EXPERIMENTAL STUDIES ON NERVE FIBERS IN LEPROSY

II. THE REACTION OF HUMAN SCHWANN CELLS TOWARD CARBON PARTICLES AND LEPROSY BACILLI[1]

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INTRODUCTION

In a previous paper Palmer, Rees and Weddell ([3]) gave an account of the reaction of rat Schwann cells toward carbon particles and two species of leprosy bacilli, *Mycobacterium lepraemurium* and *M. leprae,* injected between the stumps of divided sciatic nerves or into crushed nerves at the site of damage. Carbon particles within the endoneurium were taken up chiefly by Schwann cells and, at different periods after injection, could be traced both to the endothelial cells of intraneural blood vessels and, via the perineurium, to histiocytes in the epineurium. When *M. lepraemurium* was substituted for carbon particles, the bacilli were taken up only by Schwann cells in the absence of competing extraneous phagocytes, which are not found within the endoneurium in *crushed* nerves. When *M. leprae* was substituted for carbon particles and injected between the stumps of *divided* nerves, the bacilli evoked an intense inflammatory response in which there were numerous phagocytes containing many bacilli. A few of the organisms looked viable, but the majority were breaking up. Bacilli were found also in small numbers in many outwandering Schwann cells as well as in some within the endoneurium of both stumps. These organisms were all in an advanced state of disintegration. It was thus clear that Schwann cells had competed more successfully than inflammatory and other cells for *M. leprae,* as compared with *M. lepraemurium.*

The results encouraged us to extend our experimental work to human Schwann cells. During a visit to the Northern region of Nigeria, in an area where leprosy was endemic, patients suffering from various forms of leprosy were asked, and readily consented, to cooperate in this project. In addition the health authorities gave permission for examination of a randomly selected class of school children for any signs of infection and, in doubtful cases, for skin biopsies as well as other tests that might be helpful.

[1] Received for publication March 24, 1965.

MATERIALS AND METHODS

Series I.—In volunteers with various forms of leprosy 0.1 ml. of a suspension of carbon particles in normal saline (10 per cent India ink, C11/1431, particle size 200 Å, Gunther Wägner, Hannover) was injected into the skin, where lesions appeared to be most active, 48 hours prior to routine biopsies. The areas of skin that had been injected with carbon were removed and divided into two, one part being fixed in Zenker's fluid and the other in either Flemming's solution or 10 per cent neutral formalin. The pieces fixed in Zenker's fluid were used to assess the effectiveness of treatment and the others to determine the fate of the injected ink.

Series II.—In another series of volunteer patients with indeterminate forms of the disease, the dorsal cutaneous branch of the radial nerve was exposed at the wrist bilaterally and a fine bundle excised. On the right side 0.1 ml. of a similar suspension of carbon particles in normal saline was injected between the cut ends, and on the left side 0.1 ml. of a washed suspension of approximately 10^8/ml. autoclaved *M. leprae* in normal saline. Patients with this form of the disease were chosen in the hope that resident *M. leprae* would be few enough to permit the autoclaved bacilli to be recognized unequivocally.

Series III.—Thirty school boys in Class IV (9-12 years old) of a junior primary school were each examined physically for early signs of leprosy and in addition for palpable differences in the size and consistency of their ulnar and radial nerves at the elbow and wrist as compared with those of three observers, two European and one African. Before the investigation, each of the observers had agreed that the nerves of his colleagues were "normal" and comparable in size with his own. Each child was examined by each observer independently, who recorded his observations. Cases in which opinions were found to differ were subsequently reexamined to exclude any recording errors and the results were then pooled and averaged (Table 1).

Series IV.—Seven children from Series III with "enlarged" nerves (Nos. 8, 10, 13, 14, 19, 22 and 23), as well as one child (No. 15), whose nerves were not enlarged, were detained and the following procedures carried out: Child No. 8 was selected as a control and told to go home and report again five days later for biopsy. Child No. 10 had both his radial nerves at the wrist infiltrated with Novocain and adrenalin in the hope that the injection procedure would in itself be sufficient to stimulate neural turn-over. The areas of cutaneous anesthesia were then mapped and recorded to ensure that subsequent biopsy specimens were taken from skin supplied by the nerves that had been blocked. He also was sent home and told to report again five days later. In the remaining children the radial nerves were infiltrated at the wrists with Novocain and adrenalin and an anesthetic area at the base of the right forefinger was injected with 0.1 ml. of the same suspension of carbon particles in normal saline. In children Nos. 13, 14, 15 and 19 0.1 ml. of the suspension of autoclaved *M. leprae* in normal saline, and in Nos. 22 and 23 0.1 ml. of a comparable suspension of autoclaved *M. lepraemurium* (approximately 10^{10} bacilli/ml.), were injected into anesthetic areas at the bases of the left forefingers. The children were then told to go home and to report back for biopsy five days later. All the children reported back as requested, and under local anesthesia 2 mm. punch biopsies were taken from the areas injected with ink and autoclaved organisms. Comparable biopsies were taken from child No. 10 in zones that had been anesthetic after his radial nerves had been blocked at the wrists with Novocain and adrenalin, and 2 mm. punch biopsy specimens were removed from anesthetic zones at the bases of his right and left fingers, respectively, in positions comparable with those in which foreign matter had been injected in the other children.

After the biopsy specimens had been fixed, frozen sections 50μ thick were cut, and stained with silver by Schofield's modification of Bielschowsky's technic and counterstained by a modified Fite-Faraco technic (5). The rest of the material was embedded in paraffin and alternating 10μ sections were stained with hematoxylin and the modified Fite-Faraco method or hematoxylin and eosin.

TABLE 1.—*Size, consistency and mobility of radial and ulnar nerves in school children age 9-12 years.*

Child No.	Radial nerves at wrist		Ulnar nerves at elbow	
	Right	Left	Right	Left
1	0	0	+	0
2	0	0	+	0
3	+	0	+	0
4	thin & hard	thin & hard	thin & hard	thin & hard
5	0	0	0	0
6	0	0	0	0
7	0	0	+	0
8	+	+	+	+
9	0	0	0	0
10	+	+	+	+
11	hard	hard	hard	hard
12	very mobile	very mobile	very mobile	very mobile
13	+	+	+	+
14	++	++	+	++
15	0	0	0	0
16	0	0	0	0
17	0	0	0	0
18	+	+	+ hard	+ hard
19	+	+	0 hard	0 hard
20	0	0	0	0
21	0	0	0	0
22	+	+	+	+
23	+	+	+	+
24	0	0	0	0
25	+	+	+	+
26	0	0	0	0
27	+	+	+	+
28	0	0	0	0
29	0	+	+	+
30	++	0	0	0

OBSERVATIONS

Series I. Injection of carbon particles into the skin of patients with lepromatous, indeterminate and tuberculoid leprosy.—Table 2 shows the results in the different forms of the disease. In the four cases with lepromatous leprosy (Nos. 36, 39, 40 and 41) carbon particles were found in Schwann cells in every case. The a and b sections of Figure 1, from case No. 39, are typical of the pictures seen. Figure 1a shows a Schwann cell related to a "digestive" chamber with carbon particles in its wall (arrow). There are also carbon particles in the cytoplasm of another axon-free Schwann cell lying next to a healthy Schwann cell and its related axon (arrow). Figure 1b shows a clump of carbon particles (arrow) in a vacuole in the cytoplasm of an axon-free Schwann cell. The strand of cells of which this one was a member is lying beside another strand related to a regenerating axon. These pictures demonstrated very clearly the increased neural turnover seen everywhere in the skin of patients with lepromatous leprosy.

TABLE 2.—*Uptake of carbon particles by Schwann cells two days after injection of India ink into active skin lesions of patients with lepromatous, tuberculoid and indeterminate types of leprosy.*

	Type of leprosy			
Patient No.	Lepromatous	Tuberculoid	Indeterminate near lepromatous	Indeterminate near tuberculoid
36	+		-	
37		0		
39	+			
40	+			
41	+			
42			+	
43				+

TABLE 3.—*Uptake of carbon particles and* M. leprae *by Schwann cells five days after injection of India ink or bacilli into a cut radial nerve bundle at the wrist in patients with tuberculoid or indeterminate leprosy.*

	Type of Leprosy			
	Tuberculoid		Indeterminate	
Patient No.	Carbon	*M. leprae*	Carbon	*M. leprae*
78			+	+
79	0	0		
80	0	0		
81			+	+

Carbon particles were seen also in Schwann cells in patients No. 42 and 43, each with an indeterminate form of the disease, but in one of whom (No. 42) there was histopathologic evidence that the disease process was more toward the lepromatous end of the spectrum. By contrast, in patient No. 37, who had tuberculoid leprosy, we were unable to find any carbon particles in the few Schwann cells that remained in the anesthetic zone. In one of the few nerve bundles still present, which contained healthy nonmyelinated axons, a few carbon particles were seen in some of the perineurial cells.

In each of these cases most of the injected carbon particles filled histiocytes lying among collagen fibers in the dermis.

Series II. The reaction of Schwann cells to carbon particles and autoclaved M. leprae *injected between the ends of divided sensory nerves.*—In the four cases in which small fascicles had been excised from the cutaneous branch of the radial nerve exposed at the wrist, and carbon particles injected between the stumps, the second biopsy specimens were taken five days after the operation (Table 3). In cases Nos. 78 and 81 carbon particles were found in Schwann cells lying within both proximal and distal nerve stumps as well as in outgrowing cells from the distal stump. For example, Figure 2, from the biopsy specimen taken from case No. 81, shows a group of carbon particles in the cytoplasm of an axon-free Schwann cell lying among healthy

nerve fibers within the proximal stump. As can be seen from Table 3, each of the cases just referred to had an indeterminate form of the disease; in one it was histopathologically more toward the tuberculoid end of the spectrum than in the other. In cases Nos. 79 and 80, in which

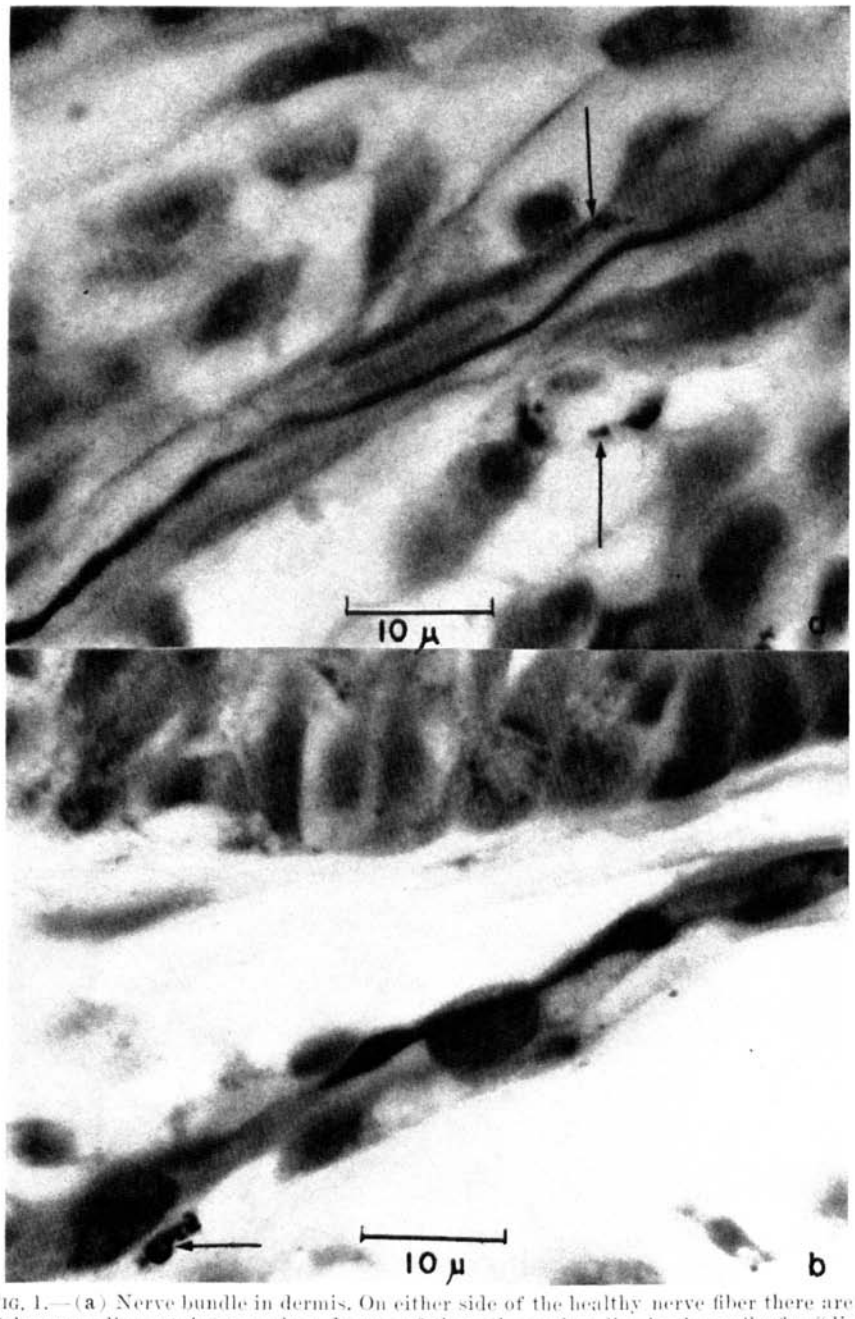


FIG. 1.—(**a**) Nerve bundle in dermis. On either side of the healthy nerve fiber there are axon-free Schwann cells containing carbon. In one of them the carbon lies in the wall of a "digestive chamber" (arrows). (**b**) Nerve bundle just below epidermis containing two strands of Schwann cells. One is related to a regenerating axon; the other is axon-free, but one of its cells has a clump of carbon particles in a vacuole in its cytoplasm (arrow). From 50μ silver-stained sections.

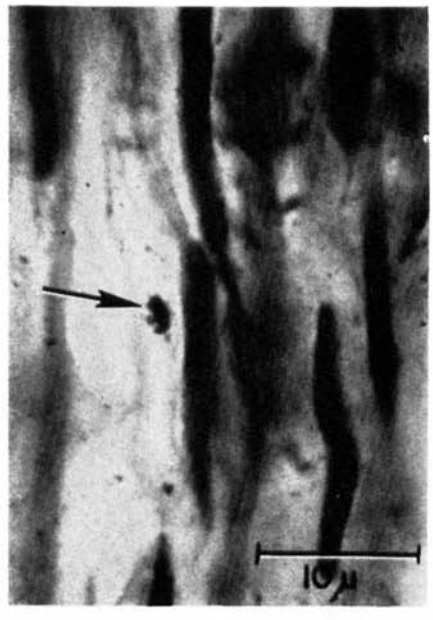


FIG. 2.—Nerve bundle in dermis. There are carbon particles (arrow) in an axon-free Schwann cell lying among healthy nerve fibers. From a 50μ silver-stained section.

no carbon was found in the second biopsies, the material obtained at the first biopsies showed that the nerves had already been destroyed by the disease. Neither specimen contained any axons, but only a few strands of nuclei, in size and shape resembling those of Schwann cells, buried in a mass of scar tissue. Nevertheless, the second biopsies revealed that a considerable amount of fibrous tissue had bridged the gaps left by the first biopsies, but the few cells present contained no carbon particles.

Before injecting washed autoclaved *M. leprae* between the stumps of divided sensory nerves of volunteer leprosy patients a pilot experiment had been performed on a normal rat, to see if material of this kind was likely (a) to survive and be recognizable after five days and (b) to evoke the same response as viable *M. leprae* had done when they were injected between the stumps of divided sciatic nerves ([3]).

One-tenth ml. of autoclaved *M. leprae* was injected between the stumps of a divided rat sciatic nerve. Five days later both stumps and the tissues between them were removed and processed as described above. There had been an intense inflammatory reaction. Nevertheless, disintegrating organisms were found in Schwann cells lying in the proximal stump 3 mm. or more from the site of section, as well as in Schwann cells within and growing out from the distal stump. Figure 3 shows disintegrating bacilli in a "digestive" chamber (arrow) as well as a single organism in the cytoplasm of another Schwann cell (arrow) which has retained its characteristic appearance. There were many organisms in the inflammatory cells in the epineurial sheath and between the nerve stumps, but they were not broken up as much as those in the Schwann cells. It was clear from this experiment that the Schwann cells had competed successfully for the autoclaved *M. leprae,*

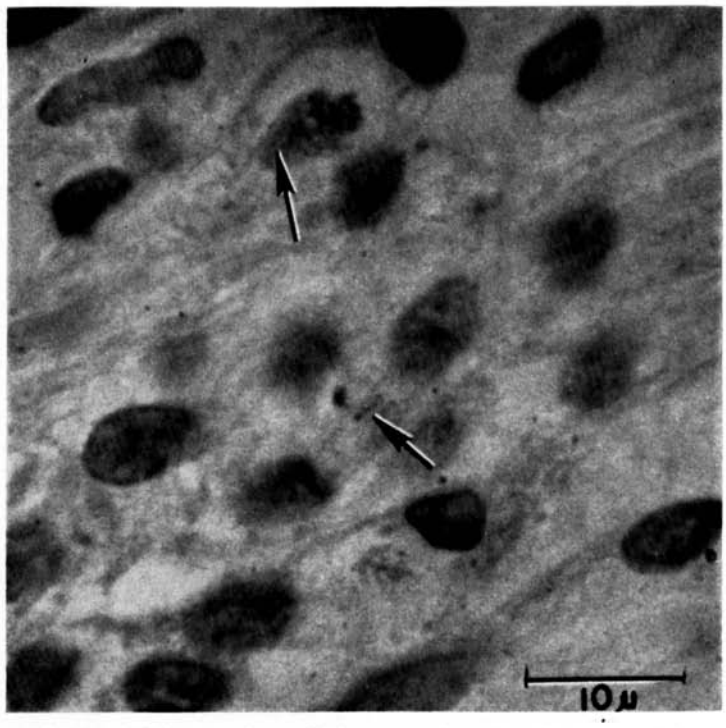


FIG. 3.—A clump of disintegrating acid-fast organisms in a Schwann cell 2 mm. *within* the proximal stump of a divided rat sciatic nerve. They are lying in a "digestive chamber" (arrow). There is a single organism, which has retained the appearance characteristic of bacilli that have been autoclaved, in the cytoplasm of another Schwann cell (arrow). From a 10μ section stained with hematoxylin and carbol fuchsin.

despite the presence of an inflammatory response in which there were many phagocytes, and that they were disposing of them more rapidly than the other cells. The response was in fact similar to that following the injection of viable *M. leprae* ([3]).

As can be seen in Table 3, in cases No. 78 and 81, with indeterminate forms of leprosy, in which carbon particles had been taken up by Schwann cells on the right side, there were inflammatory responses on the left side into which the autoclaved *M. leprae* had been injected. In case No. 81 it was of a type seen in a lepromin-positive reaction. The tissue response in case No. 78 was less intense, but in both cases the reactions were visible clinically, the bases of the fingers being tense and swollen. In each case, injected organisms were seen in Schwann cells and, as in the rat, most of the rods were breaking up more rapidly in these cells than they were in the surrounding inflammatory cells which contained more of them. In case No. 78 the Schwann cells showed unusually intense activity, to judge by the number that had proceeded from the distal stump, as well as the presence of multiple nucleoli in their nuclei. These cells contained numerous autoclaved organisms that were breaking down. There were also many characteristic organisms in the inflammatory cells as well as in the cells of the epineurial sheath, but, unlike those in Schwann cells, the majority were not under-

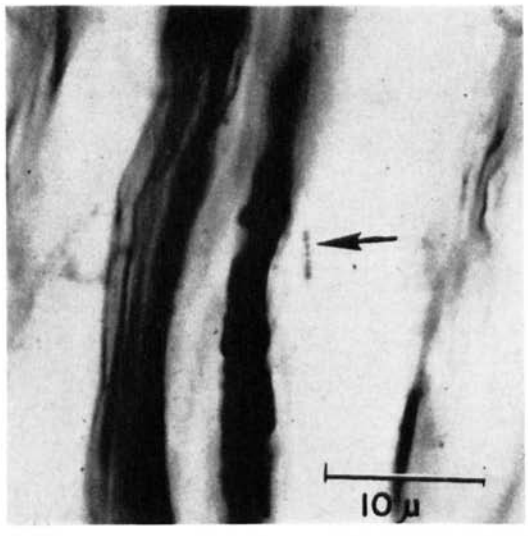
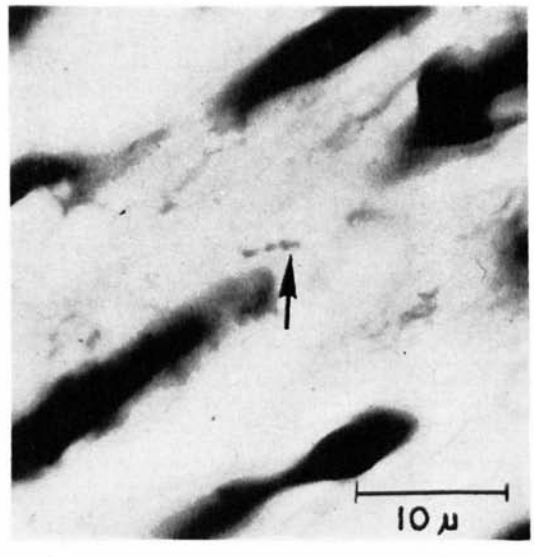


FIG. 4.—Proximal stump of radial nerve 5 days after injection of autoclaved *M. leprae* into gap between ends of divided bundle. A single organism is seen beside a relatively healthy looking axon (arrow). It is comparable in form to the autoclaved bacilli injected at operation and unlike the organisms in the nerve before it was divided (see Fig. 6). Biopsy from a patient with an indeterminate form of leprosy. 50μ section stained with silver and carbol fuchsin.

FIG. 5.—Distal stump of radial nerve 5 days after injection of autoclaved *M. leprae* into gap between ends of divided bundle. A single organism that is starting to fragment can be seen lying in the cytoplasm of a Schwann cell, which contains also a segment of swollen degenerating axon beside a relatively healthy-looking axon (arrow). It is comparable in form to the autoclaved bacilli injected at operation and unlike the organisms in the nerve before it was divided (see Fig. 6). Biopsy from a patient with an indeterminate form of leprosy. 50μ section stained with silver and carbol fuchsin.



going fragmentation. Figures 4 and 5 illustrate these observations. In particular, they show that the distinctive morphology conferred on *M. leprae* by autoclaving ([2]) can be recognized five days after their injection between the stumps of divided sensory nerves in human volunteers and that they are found in Schwann cells inside both the proximal and distal nerve stumps. Figure 6 comes from a section of the fascicle removed at the first biopsy from the same case (No. 78) and shows a viable organism within a Schwann cell lying alongside a normal looking nerve fiber (compare Figs. 4 and 5). Very few organisms were found in this nerve, but all those seen looked viable.

In cases No. 79 and 80 the areas of skin served by the radial nerves showed higher degrees of sensory impairment than those in cases No. 78 and 81. Moreover, at operation the nerve bundles were pale and sur-

TABLE 4.—*Summary of observations on the skin of children taken from the base of the index finger five days after radial nerve block at the wrist and the injection of carbon particles and autoclaved* M. leprae *or* M. lepraemurium *at the base of finger.*

Child No.	Radial nerve size		Treatment 5 days prior to biopsy		Changes in skin taken from base of index fingers		Clinical examination after 12 months
	R	L	Right	Left	Right	Left	
8	+	+	Nil	Nil	Increased neural turn-over, perineurium thickened	Increased neural turn-over, perineurium thickened	No change in size of nerves. No signs of leprosy
10	+	+	Radial nerve blocked at wrist	Radial nerve blocked at wrist	Innervation normal	Increased neural turn-over, perineurium thickened	"
13	+	+	Ditto plus carbon base index finger	Ditto plus *M. leprae* base of index finger	Innervation normal, no carbon particles	Lepromin positive (++). Increased neural turn-over at site of injection (++), elsewhere (+). No acid-fast bacilli	"
14	++	++	"	"	"	Lepromin negative. Innervation normal. No acid-fast bacilli	"
15	0	0	"	"	Increased neural turn-over and carbon in Schwann cells, site of injection only	Lepromin negative. Increased neural turn-over (+) at site of injection only. No acid-fast bacilli	"
19	+	+	"	"	Innervation normal, no carbon particles	Lepromin positive (++) Increased neural turn-over at site of injection (++), elsewhere (+). No acid-fast bacilli	"
22	+	+	"	Ditto plus *M. lepraemurium* base of index finger	Increased neural turn-over and carbon in Schwann cells, site of injection only	Extensive inflammatory reaction. Increased neural turn-over (+) at site of injection only. No acid-fast bacilli	"
23	+	+	"	"	Innervation normal, no carbon particles	"	"

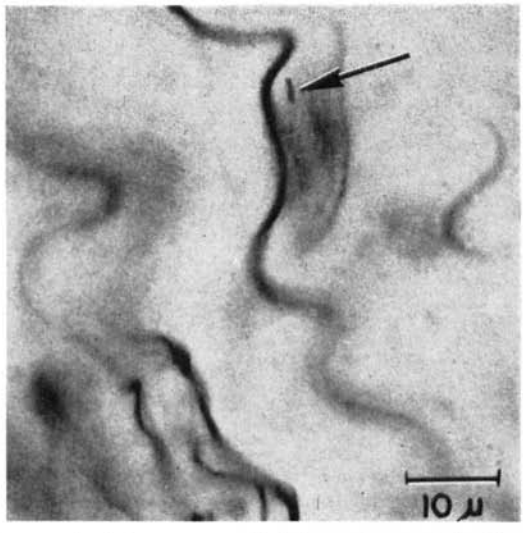


FIG. 6.—Bundle of fibers excised from the radial nerve at the wrist of a patient with an indeterminate form of leprosy. A single viable-looking organism is lying beside a healthy-looking axon (arrow). 50μ section stained with silver and carbol fuchsin (cf. Figs. 4 and 5).

rounded by very thick epineurial sheaths. The sheaths were removed and suitable fascicles excised. However, the first biopsies showed that the nerves had already been destroyed and the second biopsies showed no Schwann cell activity. No organisms were found in any of the specimens. In both cases, however, there was a profusion of newly formed fibrous tissue between the "stumps," and this is consistent with the fact that, before the second biopsy specimens were taken, hard, neuroma-like bodies could be felt beneath the skin at the sites from which the nerve fascicles had been excised.

Series III and IV.—The results of the investigations in the school children are given in Tables 1 and 4. They show that neither enlargement nor hardening of either mixed or cutaneous nerves (compared

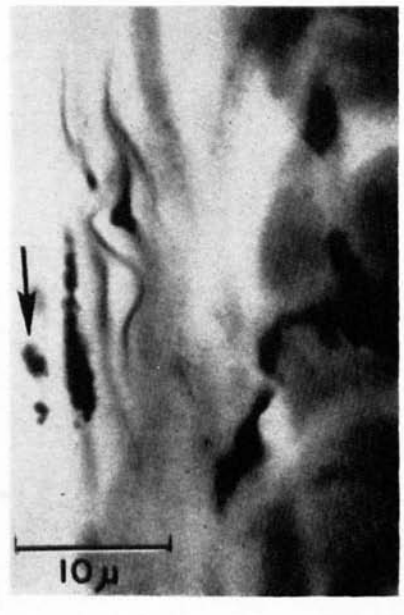


FIG. 7.—Small bundle of nerve fibers in the dermis of forefinger skin 5 days after the injection of carbon particles. An aggregation of particles can be seen in an axon-free Schwann cell pathway beside healthy-looking axons. A few particles can be seen also in the perineurium (arrow). The great majority of carbon is in nearby macrophages. 50μ silver-stained section.

with those of the examiner) permit any inferences to be drawn as to the state of the cutaneous twigs served by the nerves, let alone the likelihood that the subjects were suffering or had suffered from leprosy. Neither did they give any indication as to whether or not the subjects would develop signs of leprosy within the next twelve months, a fact which emerged on reexamination. It is also clear that blocking a nerve trunk with novocain and adrenalin *per se* did not do enough damage for this to be detected in the skin served by the nerve five days later (Table 4, case No. 14). On the other hand, intradermal injections of suspensions of either carbon particles or mycobacteria into the chosen sites did lead to local neural damage detectable five days later (Table 4, case Nos. 13, 15, 19, 22 and 27). For example, in case No. 15 neither the mixed nor the cutaneous sensory nerves were judged to be in any way clinically abnormal. Yet on the right side, but only surrounding the site of injection, there was a local increase in the turn-over of nerve fibers, and Schwann cells had ingested carbon particles (Fig. 7). Elsewhere in the specimen the nerves appeared to be entirely normal. On the left side, but again only surrounding the site of injection, there was also a local increase in neural turn-over, which was absent elsewhere in the specimen. It was of interest that there had been no cellular response to the injection of autoclaved *M. leprae* and that no organisms were seen in the sections from this biopsy. Furthermore, as noted in

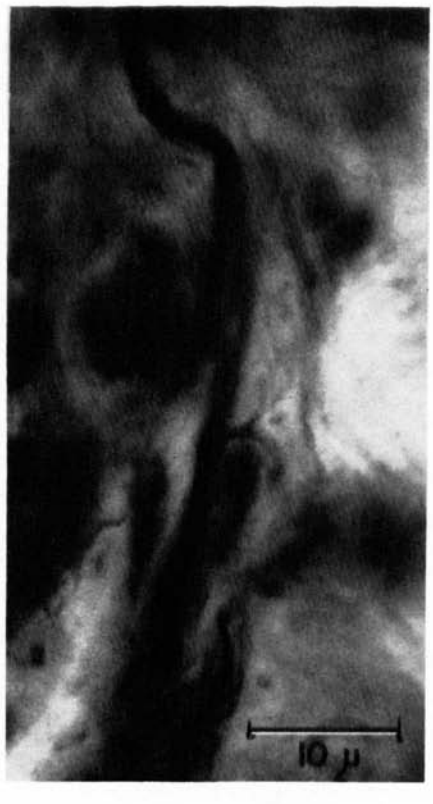


FIG. 8.—A small bundle of nerve fibers in the dermis of forefinger skin. No injections had been made in this area of skin, which looked healthy; the radial nerve was not blocked before operation. One unusually large axon is myelinated and gives off a branch which itself dichotomizes. This is typical of a regenerating nerve. The perineurial sheath is also unusually thick and all these appearances are similar to those seen in skin of patients with an indeterminate form of leprosy. 50μ silver-stained section.

Table 4, there was no clinically detectable swelling at the site of injection. It can only be supposed that this child was lepromin-negative and that the injected organisms had either been eliminated by the Schwann cells or that the zones from which the sections came were a fraction too far away from the site of injection for organisms to be recognized. In the biopsy from case No. 22, whose mixed and cutaneous sensory nerves were slightly enlarged clinically, carbon was found also in Schwann cells close to the site of injection. Elsewhere the nerves in the section were normal. In cases No. 13, 14 and 23 the biopsies showed no carbon particles, nor was there any increase in neural turn-over. It was obvious that these biopsies had failed to include the site of injection of carbon. By contrast, in skin from both sides in case No. 8 and from one side in case No. 10 all the perineurial sheaths surrounding the bundles of nerve fibers were thickened and there was a marked increase in neural turn-over (Fig. 8). In neither of these cases had local injections been made into the skin at the sites of biopsy; yet the neural changes were greater and more widespread than in any of the other specimens examined. They looked the same as those seen in indeterminate forms of leprosy except that no acid-fast organisms could be found. Moreover, there were no symptoms or clinical signs of the disease at this time nor twelve months later.

In four out of the six boys there was an inflammatory response at the site of injection of the mycobacteria, but again no organisms were found. In all cases except one, in which *M. leprae* had been injected, there was an increased turn-over of nerve fibers, and in the two cases in which *M. lepraemurium* had been injected, there had been an intense inflammatory response including the appearance of giant cells, resembling closely the cellular response seen in tuberculoid leprosy. However, the nerve fibers and Schwann cells in both these cases were quite unaffected, except immediately adjacent to the site of injection, where there was increased neural turn-over.

It should be emphasized that in the school children the amount of fluid injected was very small, as indeed was the amount of skin removed, so that in case No. 15, in which no inflammatory response was apparent in the tissues on the left side and no increase in neural turn-over detectable on either side, both biopsy specimens must have come from skin not coinciding with the sites of injection in a lepromin-negative subject.

These experiments, taken together, suggested that leprosy is probably transmitted by inoculation of *M. leprae* into the skin and that the Schwann cells of nerve fibers injured by the inoculation successfully compete for the bacilli. The bacilli may be destroyed by the Schwann cells or they may destroy the Schwann cells that ingested them. These cells would then be ingested by neighboring Schwann cells, which in their turn might succumb, and in this way the disease could spread centrifugally along a nerve bundle until the organisms were finally destroyed. Such a sequence of events would certainly account for the

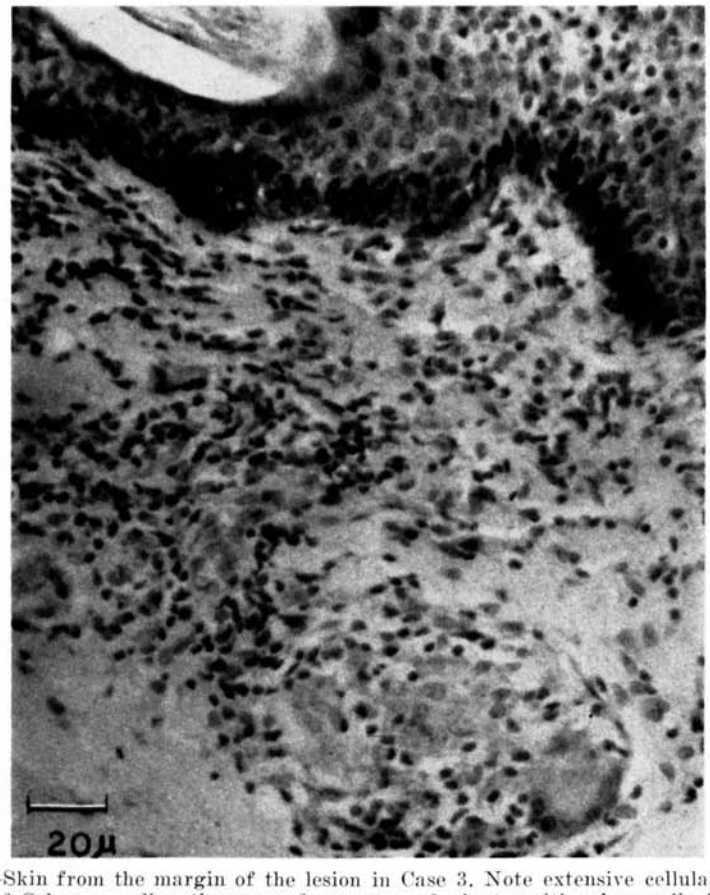


FIG. 9.—Skin from the margin of the lesion in Case 3. Note extensive cellular infiltration, destruction of Schwann cell pathways and presence of giant multinuclear cells in the neurovascular bundles. The picture is typical of a self-limiting tuberculoid lesion. No acid-fast bacilli were seen. 10μ section, stained with hematoxylin and carbol fuchsin.

clinical observations in the so-called "self-limiting" form of the disease, i.e., tuberculoid leprosy.

Evidence that spread of injection can and does take place in this manner in certain regions of the body is afforded by the following observations. A Nigerian boy aged 14 years was sent by his family to a center for treatment because six months previously two areas of skin over his chest wall had first become reddened and then become depigmented and anesthetic. Since then the patches had not become any bigger, but equally they showed no signs of getting smaller or becoming repigmented. He had received no treatment of any kind.

On clinical examination the lesions were found to be typical of tuberculoid leprosy, and there were thickened cutaneous branches of an intercostal nerve going to each of them. Both affected areas were depigmented and anesthetic. There was a very slightly raised margin around each area, but it was not reddened and there was no clinical indication that the disease was progressing, but rather that it was resolving. Moreover, each of the thickened nerves could be palpated without giving rise to pain. Skin biopsy specimens were taken from the

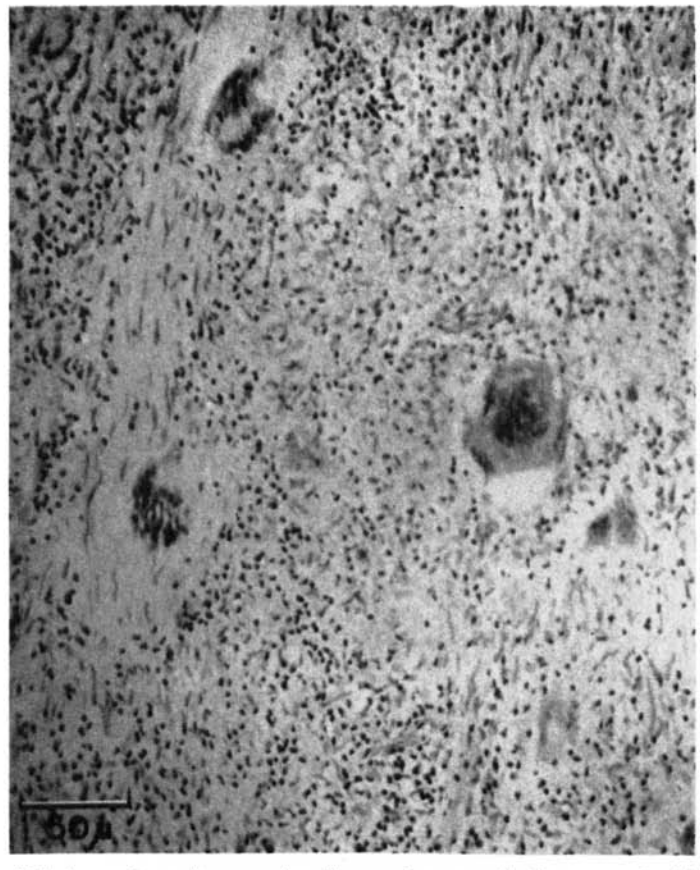


FIG. 10.—Distal portion of nerve bundle serving anesthetic area of skin in Case 3. It shows massive cellular infiltration, multinuclear giant cells and complete disorganization of neural structures. 10μ section, stained with hematoxylin and carbol fuchsin.

center and margins of each lesion. No acid-fast bacilli were seen in any of these specimens. Specimens from the center of the areas contained no nerve fibers and only a few poorly organized Schwann cell pathways. At the margins of both lesions there was an inflammatory response containing giant cells, typical of tuberculoid leprosy; this is illustrated in Figure 9. No nerve fibers were seen toward the anesthetic side of the marginal zone, but a few regenerating axons were seen advancing toward the denervated area from adjacent normal skin that had been included in the biopsy specimens. The skin beyond the lesions showed neither neural nor other histopathologic changes and no acid-fast organisms could be found in these regions.

A biopsy specimen 1 cm. in length was taken also from the cutaneous nerve serving one of the patches, about 5 cm. distant from the lesion. As can be seen in Figure 10, a typical tuberculoid response had destroyed the distal part of the nerve bundle, but more proximally a few intact healthy-looking axons were still present and related to equally healthy-looking Schwann cells, except for the fact that they contained viable *M. leprae* in their cytoplasm (Fig. 11).

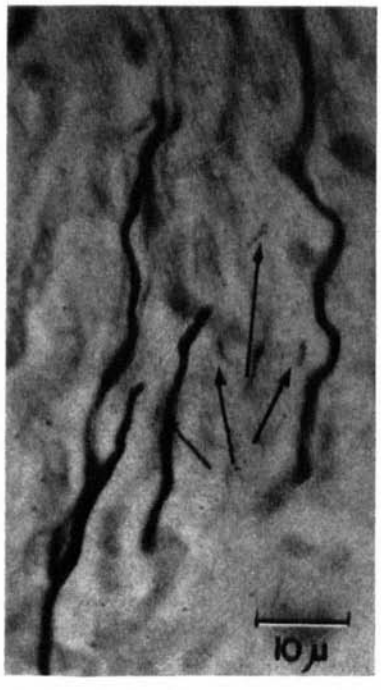


FIG. 11.—Proximal portion of nerve bundle serving anesthetic area of skin in Case 3. It shows a few healthy-looking axons, lying among degenerating nerve fibers. Here and there (arrows) acid-fast bacilli, some apparently viable, are seen in Schwann cells. 50μ section, stained with silver and carbol fuchsin.

DISCUSSION

It has been demonstrated that Schwann cells of cutaneous nerves take up carbon particles injected into their neighborhood under certain conditions: (a) in the presence of de- and regenerating nerve fibers, and (b) when growing out from the stumps of recently divided nerves.

Since neural turn-over in the skin of patients with lepromatous and some forms of indeterminate leprosy is always greater than that in healthy subjects ([7]), it was not surprising that a number of cutaneous nerve fiber Schwann cells had carbon particles in their cytoplasm 24 hours after it had been injected. Carbon particles were found also in healthy finger skin close to the site of injection five days previously. Their presence in Schwann cells was clearly due to the locally increased neural turn-over, which in turn must have been due to the trauma caused by the injection of the fluid containing the carbon, since at these sites it had to be injected under pressure.

To explain these observations it may be assumed that either the perineurial sheath, consisting of interlocked cells, ends before nerve fibers terminate in the skin or that it is easily breached by trauma or disease. In fact, electron microscope studies have demonstrated that the cellular perineurial sheath *does* change its form radically in relation to nerves forming the cutaneous plexus. In this region there is no complete sheath of interlocked cells, but rather a series of perineurial satellite cells that accompany the Schwann cells and neurites towards their termination, but stop short of it ([8]).

Carbon particles were found in the cytoplasm of the Schwann cells

bridging the gaps between the stumps of the excised nerve fascicles in the volunteer patients with indeterminate forms of leprosy. It should also be stressed that carbon particles were found in the cytoplasm of Schwann cells lying well within both nerve stumps. To have reached these positions they must have been transported by the cells themselves, for there are no lymphatics or other channels in the stumps along which the carbon could have either traveled passively or been pushed. When autoclaved *M. leprae* were substituted for carbon particles in such patients, even though washed, they evoked a lepromin-like reaction. Moreover, even in the presence of an inflammatory response with many phagocytes, Schwann cells had competed for them successfully and were destroying them more rapidly. Disintegrating bacilli were found within Schwann cells inside both nerve stumps, as well as in outwandering cells from the distal stump.

These findings paralleled comparable experiments in rats ([3]) in which it has been shown that Schwann cells behave as phagocytes toward carbon particles and compete successfully against other phagocytes for both viable and autoclaved *M. leprae* injected between the stumps of sciatic nerves divided five days previously. In rats also, both viable and autoclaved *M. leprae* were found in Schwann cells well inside both nerve stumps, although neither viable nor autoclaved *M. lepraemurium* were ever seen in this position.

In the series of investigations in which saline suspensions of either washed autoclaved *M. leprae* or *M. lepraemurium* were injected into healthy finger skin of children living in a region where leprosy was endemic, the *M. leprae* either caused a lepromin reaction or failed to cause any reaction at all. *M. lepraemurium*, on the other hand, caused a severe reaction (including the appearance of giant cells), with considerable swelling and edema. After five days no acid-fast organisms could be found in any of the specimens, but in the skin in which *M. leprae* had been injected there was a marked increase in the turn-over of the nerve fibers, which was only roughly localized around the site of injection. By contrast, in skin into which *M. lepraemurium* had been injected, there was a small sharply localized zone of increased neural turn-over at the site of injection, but none elsewhere, despite the very much greater inflammatory response that took place both at, and at a distance from, the site of injection.

These investigations on human subjects confirmed the results of the experimental work reported above and in the first of these papers ([3]): It is clear that Schwann cells react quite differently toward *M. leprae* and *M. lepraemurium*. They compete successfully against other cells for *M. leprae*, and five days after the inoculation of bacilli, were in a state of activity normally only seen at a later date following nerve damage. They were quite unaffected by *M. lepraemurium*, and it was remarkable also that, despite the intense inflammatory reaction evoked by these bacilli, nerves surrounded by inflammatory cells, but not immediately adjacent to the site of injection, were quite unaffected. An-

other gratifying finding was that autoclaved *M. leprae* and *lepraemurium* were capable of evoking specific cellular responses, in all respects comparable with their viable counterparts, in both rats and human subjects.

As stated in our first paper on this subject, it has been known for a very long time that *M. leprae* and nerve fibers are closely associated. Here we were concerned specifically to determine which neural element was chiefly involved. It proved to be a Schwann cell.

This finding we consider to be of importance in understanding the disease process, for we are beginning to know more about the activity of nerve fibers and Schwann cells in the skin as the result of studies on other aspects of this problem. It is now quite clear that they differ both with regard to number and turn-over in different people and in different races. In all persons and at all ages there is a turnover of both these elements in skin, which tends to increase with increasing age ([8]). On the other hand, in some individuals, e.g., those with psoriasis, a disease that indeed may exist in subclinical form, the turn-over is enormously accelerated ([6]).

It must be presumed that leprosy is caused by the inoculation of mycobacteria into the skin, although it is of course theoretically possible that they might get into Schwann cells by inunction, or even by other routes. We ourselves have never seen *M. leprae* lying in the epidermis, although we have examined thousands of sections of lepromatous skin. Occasionally organisms were thought to have been in the epidermis, but this has always proved to be wrong and either an optical illusion due to the angle of the section or to the fact that they had been displaced from elsewhere by the microtome knife. Moreover, we have come across only one statement in the literature claiming that these organisms were seen in the epidermis ([1]). Whatever the route, they will eventually reach Schwann cells related to damaged nerve fibers, which will compete for them. Further developments must depend upon (a) the rate of exposure to infection and the number of organisms injected at each exposure, and (b) the state of the subject's immunity. In lepromatous leprosy there is now good evidence that it is contracted most commonly when subjects are exposed to massive doses of infection, for the lepromatous rate falls when such patients are either segregated or treated successfully ([4]). Nevertheless, in some persons lepromatous leprosy apparently develops far more readily than in others when exposed to the same environmental hazards. It is reasonable to propose that in lepromatous leprosy the organisms are harbored by Schwann cells. Only when they have multiplied sufficiently do they burst into the endoneurium, whence they are taken to blood vessels, and so get into the bloodstream ([9]), or are carried through the perineurium into macrophages in the epineurium, just as in the case of carbon particles ([3]). From the bloodstream they are disseminated to other tissues and eventually those accumulating in the epineurium cause strangulation of nerve trunks. Thus the well-known clinical con-

dition of lepromatous leprosy develops. From what has been said it is reasonable to speculate that, since the treatment of these cases despite "sulfones" is still prolonged and not always successful, drugs currently in use do not reach the organisms in Schwann cells in the endoneurium in sufficient concentration to kill them. It is usually possible to destroy organisms lying in other cells with sulfones. Now that we know that Schwann cells harbor viable organisms, it follows that progress in treatment might lie in a determined effort to find drugs that can reach the organisms in these cells.

SUMMARY

The Schwann cells of sensory nerves in man behave in a manner analogous to that of Schwann cells of rat sciatic nerves toward carbon particles, and *autoclaved M. leprae* and *M. lepraemurium*.

Human Schwann cells take up carbon particles for disposal outside the endoneurium and compete successfully against inflammatory cells for *M. leprae*, but do not do so in the case of *M. lepraemurium*. The cellular response in man toward washed autoclaved *M. leprae* suspended in saline is similar to that in the rat toward both viable and autoclaved organisms.

These observations suggest that every effort should be made to discover some drug that will enable *M. leprae* harbored within Schwann cells to be destroyed without injury of the Schwann cell at the same time.

RESUMEN

Las células de Schwann de los nervios sensoriales en el hombre, se comportan de una manera análoga a aquellas de los nervios ciáticos de las ratas con respecto a las partículas de carbón y a los *M. leprae* y *M. lepraemurium* post-autoclave. Las células de Schwann humanas toman las partículas de carbón para su disposición fuera del endoneurium y compiten existosamente con las células inflammatorias en cuanto al *M. leprae*, pero no lo hacen así en el caso del *M. lepraemurium*. La respuesta celular en el hombre hacia los *M. leprae* de autoclave y lavados, suspendidos en solución salina, es similar a aquellos en las ratas hacia ambos organismos viables y de autoclave.

Estas observaciones sugieren que debe ser hecho el mayor esfuerzo para descubrir alguna droga que permita destruír al *M. leprae* localizado dentro de las células de Schwann, sin injuria para estas mismas.

RESUMÉ

Les cellules de Schwann des nerfs sensitifs de l'homme se comportent d'une manière analogue aux cellules de Schwann des nerfs sciatiques du rat envers les particules de charbon, envers les *bacilles M. leprae* autoclavés ainsi qu'envers les *bacilles M. lepraemurium* ègalement autoclavés.

Les cellules de Schwann humaines absorbent les particules de charbon pour les évacuer hors de l'endonèvre et entrent en compétition avec les cellules inflammatoires en ce qui concerne *M. leprae*, mais ne se comportent pas de la même manière dans le cas de *M. lepraemurium*. Chez l'homme, la réponse cellulaire à une suspension saline de *M. leprae* lavés est similaire à la réponse observée chez le rat à l'égard des organismes vivants et des organismes autoclavés.

Ces observations suggèrent que tous les efforts devraient tendre vers la découverte de quelque médicament pouvant permettre la destruction de *M. leprae* lorsqu'il est contenu dans les cellules de Schwann sans causer en même temps de dommage à cette cellule de Schwann.

Acknowledgements.—Our grateful thanks are due to the following organizations which have given us financial aid: Colonial Medical Research Council—"Lepra," and The Rockefeller Foundation. The cost of 7 black and white illustrations has been met by the Mission to Lepers, to whom we would like to offer our sincere thanks. Two of us (D.G.J. and G.W.) would also like to thank the Emir of Katsina for his help and cooperation during our visit to the Northern Region of Nigeria, without which this investigation would not have been possible.

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