trunks (16 cases; TT-2, BT-14).* There was no significant difference between the TT and BT cases: nor between the radial nerve biopsies (8 cases) and the subcutaneous nerves close to skin lesions (8 cases). Two radial nerves were normal, and a third normal apart from the presence of very scanty bacilli in the Schwann cells.

The changes in these biopsies were much the same as those seen in the severely damaged dermal nerves; and though the inflammatory response chiefly affected the endoneurium, the perineurium did not escape unscathed. It was usually fused with the epineurium into a thick fibrotic cellular collar; the whole structure was highly vascular, and many inflammatory cells, predominantly lymphocytes, appeared to be passing out of the blood vessels into the perineurial zone. Moreover, the boundary between peri and endoneurium was often indistinct; all the components of the nerve tended to coalesce, and lymphocytes appeared to be entering the endoneurium via the perineurium as well as through the endoneurial blood vessels. In two biopsies epithelioid granulomata could be seen in the perineurial zone.

The endoneurium was usually wholly replaced by an epithelioid granuloma, but

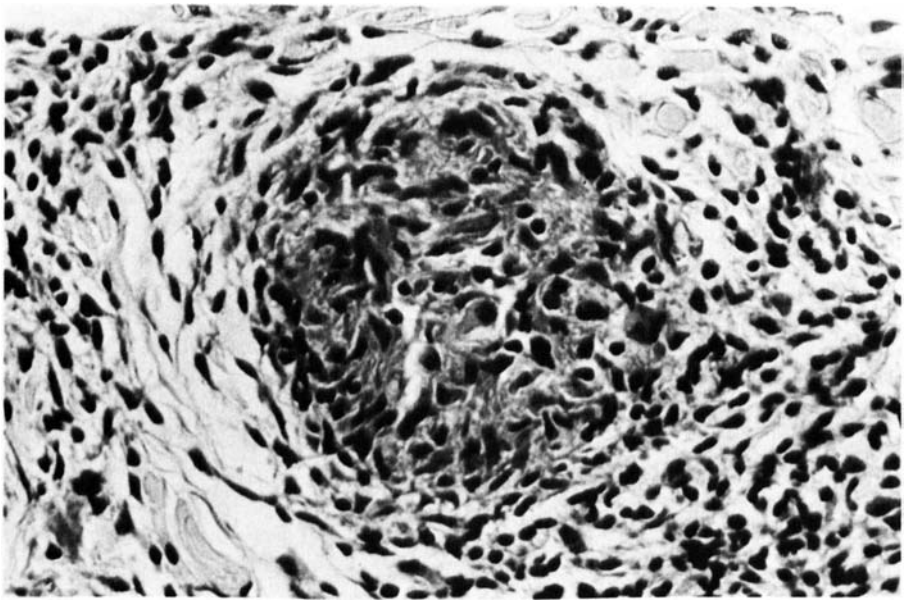

Fig. 3. Tuberculoid (BT) leprosy. The endoneurium of this dermal nerve is replaced by epithelioid granuloma, and there is a loose focus of lymphocytes occupying a sector of the perineurial zone at the site of entry of a blood vessel. (x500.)

the inflammation sometimes showed a focal quality. Occasionally surviving strands of Schwann cells could be identified, and in one instance a radial nerve biopsy included two fascicles, one grossly swollen with epithelioid cell change, the other normal.

LEPROMATOUS LEPROSY

(1) *LL Cases.* Three biopsies (from 2 patients) were in this category, but dermal nerves were only recognizable in two of them. The perineurium had lost its compact appearance and formed a perineurial zone, a multilayered structure many times wider than normal (Fig. 4). This zone was not clearly demarcated from either epi- or endoneurium; the three layers merged into one another with no definite lines of transition. The perineurial layers were thick, and appeared to be cytoplasmic rather than fibrotic; their nuclei resembled those of the Schwann cells of the endoneurium, and were quite unlike those of normal perineurium. Histiocytes and plasma cells were seen both among the perineurial layers and in the endoneurium, but lymphocytes were scanty or absent.

Bacilli, including globi, were present in very large numbers in the perineurial layers and in the histiocytes among them. The bacillary concentration in the perineurial zone was markedly higher than in the endoneurium, which, though hypercellular in one biopsy and rather fibrotic looking in the other, was relatively much more normal in appearance.

(2) *BL Cases; Early Lesions.* Three biopsies were included in this group. The general appearance of the nerves was rather similar to that of the early BT lesions,

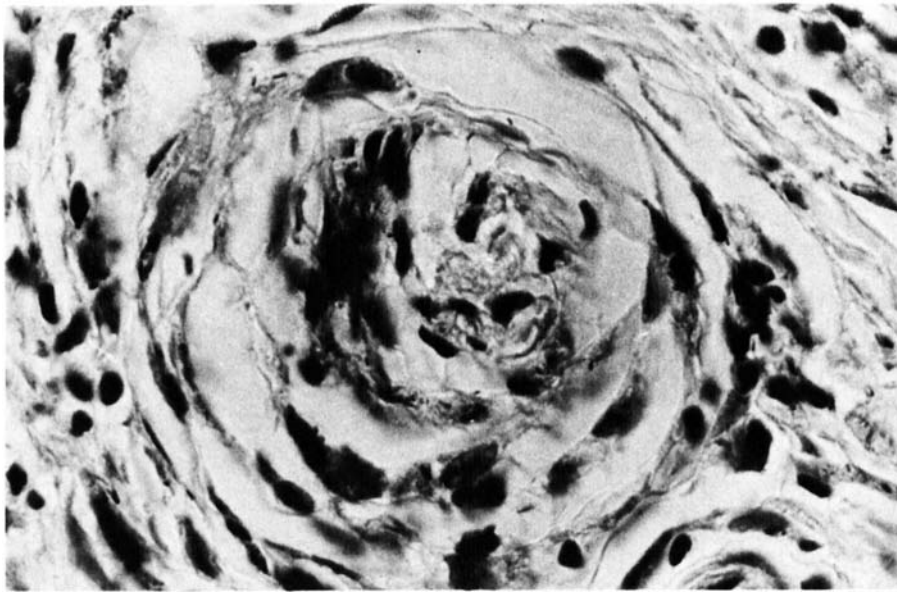

Fig. 4. Lepromatous (LL) leprosy. This dermal nerve shows thickened perineurial strands, containing large numbers of leprosy bacilli, encircling a few surviving Schwann cells. (×1500.)

though there were far more bacilli in the Schwann cells. However, the perineurial zone was less cellular and bacilli could be found in probable histiocytes among its rather scanty layers. The bacilli often lay in cells, including small foci of macrophages, which appeared to be related to blood vessels, rather than nerves, in the neurovascular bundles.

(3) *BL Cases; Mature Lesions (5 biopsies).* The general appearance of the dermal nerves, which could be identified in all 5 biopsies, was similar to that of the LL cases, but the strands which formed the perineurial zone (in 3 cases) were thinner and appeared somewhat more fibrotic and less cytoplasmic (Fig. 5), and lymphocytes were seen in considerable numbers among the layers in 2 biopsies. In the remaining 2 biopsies the perineurium appeared normal.

Bacilli were seen in all 5 cases, but the preference for perineurium seen in LL cases was not evident, being present in 1 case only. In another case the concentration was less in the perineurium, and in the remainder it was much the same as in the endoneurium.

(4) *LI Cases (17 biopsies. (For definition of LI see Ridley and Waters 1969.)* Dermal nerves could be identified in all but 3 biopsies, including 2 cases where the skin was anaesthetic at the biopsy site. The perineurial changes were most advanced in these 2 cases, with a higher bacillary concentration in the perineurial zone than in the endoneurium.

The other 12 biopsies showed a wide range of appearances. The earliest lesion (and the only one with normal sensory acuity) showed no perineurial "layering," but there were clumps of bacilli in the Schwann cells (whose nuclei appeared increased in number) and rather fewer in the perineurial cells. The rest of the

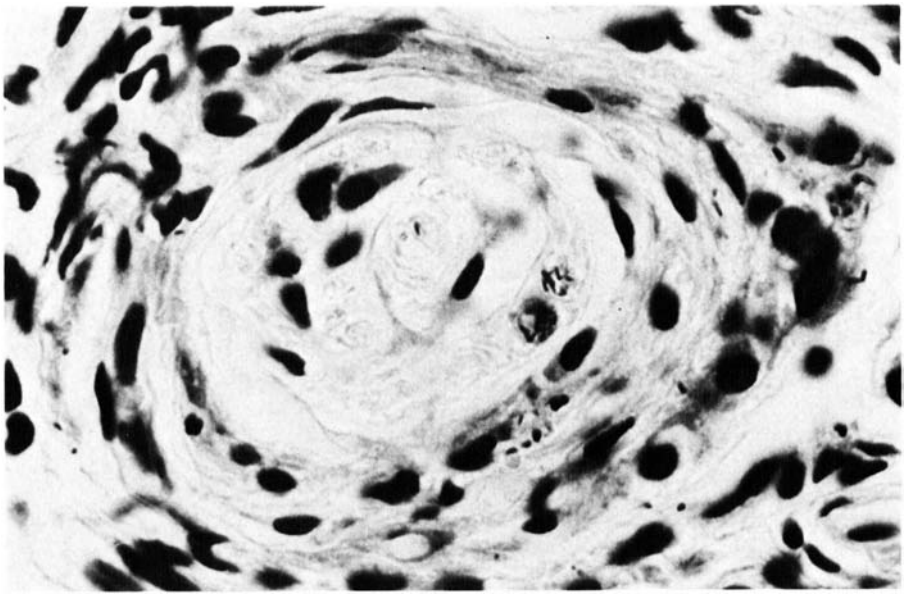

Fig. 5. Lepromatous (BL) leprosy. The perineurial layers of this dermal nerve are somewhat less thick than those seen in LL. The perineurial zone contains some infiltrating inflammatory cells, and its bacillary concentration is much the same as that of the endoneurium. (×1500.)

biopsies showed varying degrees of layering which was imprecisely related to the degree of sensory loss (Fig. 6) and could not be related to the probable duration of the disease. The appearance of the perineurial layers usually fell between the rather fibrotic strands of the BL cases and the thicker fleshier form of the LL cases. There were always some macrophages among the layers and, commonly, also a small number of lymphocytes and plasma cells. Acid-fast bacilli were always present in greater (5 biopsies) or similar (6 biopsies) concentration in the perineurial layers compared to the endoneurium.

(5) *Nerve Trunk Lesions.* Thirteen biopsies of nerve trunks were available, and though they included LL (1 case), LI (8 cases) and BL (4 cases) there were no major differences that could be associated with the classification. They are therefore considered together as a group and demonstrate the evolution of the infection; 4 biopsies were early, 2 advanced, and the remaining 7 lay somewhere between.

In the early biopsies (BL, 2; LI, 2) the bacilli lay singly and in clumps in the Schwann cells of the endoneurium. They tended to be linearly arranged, so that long thin segments of nerve were affected and larger zones between them contained no bacilli. It was often possible to see small endoneurial blood vessels close to these foci of infection. In the areas where the bacilli were more dense and where small globi might be present, the number of nuclei was increased. This appeared to be due chiefly to Schwann cell proliferation, but the nuclei were less elongated than normal, and some could have been inflammatory cells entering via the blood vessels. In all these cases there were already considerable numbers of bacilli, but there was minimal cell response to their presence. Most of the

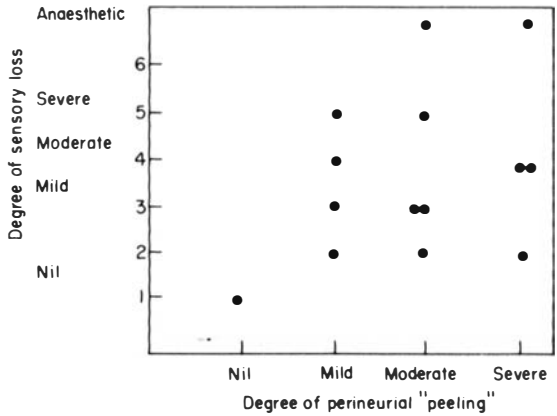

Fig. 6. The relationship between the degree of perineurial "peeling" and the sensory acuity of the biopsied area in fourteen biopsies from patients with lepromatous (LI) leprosy. Sensory testing was performed using graded nylon bristles (Pearson and Weddell, 1971); the figures in the vertical axis represent the finest bristle the patient could feel ranging from 1 (very fine) to 6 (coarse).

Schwann cells appeared intact, even though bacillated, and and the sensory acuity in the area supplied by the radial nerve was normal in the 3 cases in which it was tested.

Even at this early stage the perineurium was also affected. The two earliest cases (both BL) showed bacilli present in perineurium in about the same concentration as endoneurium. In the two more heavily infected cases (both LI) the concentration was greater in the perineurial zone, which showed the same multilayered appearance as was seen in the dermal nerves. Moreover, bacilli were seen in large numbers in the endothelial cells of the endoneurial blood vessels in these two biopsies.

As the infection progressed the endoneurium became more cellular, till in moderately advanced cases no Schwann cells of normal configuration survived. Before this stage, however, they formed strands between zones of more abnormal looking endoneurium. These zones were centred on blood vessels, and were more cellular than the remainder. The cells could not always be identified but there appeared to be monocytes and, in the more subpolar cases, lymphocytes, which clearly delineated the blood vessels, forming a cuff around them. Plasma cells were often seen, and in one case small foci of polymorphs. In addition to the cellular infiltration, however, the tissues had a cloudy appearance and took up stains more readily than usual. This abnormality was also present in a zone just within the perineurium. It was as if some abnormal material was leaking into the nerves through both the perineurium and the endoneurial blood vessels. The contrast between the two zones was most clearly evident by phase contrast microscopy.

There were also abnormalities in the other parts of these nerves. Here the myelin was very markedly swollen and took up stains fairly readily, so that nodes of Ranvier and even Schmidt Lantermann clefts could be identified in haematoxylin and eosin stained sections. These abnormalities were probably reversible, as the 3 cases where such axons were present in large numbers all had

normal sensation on follow up after 6–12 months' treatment, though one of them was anaesthetic at the time of biopsy (the other two were not tested initially).

In the most advanced cases the structure of the nerve was almost completely obliterated, and only strands of foam cells, in places encircled by strands of collagen which appeared perineurial in origin, could be seen. The bulk of such "nerves" consisted of collagen bundles, with occasional inflammatory cells and bacilli among them. In slightly less advanced cases, where there was readily recognizable endoneurium, the number of blood vessels in this zone was increased. The increase was recognizable, though less marked, in the earlier stages of the infection.

The multilayered appearance of the perineurium was seen in all these biopsies, and appeared to be maximal in the more advanced cases. The perineurial zone showed infiltration (with lymphocytes mostly in BL cases, histiocytes mostly in LI and LL cases) similar to that seen in the dermal nerves. Bacilli were always present in both endo- and perineurium; in the BL cases they tended to be more concentrated in the endoneurium, whereas in LI and LL there were as many or more bacilli in the perineurial layers.

BORDERLINE LEPROSY

(1) *Skin Lesions.* Six biopsies were classified as BB, and nerves were identified in every case. They showed two characteristic features, one or both of which were present in every biopsy.

(a) In 4 cases the Schwann cells were bacillated but otherwise almost normal.
(b) In 4 cases there were strands of bacillated Schwann cells, and also zones of epithelioid cells, which tended to lie at the periphery of the nerves.

Silver stains showed that axons were only present in the relatively intact Schwann cell strands, and even there they were reduced in number.

The perineurium always showed a multilayered fibrotic appearance with many lymphocytes among the strands. In three cases macrophages were also present. Nuclei within the strands themselves were very much less elongated than normal perineurial nuclei (Fig. 7). Bacilli were usually present in the perineurial zone, but the concentration was always less than in the relatively preserved Schwann cell strands. In two cases epithelioid granulomata were present in the perineurial zone; they were usually in continuity with the endoneurial granuloma.

The nerves of the two patients classified BB/BL were very similar to each other. The Schwann cells of the endoneurium contained many acid-fast bacilli, but their concentration was almost as high in the perineurial zone. The perineurium showed moderate layering, with strands which usually looked fibrotic, but in a few places were thicker and contained bacilli. Most nuclei lay within the perineurial strands, but were more rounded than normal perineurial nuclei and much greater in number (Fig. 8). There were also occasional lymphocytes and histiocytes among the layers. In both cases the perineurial strands were not confined to the normal perineurial zone, but appeared also to have proliferated within the endoneurium, which was split into multiple small compartments, each containing at least one Schwann cell.

Three patients were classified BB/BT; scanty dermal nerves could be identified in two of them; and the Schwann cells contained very few bacilli. The perineurial zone was replaced by a wide dense band of lymphocytes, which almost

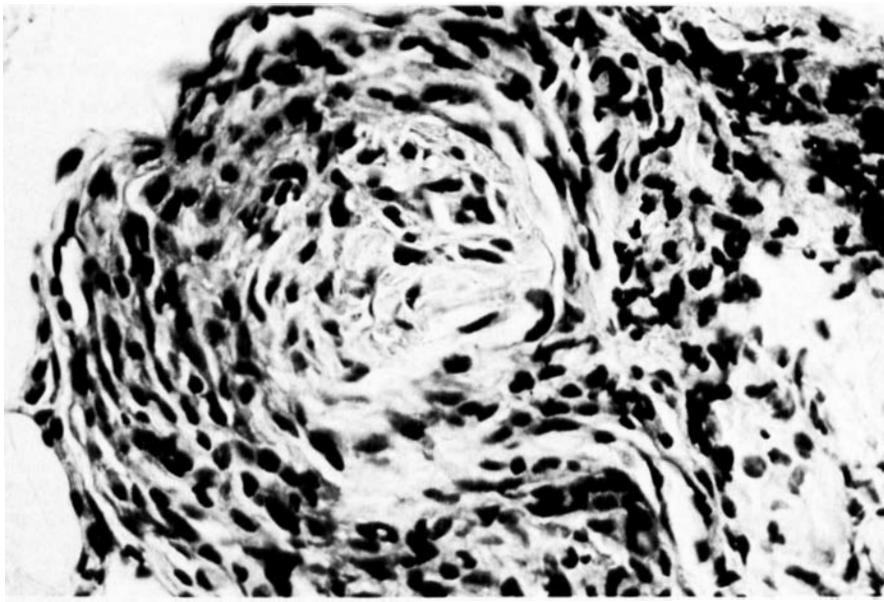

Fig. 7. Borderline (BB) leprosy. A dermal nerve showing a multilayered perineurium with rounded nuclei and some infiltrating cells. Epithelioid granuloma has formed in the outer part of the endoneurium, and in one sector appears to involve the perineurium. (x600.)

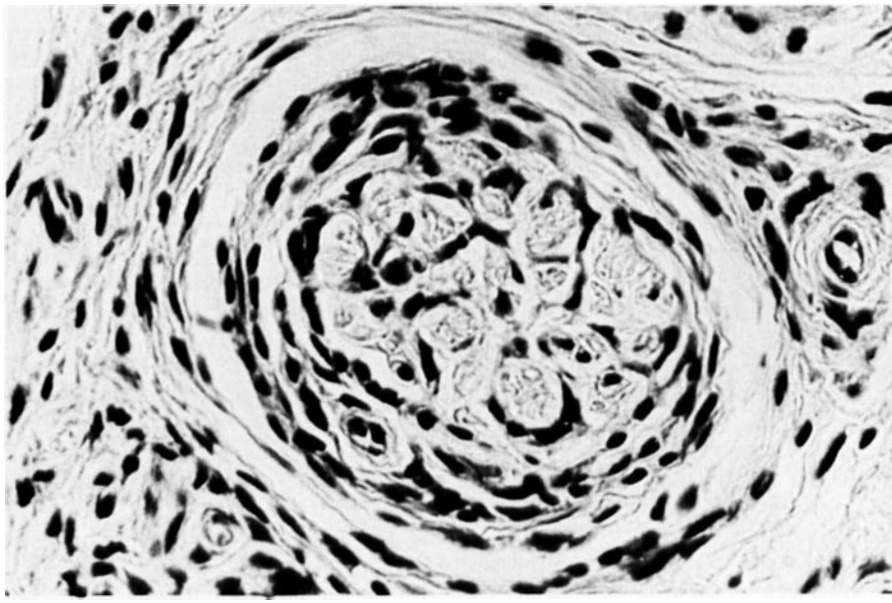

Fig. 8. Borderline(BL/BL) leprosy. The perineurial strands of this dermal nerve contain many well rounded nuclei, and the endoneurium is split into multiple compartments by similar looking strands. (x750.)

obliterated the fibrotic strands of perineurium. There were no bacilli in the perineurial zone.

(2) *Nerve Trunk Lesions.* Six biopsies were available (BB, 4; BB/BL, 1; BB/BT, 1), but were normal in two of the BB cases. The remaining four can be considered as a group.

The endoneurium was affected in a strikingly focal manner. There were strands of normal looking Schwann cells which, however, contained many acid-fast bacilli, including even some small globi. There was some increase in the number of Schwann cell nuclei in these zones. Surrounding these strands were areas where the normal neural architecture was lost and replaced by a cellular granuloma which in some places had matured to fully differentiated epithelioid cells. Lymphocytes were rather scanty except round the endoneurial blood vessels, where they sometimes formed loose foci. The number of blood vessels was markedly greater than normal.

The granulomatous zones usually lay adjacent to perineurium, which formed a widened and highly vascular perineurial zone. There was a moderate degree of layering, and the perineurial strands usually had a fibrotic appearance. In some places however, they were obliterated by dense foci of lymphocytes, which tended to be situated at positions where the larger perineurial blood vessels penetrated into the endoneurium. Acid-fast bacilli were present in the perineurium, although they were scantier than in the relatively normal sectors of endoneurium. However, in one case there was a thin, but very dense, zone of bacilli in the outer part of the perineurial zone. One biopsy showed areas of epithelioid cells in the perineurial zone.

Discussion

Previous comments on the perineurial changes seen in leprosy have been largely confined to observations on the multilayered appearance which can be observed in all types of the disease. However, even this appearance is not absolutely uniform, and the perineurial changes in leprosy form a spectrum of their own, which can be related to the classification of the disease.

(1) THE MULTILAYERED APPEARANCE

(a) The "quality" of the layering was by no means uniform: in LL and many LI cases the layers took on a thick, cytoplasmic appearance. This appearance was less marked in the BL cases, and in BB and tuberculoid lesions the layers took the form of thin fibrotic strands.

(b) In lepromatous leprosy the nuclei in the layers appeared identical to those of Schwann cells of the endoneurium. In BL/BB and BB cases there were nuclei within the layers but they were more elongated, though still much rounder and greater in number than normal perineurial nuclei. At the tuberculoid end of the spectrum there was too much cellular infiltration for perineurial nuclei to be identified or localized.

(c) The degree of "layering" was generally related to the maturity of the lesion—the earlier lesions showed it less intensely than the more advanced ones.

(d) In lepromatous cases the amount of layering was also roughly proportional to the degree of sensory loss. This may be a causal relationship, or may simply mean that both develop slowly in lepromatous leprosy.

(2) CELLULAR INFILTRATION

In most cases infiltrating cells were seen among the perineurial layers. At the tuberculoid end of the spectrum they were almost all lymphocytes: in borderline cases a few macrophages were sometimes identified, and in a few BT and BB cases frank epithelioid changes could be observed: in lepromatous leprosy histiocytes and plasma cells predominated, though there were considerable numbers of lymphocytes in BL cases, and a few in the LI group.

(3) ACID-FAST BACILLI

No bacilli were found in the perineurial zone in TT or BT cases, or in the patients classified BB/BT. They were, however, present in the remainder and could usually be seen both in the perineurial strands and in infiltrating histiocytes. (In the LL and LI cases, however, it was often impossible to be certain of their cellular location.) The bacillary concentration was higher in the endoneurium in the borderline cases, but in the two LL biopsies and six of the LI cases there were markedly more in the perineurium. The concentrations were much the same in both sites in the remaining LI and BL patients.

(4) ENDONEURIAL "INVASION" BY THE PERINEURIUM

This appearance was seen in a few BT and BB/BL cases, but it is hard to assess from this rather small series just how commonly it occurs. Fibrotic strands of tissue extended inwards from the perineurium, giving a reticular appearance to the endoneurium, and dividing it into multiple small compartments. We have seen, in two biopsies from borderline leprosy (not included in this series) what may be a more advanced stage of this change, when a small nerve that should have been monofascicular was split into many tiny fascicles, each comprising one or several Schwann cells surrounded by well organized perineurium.

(5) ENDONEURIAL CHANGES

Two findings deserve special comment:

(a) The survival of bacilli in the endoneurium in the tuberculoid and borderline cases, and particularly their presence in higher concentration than in extraneural sites, offered good evidence that the protection within peripheral nerves was immunological as well as, perhaps, histochemical.
(b) In some lepromatous cases the cloudy appearance of the endoneurium, and the swelling and altered staining quality of the myelinated axons, suggested very strongly that in such cases the perineurial and vascular barriers had been breached.

In speculating about the way in which these striking perineurial changes may be brought about, it is convenient to consider first, the effect of perineurial damage on the endoneurium, and secondly the effects of endoneurial damage on the perineurium.

The Effect of Perineurial Damage on the Endoneurium. The function of the perineurium appears to be to act as a semi-permeable barrier between the extraneural fluid and the endoneurium (Shantha and Bourne, 1968); it provides a degree of chemical isolation for the Schwann cells. Thus, it has been shown that ferritin applied around a nerve will not pass into the perineurium (Waggener *et al.*, 1965). Any failure of this barrier function is therefore liable, by allowing ingress of abnormal constituents to the endoneurium, to cause alterations of structure and function of the nerve.

Morris *et al.* (1972) have coined the colourful phrase "environmental draught" to describe this endoneurial exposure, and investigated (in the rat sciatic nerve) the sequence of events following the deprivation of endoneurium of its perineurial protection. Within a few hours an abnormal floccular substance was seen in the endoneurium. During the next few days a fine meshwork of fibres developed around the Schwann cells, which in the next 3–4 weeks became organized to form many small fascicles of Schwann cells each encircled by tissue morphologically identical with perineurium. The floccular substance was seen only outside the new fascicles. By electron microscopy they demonstrated that the new perineurium was in part at least derived from Schwann cells, which divested themselves of their axons and extended lateral processes enclosing groups of other Schwann cells. Thus, under these experimental conditions, Schwann cells could metamorphose to take the form and function of perineurial cells.

Lepromatous leprosy partly reproduces the experimental conditions of Morris *et al.*, in that the perineurial cells are always colonized by *Mycobacterium leprae,* and in experimental leprosy the perineurial layers have been shown to be abnormally permeable (Boddingius *et al.*, 1972). Thus the endoneurium is exposed to environmental draught, and it seems likely that the swollen axons seen in some biopsies from lepromatous leprosy are the result of this exposure, and that the altered staining quality of the endoneurium represents the floccular substance seen by electron microscopy. Furthermore, changes very similar to the compartmentation reported by Morris *et al.* have been observed in some patients in the borderline range (this is the type of leprosy in which sudden nerve destruction is most likely to occur, i.e. the type that might most closely reproduce their experimental conditions).

The concentric multilayered appearance of the perineurium is, however, much more commonly seen. To account for its development in terms of the processes described by Morris *et al.*, it is necessary to take into account two major differences between lepromatous leprosy and their experimental situation:

(1) Their injury was a single acute insult. In leprosy the processes occur slowly, and the final situation is the end result of months or years of bacillary multiplication.

(2) In their experiments the whole nerve was exposed to an environmental draught which could reasonably be described as gale force. In leprosy the perineurial changes are best considered as "micropunctures," chiefly affecting the immediately adjacent endoneurium, and having a directional element lacking in the experimental model.

Our hypothesis to account for the development of the multilayered appearance of the perineurium in lepromatous leprosy is that, under these circumstances, the endoneurial response to perineurial injury is for cells, including Schwann cells, to go through the same processes. However, instead of encircling other Schwann cells they respond to the directional element of the environmental draught by applying themselves as "patches" to the perineurium. The cells involved would probably themselves be bacillated, and the patches liable to break down sooner or later and need further patching. The stage is thus set for the development of the multilayered appearance characteristic of the bacillated perineurial zone in lepromatous leprosy (see Fig. 9). But whether or not this concept is correct, in lepromatous leprosy the nerve damage is, in part at least, brought about by a perineuropathy, though this does not exclude the effects of other processes, such as the direct damage to Schwann cells by contained bacilli, the possible strangling

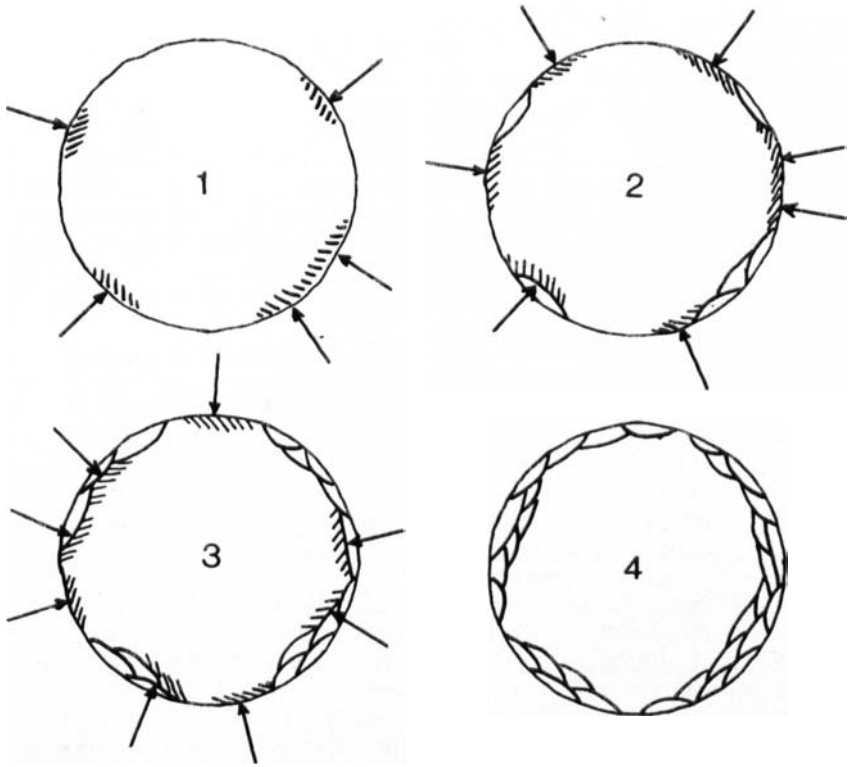

Fig. 9. Diagrammatic representation of the processes which may be involved in the development of "multilayering" of the perineurium in lepromatous leprosy. → = site of "micropuncture"; //// = zone of "endoneurial draught".

effects of intraneural and perineurial collagen formation, and limitation of blood supply.

The Effects of Endoneurial Inflammation on the Perineurium. In tuberculoid leprosy the nerve damage is primarily endoneurial in origin. Bacilli enter Schwann cells, multiply in them for a period, and then the mycobacterial antigen reaches a critical level at which, despite the protected environment, it is recognized by the tissue defences. A delayed hypersensitivity response (cell mediated immune response) ensues, in which the bacilli are attacked and largely destroyed. In the process the cells which contain them, and indeed the whole nerve, are also destroyed, and replaced by epithelioid granuloma, and the inflamed nerve becomes grossly swollen.

It is hard to assess how efficiently the perineurium functions in borderline (and tuberculoid) leprosy. In this series of patients the two classified BB/BL showed early changes of "compartmentation," indicating that the perineurial barrier was not fully effective. There were considerable numbers of bacilli in the perineurial layers of these biopsies. This was not so in those classified BB, where very few bacilli were seen in this zone; and none were present in the more tuberculoid cases. Moreover, it was in the BB and BT group of patients that bacilli could be

seen preserved in Schwann cells, despite the presence of perineurial layering and lymphocytic infiltration of the perineurial zone. In such cases, there appeared to be at least partial preservation of barrier function.

Frank epithelioid changes were probably observed in the perineurium of a few BB and BT cases, though the distorted neural architecture in these biopsies makes the site of any changes hard to define. In such zones it is most unlikely that the perineurium is functioning effectively, and not, therefore, surprising that epithelioid changes were to be found in the endoneurium adjacent to the affected perineurium. The infrequency of such findings, however, argues against this being the usual way in which the perineurium is breached and the endoneurium damaged. Certainly in the larger nerve trunks some foci of epithelioid change were centred on endoneurial blood vessels.

It seems then that in borderline and tuberculoid leprosy the perineurial layers tend to look fibrotic, have few or no bacilli, and retain some function, whereas in lepromatous cases they look more cytoplasmic, contain many bacilli, and have little or no barrier function. These are major differences, and raise the question of whether the layering of non-lepromatous leprosy has the same pathogenesis as that described for lepromatous leprosy.

It is very difficult to apply the hypothesis of Schwann cell metamorphosis to explain the multilayering seen in borderline and tuberculoid cases. The theory presupposes the presence of bacilli in the perineurium with consequent functional impairment, whereas the perineurial barrier is, to some extent, at least, intact, and bacilli are very seldom seen in the perineurial zone. Epithelioid cells (which would provide evidence of bacillation at an earlier stage, before an immune response had fully developed), are seldom seen. It seems more likely that the perineurial changes seen in borderline and tuberculoid cases represent a perineurial response to endoneurial inflammation (just as Schwann cell metamorphosis can be regarded as an endoneurial response to perineurial injury). This hypothesis also presents problems, for there can be marked perineurial layering when the endoneurium shows only slight inflammatory changes, and (less commonly) very little perineurial change despite epithelioid cell formation in the endoneurium. This inconstancy implies that the factors initiating the response have yet to be defined. An important argument in favour of such a process is that it might be expected, on the whole, to preserve the function of the perineurium. The same argument can be applied against such a process being solely responsible for the changes of lepromatous leprosy.

For the time being, therefore, it seems impossible to go beyond attributing these changes to a perineurial response to endoneurial inflammation. When there is also perineurial damage endoneurial changes ensue. The situation is a complex one, and further progress is likely to require electron microscopy, and the development of experimental models which will reproduce the inflammation of leprosy independently in endoneurium and perineurium. It is clear however, that perineurial involvement deserves more attention than it has hitherto received in studies of the pathogenesis of nerve damage in leprosy.

Acknowledgements

We are grateful to Dr D. S. Ridley for independent histological classification of these patients, and to the British Leprosy Relief Association (LEPRA) for their support to one of us

(A. G. M. W.). The electron micrograph in Fig. 1 was supplied by Dr J. Boddingius. The Leprosy Research Unit is jointly administered by the (British) Medical Research Council and the Malaysian Ministry of Health.

References

Boddingius, J., Rees, R. J. W. and Weddell, A. G. M. (1972). Defects in the "Blood-nerve barrier" in mice with leprosy neuropathy. *Nature, New Biology* **237**, 190.

Danielssen, D. C. and Boeck, C. W. (1847). *Om Spedalskhed.* Christiana.

Dastur, D. K., Ramamohan, Y. and Shah, J. S. (1973). Ultrastructure of lepromatous nerves. Neural pathogenesis in leprosy. *Int. J. Lepr.* **41**, 47.

Iyer, C. G. S. and Desikan, K. V. (1968). Nerve involvement in leprosy; Pathogenesis and significance. *Neurology (India)* **16**, 89.

Job, C. K. (1971). Pathology of peripheral nerve lesions in lepromatous leprosy—A light and electron microscopic study. *Int. J. Lepr.* **39**, 251.

Morris, J. H., Hudson, A. R. and Weddell, G. (1972). A study of degeneration and regeneration in the divided rat sciatic nerve based on electron microscopy. 4. Changes in fascicular microtopography, perineurium, and endoneurial fibroblasts. *Z. Zellforsch.* **124**, 165.

Pearson, J. M. H. (1972). Mechanisms of nerve damage in leprosy. *Annals of the Academy of Medicine, Singapore. (New series)* **1**, 156.

Pearson, J. M. H. and Weddell, A. G. M; (1971). Changes in sensory acuity following radial nerve biopsy in patients with leprosy. *Brain* **94**, 43.

Ridley, D. S. and Jopling, W. H. (1966). Classification of leprosy according to immunity. A five group system. *Int. J. Lepr.* **34**, 255.

Ridley, D. S, abd Waters, M. F. R. (1969). Significance of variations within the lepromatous group. *Lepr. Rev.* **40**, 143.

Shantha, T. R. and Bourne, G. H. (1968). *The Structure and Function of Nervous Tissue.* (Ed. G. H. Bourne). New York and London: Academic Press.

Virchow, R. (1864). *Die Krankhaften Geschwulste*, Vol. 2. Berlin: A. Hirschwald.

Waggener, J. D., Bunn, S. M. and Beggs, J. (1965). The diffusion of ferritin within the peripheral nerve sheath. An electron microscopic study. *J. Neuropath. Exp. Neurol.* **24**, 430.