SESSION 6—13 MAY 1965

Dr. Binford. We had hoped to have Dr. C. G. S. Iyer of Dr. Dharmendra's staff with us at this meeting, but he was unable to leave India in time to come to this conference. He will arrive in Washington soon for a stay of several weeks at the Armed Forces Institute of Pathology. Dr. Iyer is a neuropathologist engaged in research with Dr. Dharmendra. We hope he will have a chance to visit institutions in this area that are carrying on leprosy research. The paper he was to give at this conference will be published in its *Proceedings*.

Predilection of *M. leprae* for Nerves

Neurohistopathologic Observations[1]

C. G. S. Iyer, M.D.[2]

Involvement of the cutaneous and peripheral nerves constitutes the most unique feature of the pathologic changes in leprosy. Besides raising the question of selective predilection of *M. leprae* for the peripheral nervous system([11]), this feature lends itself to application in the study of leprosy in several ways. Reports on the histopathologic features of leprosy mention involvement of nerves in all stages and types of the disease. The object of this paper is to attempt to define the sequence of the pathologic changes in nerves, in as nearly complete a manner as possible with the material available for study.

MATERIALS AND METHODS

The materials on which this study has been based include numerous specimens of cutaneous lesions of leprosy, and about 20 cutaneous nerves removed for biopsy because of palpable thickening. The cutaneous lesions represent both macular and infiltrative forms of leprosy, and nerves were taken for biopsy from patients with tuberculoid, borderline, or lepromatous leprosy respectively. Specimens were fixed suitably, and paraffin sections were stained routinely with hematoxylin and eosin, Mallory's aniline blue for connective tissue, and the Fite-Faraco modification of the Ziehl-Neelsen stain for acid-fast bacilli. Special fixing, sectioning, and staining procedures were employed, in addition, in a few cases.

The clinical classification of the type of leprosy was determined by the usual criteria employed for this purpose. It was almost invariably substantiated by the pattern of tissue response observed in the biopsy specimens.

OBSERVATIONS

The pattern of pathologic change was generally similar within both the dermal and the deeper cutaneous nerves, but cer-

[1]Part of this material was presented as a lecture at the Armed Forces Institute of Pathology, Washington, D.C., and at the Department of Pathobiology, School of Public Health, Johns Hopkins University, Baltimore, Md. in June 1965 during a visit to the United States sponsored by the Leonard Wood Memorial.

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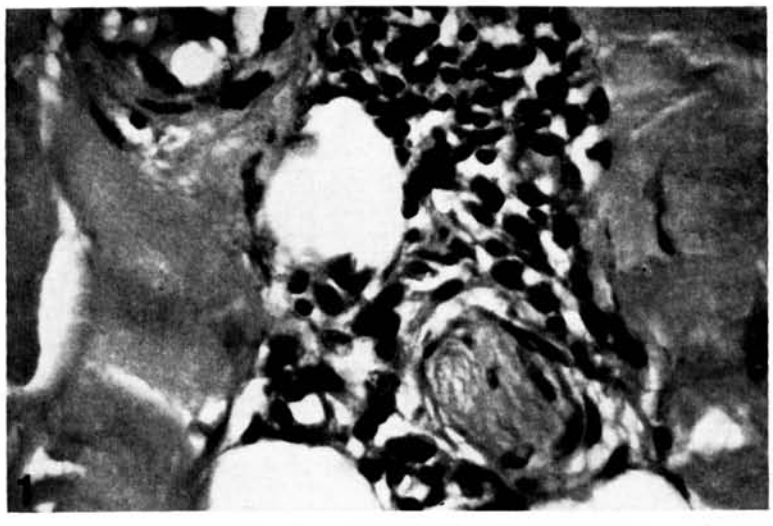

FIG. 1. Part of a neurovascular bundle from a case of indeterminate leprosy. Note the intact small nerve twig and the mononuclear cells infiltrating the neurovascular space (H & E, × 480).

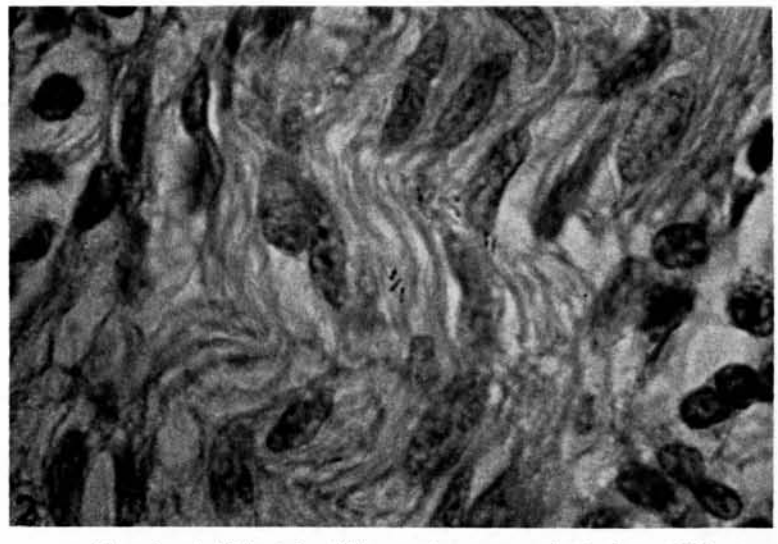

FIG. 2. Acid-fast bacilli seen in scattered singles within an intact small nerve similar to the one seen in Fig 1. (Fite-Faraco × approx. 1000).

tain stages of the process were seen better in one or the other. What appeared to be the earliest changes in the nerves were encountered within some of the macular lesions of leprosy. Nerve twigs of apparently normal appearance were seen in the neurovascular spaces of the dermis, surrounded by a moderate number of lymphocytes and other small mononuclears (Fig. 1). Acid-fast bacilli were seen within these nerves singly and in small groups (Fig. 2). A slightly more advanced phase of this process was represented by the impingement of such inflammatory cells upon and infiltration into the margins of these nerves, the bulk of the nerve parenchyma remaining unaffected, and revealing the presence of acid-fast bacilli. Such findings were encountered in approximately 50 per cent of indeterminate and maculoanesthetic skin

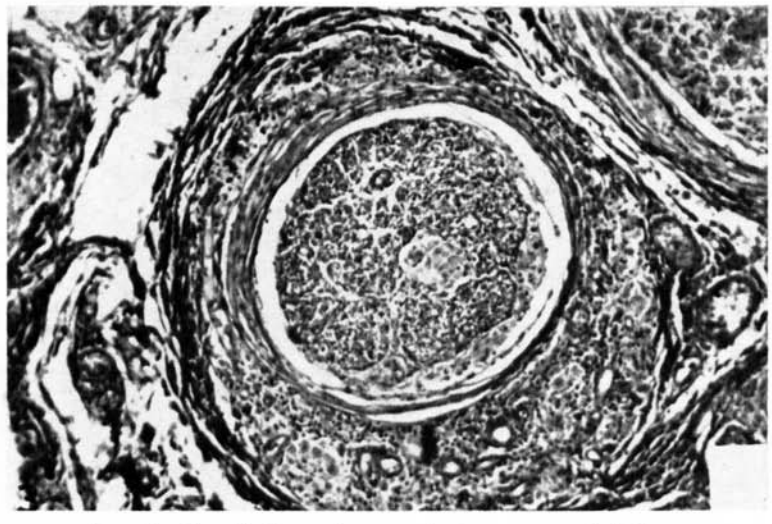

FIG. 3. Small dermal nerve twig, surrounded by exudate, from a case of tuberculoid leprosy. Note marginal infiltration by, and a small focus of, epithelioid cells, in the center of the nerve twig (Mallory's aniline blue, × 100).

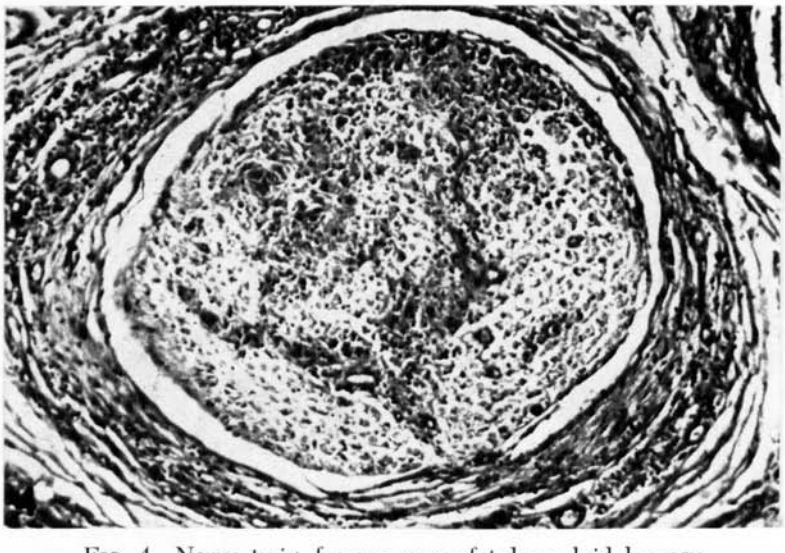

FIG. 4 Nerve twig, from a case of tuberculoid leprosy, surrounded by exudate and showing further replacement of nerve parenchyma by epithelioid cell groups (H & E, × 100).

lesions respectively, and have been termed as banal or nonspecific([4]). Further advancement of the process was represented by inflammatory cellular infiltration of the interstitial tissue with focalized collections of epithelioid cells partially replacing the dermal nerve twigs (Figs. 3 & 4). This stage proceeds toward a more nearly complete replacement of the nerve by a tuberculoid granuloma with focalized epithelioid cells and one or more Langhans' giant cells. Fragmented endoneurium was detected within this granuloma in appropriately stained preparations. Further stages of the tuberculoid process in the dermal nerves were difficult to define.

The sequence of events in lepromatous leprosy is not so clearly definable, because

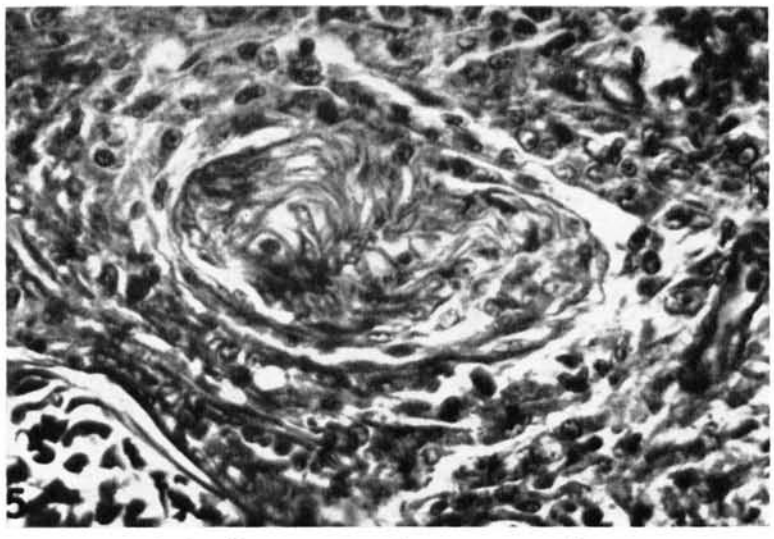

FIG. 5. Small nerve twig from a case of lepromatous leprosy. Note the exudate surrounding the nerve twig, which appears intact (H & E, × 480).

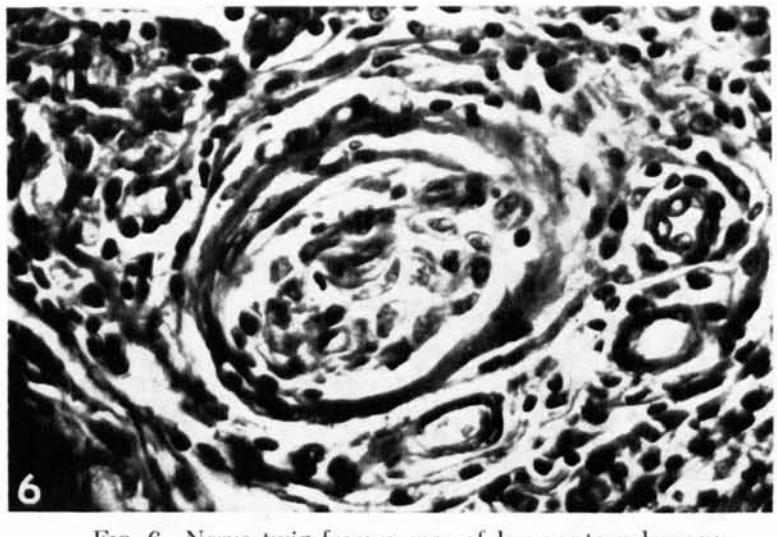

FIG. 6. Nerve twig from a case of lepromatous leprosy showing commencing proliferation of the perineurium. The nerve parenchyma appears relatively well preserved (H & E, × 480).

of the difficulty of obtaining biopsy specimens at appropriate stages. From the picture described earlier as a nonspecific lesion, it was possible to visualize a process where the nerve twig becomes surrounded by numerous adventitious elements, some of which reveal commencing vacuolation of the cytoplasm (Fig. 5). In further stages of this process such cells were seen to infiltrate between the nerve fibers, still permitting clear demonstration of the architecture of the nerve in appropriately stained preparations (Fig. 6). Numerous bacilli, generally in groups and occasionally in globi, were seen both within the vacuolated cells and along the course of uninvolved neural parenchyma. A progressive thickening and prominence of the connective tissue of the nerve was seen, manifesting itself as hyalinization of the endoneurium and a multilayer proliferation of the perineurium (Fig. 7). Either a part or the

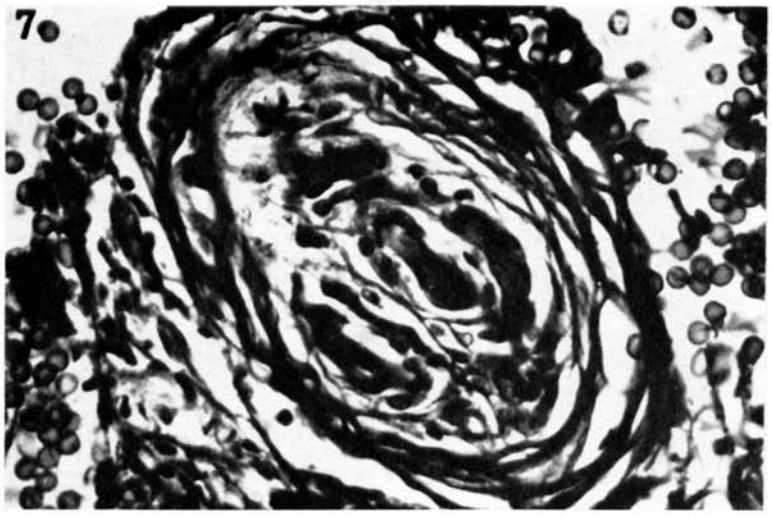

FIG. 7. A deeply situated nerve twig from a case of lepromatous leprosy. Note the prominence of the perineurium and thickening and hyalinization of endoneurial connective tissue (Mallory's aniline blue, × 480).

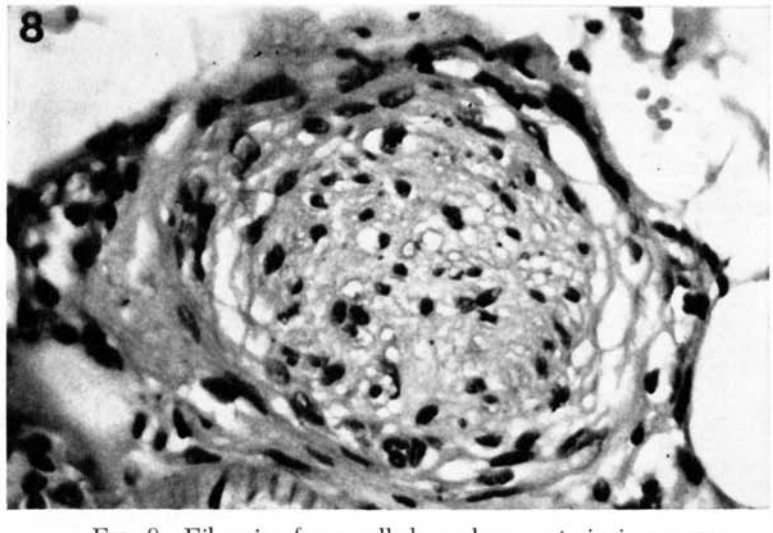

FIG. 8. Fibrosis of a small dermal nerve twig in a case of advanced lepromatous leprosy (H & E, × 480).

whole of the nerve twig may be thus involved, so that occasionally one may see a nerve, a portion of which is replaced by foamy histiocytes while the adjacent portion appears normal in conventional staining procedures. The end result of this process is a gradual replacement of neural parenchyma by the proliferated connective tissue (Fig. 8). Even at this stage small groups of fragmented acid-fast bacilli are recognizable within the nerve.

The changes in the deeper nerves in the various types of leprosy generally appear to follow the same sequence of events as those described in the dermal nerve twigs. The very early stages of this process cannot be verified histologically in biopsy material, because by the time the nerve is so thickened as to justify biopsy certain definite alterations have already occurred. As in the case of the dermal nerve twigs, the earlier stages of the changes in tuberculoid leprosy appear to consist of marginal or partial replacement of nerve parenchyma

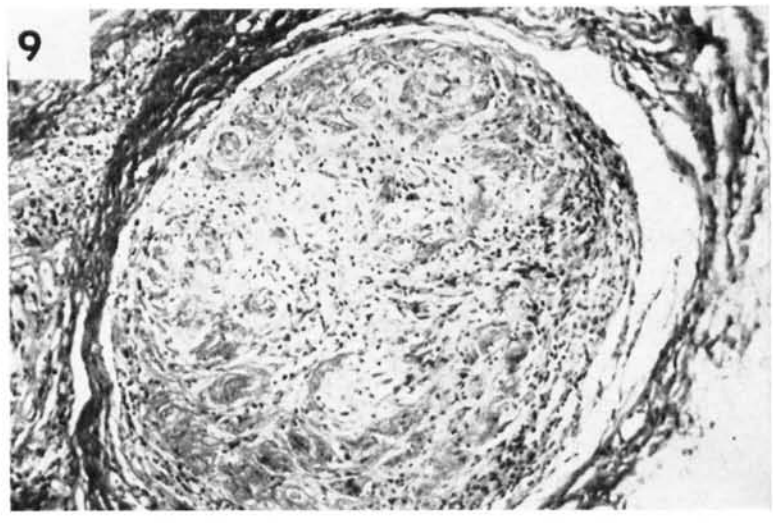

FIG. 9. Portion of a subcutaneous nerve from a case of tuberculoid leprosy showing extensive destruction of the center of the nerve, only the margins being preserved (Mallory's aniline blue, × 100).

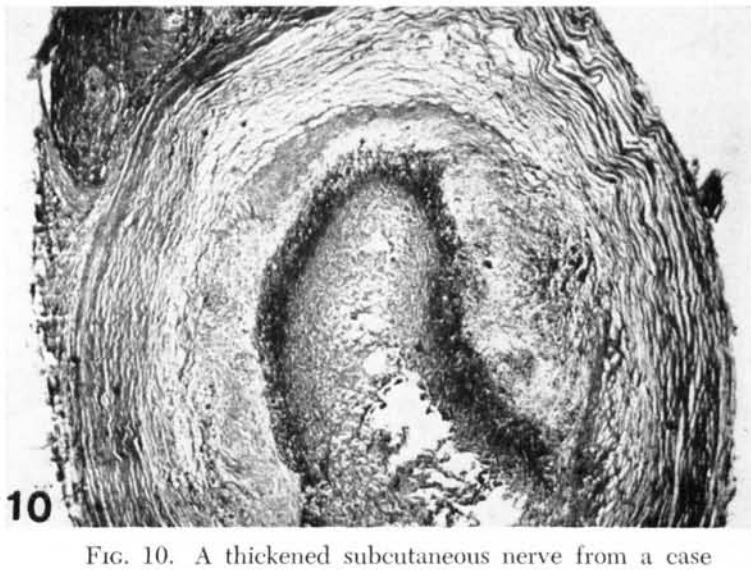

FIG. 10. A thickened subcutaneous nerve from a case of polyneuritic leprosy showing caseation of the center of the nerve and marked proliferation of the perineurium (H & E, × 24).

by small groups of epithelioid cells. Mallory-staining demonstrates endoneurial fragmentation within the extent of this replacement, while the rest of the nerve appears morphologically normal. With extension of this process a larger cross-sectional area of the nerve is replaced by granuloma (Fig. 9), but acid-fast bacilli are demonstrable as long as viable portions of the nerve are present. Eventually there is complete replacement of the nerve bundle by an epithelioid cell granuloma that often shows one or more multinucleated giant cells. There is a moderate thickening of the perineurium, and/or the epineurium, but the endoneurial connective tissue undergoes complete fragmentation, and in the late stages is unrecognizable. Such completely involved nerve bundles are often the seat of superadded processes,

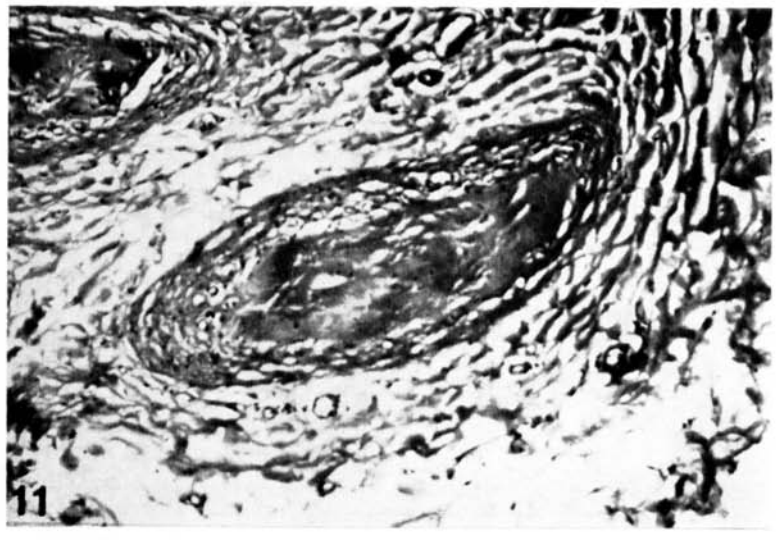

FIG. 11. Collagenous transformation of a deep nerve twig from a case of tuberculoid leprosy (H & E, × 100).

which result in caseous necrosis of the granuloma, leading to formation of the so-called tuberculoid nerve abscesses (Fig. 10). At a later stage the liquefied material appears to be removed, and the entire extent of the involved bundle is converted into a dense, almost acellular, collagenous scar (Fig. 11). Various stages and grades of this process can often be seen within the extent of a single involved nerve. Needless to say, acid-fast bacilli are difficult to demonstrate once the process has reached the stage of complete granulomatous replacement and of caseation and scarring.

Changes in nerve trunks in borderline leprosy, of which a few samples have been studied, are generally similar to those encountered in the skin lesions. Thus one may find, within the extent of a single nerve, areas completely replaced by tuberculoid granuloma, showing few or no bacilli, side by side with other areas characterized by either interstitial inflammation or moderate granulomatous replacement, but with bacilli visible more frequently, principally in the form of small groups. In borderline leprosy near the lepromatous end of the leprosy spectrum, changes resemble those of lepromatous leprosy.

The most striking feature of the involvement of peripheral nerves in lepromatous leprosy is a progressively increasing interstitial cellular infiltration by histiocytes showing various degrees of granularity and vacuolation of their cytoplasm, and containing an abundant number of bacilli. The constituent bundles of the nerve stand out prominently in the background of this cellular exudate. There is a progressive increase in the connective tissue of epineurium and perineurium, and thickening and hyalinization of the endoneurium. At the height of the process the bulk of the thickened nerve is made up of proliferated connective tissue and an extensive interstitial cellular infiltrate (Fig. 12). The neural parenchyma appears to suffer as a result of slow compression due to increased pressure within the nerve, so that, in the late stages of the process, both myelin and axon fail to be demonstrable by appropriate technic. Finally there is a gradual replacement of exudate cells and the nerve bundles by fibrous connective tissue. Even at this stage small groups of bacilli may be demonstrated occasionally within pockets in the connective tissue.

DISCUSSION

The foregoing descriptions have been given in an attempt to define the nature and possible sequence of changes in the dermal and deeper nerves in the principal types of leprosy. Perhaps the earliest stage of parasitization of nerves in the skin by *M. leprae* is that demonstrated by Khanolkar[8] in

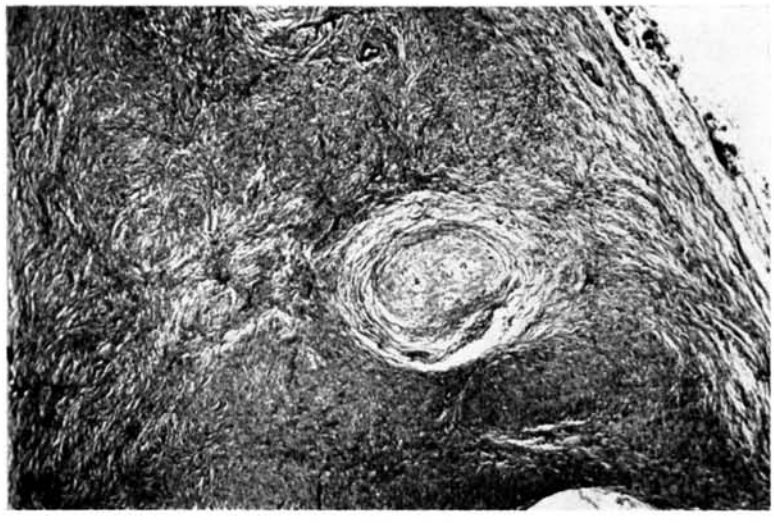

FIG. 12. Portion of a thickened cutaneous nerve from a case of advanced lepromatous leprosy. An intact nerve bundle is seen in the center of the picture surrounded by an extensive interstitial infiltrate and fibrous tissue (Mallory's aniline blue, × 24).

a study of contacts of leprosy patients. While the significance of these observations has not found universal acceptance (Dharmendra[3]), it is worthwhile to note that during a follow-up, definite lesions of leprosy occurred in some of these contacts[5,6].

In our material acid-fast bacilli that were unquestionably *M. leprae* have been found within intact small nerve twigs in macular lesions of leprosy. Although we have not had the opportunity to confirm by repetition the studies of Khanolkar and his colleagues, it is felt that some of the changes, including the presence of acid-fast bacilli within small dermal nerves, could logically be the sequence of the early parasitization demonstrated by Khanolkar.

It would be instructive to define, if possible, the relationship of nerve changes to changes in other areas of the skin in the same cases. From certain observations on the earlier stages of leprosy, including those on early tuberculoid leprosy, it is evident that the bulk of the cellular exudates in the skin are related intimately to the neurovascular bundle and particularly to the neural element in this structure. It would thus appear reasonable to surmise that progressive cellular infiltration and replacement of the smaller dermal nerves form the source of the focal and linear exudates that one sees in the dermis in early tuberculoid leprosy[10]. Nerves are constantly involved within such exudates, and often are not recognizable in areas where they are normally expected to be. Furthermore, the configuration of some of the superficial and many of the deeper exudates in quiescent tuberculoid leprosy strongly suggests the possibility of their origin from infiltration and expansion of nerve twigs, a view that finds support in the occasional finding of small fragments of preserved nerve elements within these exudates. Besides, study of consecutive sections of lesions of tuberculoid leprosy, along with a thickened nerve seen in continuity with the lesion, also supports this contention. The following case illustrates this point. J. (L-38), a 35 year old male, had an infiltrated anesthetic lesion over his left forearm, and a thickened cutaneous nerve was observed in continuity with this lesion. The lepromin test was 3+. A clinical diagnosis of tuberculoid leprosy was made and biopsy of a portion of the skin lesion, along with the thickened cutaneous nerves in continuity, was done. Histologic examination revealed that the bulk of the exudate in the dermis consisted of numerous rounded and fairly well circumscribed

granulomatous zones. The configuration of these granulomata in the dermis strongly suggested their origin from replacement and expansion of dermal nerve twigs, and this view was substantiated by observing a continuity of these granulomata with similar granulomata that had replaced the thickened cutaneous nerve taken for biopsy along with the lesion.

Even when a granuloma extends beyond the confines of the nerve, infiltrating other structures, such as sweat glands, hair follicles, and the arrectores pilorum muscles, and, rarely, the walls of blood vessels, it is not possible to be certain that such extension is not along the course of neural elements innervating these structures. Preparations of skin subjected to silver impregnation reveal a remarkable paucity of neural elements related to these structures in tuberculoid leprosy.

There are also instances where pathologic changes occur in the deeper cutaneous or peripheral nerve trunks, without any manifest lesions on the skin surface. These constitute the purely neuritic or polyneuritic variety of the disease. The only evidence of involvement of skin in these cases is anesthesia or hypesthesia in the skin whose sensations are subserved by the nerve or nerves in question. The following case is an illustrative example. A 22 year old male student (L-508-R) was examined at the Central Leprosy Teaching and Research Institute for left-sided foot-drop apparently of six months' duration. Physical examination revealed complete absence of skin lesions, a patch of anesthesia over the dorsum of the left foot, and palpable nontender thickening of the left lateral popliteal and left musculocutaneous nerves. Routine skin smears, including smears from the anesthetic area, revealed no acid-fast bacilli. A portion of the left musculocutaneous nerve, taken for biopsy, revealed the classic changes of tuberculoid leprosy. Careful search of numerous sections failed to reveal any acid-fast bacilli. The histopathologic features, however, left no doubt that the nerve involvement was the result of tuberculoid leprosy.

The constant relationship of the presence of bacilli to the development of lesions in nerves, raises question as to the manner in which these nerve changes are brought about, and the reasons for the different types of response in the different clinical and immunologic types of leprosy. The characteristic histopathologic reaction in tuberculoid leprosy, where there is a high degree of allergy, as revealed by the lepromin test, is the formation of an epithelioid cell granuloma that sometimes proceeds to caseation necrosis. The histopathologic features are those of a delayed hypersensitivity reaction([12]); according to Waksman([14]) they are brought about by actual invasion and destruction of antigen-containing parenchyma by histiocytes that constitute the main cellular element in this reaction. It is reasonable to expect that this reaction would take place at sites where antigen *(M. leprae)* is readily available, and the destructive nature of this reaction is exemplified by the extensive damage to all the parenchymal elements within the area of the reaction. The advent of this reaction is thus among other factors determined by the avaílability of the antigen, and, once it has set in, it progresses inexorably to its termination. Doubtless superadded factors, like anoxia([1]), local enzymatic digestion, and the processes of repair, play a part in the final resolution and fibrosis.

That an alternate hypothesis for the production of such a lesion is possible, is evident([9]) from a consideration of the pathogenesis of experimental allergic neuritis([15]), in which selective peripheral nerve damage has been demonstrated in certain species of mammals. According to Lumsden([9]) nerves damaged in leprosy may liberate autoantigens that can later sensitize and damage other nerve trunks without the actual presence of leprosy bacilli in these later affected nerves. It is pertinent in this respect to recall the observations of Weddell and Glees([16]), who found a continuous process of degeneration and regeneration among fibers of cutaneous nerve plexuses, even in the healthy skin of man and animals. In translating this observation to the hypothesis suggested by Lumsden([9]) it might well be that antigens liberated by such continuously degenerating nerve fibers could, in susceptible indi-

viduals, act to produce nerve damage, especially at sites where an adjuvant, in this case *M. leprae,* is present. Experimental demonstration of the actual operation of such factors in the genesis of nerve lesions of tuberculoid leprosy is still wanting, but the hypothesis is certainly worth consideration.

The chronic and gradually progressive pattern of nerve changes in lepromatous leprosy is reflected in the type of pathologic change in the nerves, detailed earlier. From a consideration of these findings, it would appear that the increasing interstitial cellular infiltration, which serves in the beginning to separate the nerve bundles, leads at a later stage to compression of the nerve parenchyma. The proliferated epineurium, perineurium, and endoneurium are doubtless able to resist more than a certain minimum increase of the volume of the nerve, beyond which the effect of compression would be expected to set in. At any stage during this process an episode of reaction, with its attendant cellular, vascular, and edematous components, is likely to produce an acute upset of the threshold of tolerance, and precipitate neural symptomatology. It may also be that excessive operation of some of these factors would so upset vascular and metabolic requirements that liquefaction, with the production of the rare lepromatous nerve abscess, results. The end stage is similar to that in tuberculoid leprosy, inasmuch as the nerve is completely replaced by connective tissue.

One feature of the lesion in nerves in this form of leprosy requires special mention. With the aid of preparations stained by the Mallory technic, an excessive overgrowth of connective tissue has been observed, not only within the nerves, but also within the cellular exudates in the skin lesions of the disease. This feature is in sharp contrast to what obtains in tuberculoid leprosy, where reticulum is seen to be considerably fragmented and almost absent within the extent of the lesions and between the individual cells. Apparently at that point the histiocyte that develops into the lepra cell has retained the ability to lay down a considerable amount of reticulum, whereas in its progression toward the epithelioid cell of tuberculoid leprosy this property is not so manifest. During the resolution of skin lesions of lepromatous leprosy a condensation of this intercellular reticulum can be demonstrated. It seems reasonable, therefore, to state that some of this proliferated mesenchyme is derived from that laid down by the interstitially infiltrating cells. It is also possible that connective tissue may be laid down by Schwann cells([7]), and this may explain the prominence of the so-called endoneurial connective tissue in lepromatous leprosy.

One of the tragic consequences of the involvement of nerves in leprosy is the development of deformities and other trophic alterations. In an epidemiologic study([13]) it was seen that deformities were generally more frequent in lepromatous than in nonlepromatous leprosy. The reason for this may lie in the fact that lepromatous leprosy involves a larger extent of the body than nonlepromatous leprosy, with, therefore, greater chances of nerve involvement. It was also seen that, in any large series of such patients, the average mean duration of the development of the deformity is greater in lepromatous than in nonlepromatous leprosy. The differences in the tempo of the process in these two types of the disease may explain these findings. Besides the slowly progressive type of deformity, there is another variety, which is seen typically during an episode of acute reaction. Both the abrupt onset and the reversible nature of the paralysis suggest edema and/or superadded ischemia as the most likely explanation for this process. The fact that recovery in these cases is hastened by measures directed to alleviation of edema and/or ischemia, would support this contention. Finally, a type of deformity is seen in patients with repeated severe lepra reaction, associated with recurrent edema of the terminal segments of the extremities. A localized scleroderma-like state develops, in which the atrophic skin is bound down firmly to underlying excessively collagenized subcutaneous tissue. This type of deformity thus appears to result from local mechanical factors, and movements are limited even in the absence of any motor paralysis.

From the foregoing it will be evident that a variety of changes occur in the

nerves at all levels in all forms and types of leprosy. When these changes reach critical proportions they lead to the development of trophic alterations and disabling deformities, which are the most serious consequences of this disease. However, there are earlier stages of such nerve involvement, manifested simply by loss of sensation in areas of the body skin, or which may be insufficient to produce any such clinical changes. One of the cardinal features of the cutaneous lesion of leprosy is an alteration of sensibility, and, in the absence of demonstrable acid-fast bacilli, this clinical feature, substantiated if possible, by histologic observations, serves to clinch the diagnosis. The presence of acid-fast bacilli within skin lesions is another feature supporting the diagnosis of leprosy, and in the current state of our knowledge the occurrence of such organisms within dermal and/or deeper nerves appears to be the most certain criterion, identifying them, in the human being, as *M. leprae*. Finally, it may not be inappropriate to refer once again to this unique feature of leprosy in which circumscript and often disseminated lesions of varying severity are produced within the dermal and cutaneous nerves.

The presence of these lesions in a host with such exquisite subjective responses as man, makes this situation suitable for investigations on cutaneous neurohistology and neurophysiology([2,17]). The study of medicine is ultimately an aspect of the study of human biology, and such investigations on abnormal states could serve to bridge the gaps that are inevitable during study of the normal.

SUMMARY

This paper deals with the sequence of histopathologic changes in nerves in leprosy. Nerves within skin lesions, as well as within cutaneous and deeper nerve trunks obtained from a variety of cases of leprosy, have been examined. Differences in the type of nerve change in the two polar forms of leprosy have been detailed. The pathogenesis of these changes has been discussed, and attention is drawn to some of the consequences of these nerve changes. Certain aspects of the significance of nerve involvement in leprosy have been discussed.

Acknowledgment. The author is grateful to Dr. Dharmendra, Director, Central Leprosy Teaching and Research Institute, for his interest in these studies, Dr. K. Ramanujam for making available suitable biopsy material, Dr. H. Srinivasan for helpful discussions on deformities in leprosy, Mr. P. B. Nath and D. Yesudos for the histologic preparations, and Mr. C. Samuel for the photomicrographs.

REFERENCES

1. Chatterjee, S. N. Mechanism of the neural signs and symptoms of leprosy. Internat. J. Leprosy **23** (1955) 1-18.
2. Dastur, D. K. Cutaneous nerves in leprosy. Brain **78** (1955) 615-633.
3. Dharmendra. The affection of the peripheral nerves in leprosy. Leprosy in India **24** (1952) 35-45. (*Editorial*)
4. Dharmendra. The maculo-anesthetic form of leprosy. Internat. J. Leprosy **31** 1963) 161-177.
5. Figueredo, N. and Desai, S. D. Positive bacillary findings in the skin of contacts of leprosy patients. Internat. J. Leprosy **18** (1950) 59-66.
6. Figueredo, N. and Desai, S. D. Lepromin test in contacts with particular reference to positive bacteriological findings. Internat. J. Leprosy **19** (1951) 165-174.
7. Harkin, J. C. Localisation of the cellular site of collagen synthesis in peripheral nerves by electron microscopic auto-radiography using 3H-proline. Proc. Vth Internat. Congr. Neuropath. 1965 (*in press*).
8. Khanolkar, V. R. Studies in the histology of early lesions in leprosy. Leprosy in India **24** (1952) 62-77.
9. Lumsden, C. E. *Discussion:* Experimental observations related to the histopathology of leprosy by Weddell, Palmer, Rees and Jameson, Ciba Foundation Study Group No. 15. Monograph on the Pathogenesis of Leprosy, 1963, pp. 15-30.
10. Muir, E. and Chatterjee, S. N. Leprous nerve lesions of cutis and subcutis. Internat. J. Leprosy **1** (1933) 129-148.
11. Muir, E. and Chatterjee, S. N. A study of nerve leprosy. Indian J. Med. Res. **24** (1936) 119-138.

12. Skinsnes, Olaf K. Immunological spectrum of leprosy. *In* Leprosy in Theory and Practice. Eds. Cochrane, R. G. & Davey, T. F. Bristol, John Wright & Sons Ltd.; Baltimore, Williams and Wilkins Co., 1964, 2nd ed. pp. 156-282.
13. Srinivasan, H. Personal communication.
14. Waksman, B. H. A comparative histopathological study of delayed hypersensitive reactions. *In* Ciba Foundation Symposium, Cellular Aspects of Immunity. Boston, Little, Brown, and Co. (1959) 280.
15. Waksman, B. H. and Adams, R. D. Allergic neuritis and experimental disease of rabbits induced by the injection of peripheral nervous tissue and admants. J. Exper. Med. **102** (1955) 213-236.
16. Weddell, A. G. M. and Glees, P. Early stages in degeneration of cutaneous nerve fibres. J. Anat. **76** (1951) 65-93.
17. Weddell, A. G. M., Jameson, D. G. and Palmer, E. Recent investigations into sensory and neurohistological changes in leprosy. *In* Leprosy in Theory and Practice. Eds. Cochrane, R. G. & Davey, T. F. Bristol, John Wright & Sons Ltd.; Baltimore, Williams & Wilkins Co., 2nd ed. 1964, pp. 205-220.