

# Study of Apparently Normal Skin in Leprosy<sup>1,2</sup>

R. Ganapati, K. V. Desikan and C. G. S. Iyer<sup>3</sup>

The importance of the study of apparently uninvolved or unaffected tissues in the understanding of the pathogenesis and the evolution of a disease process needs no emphasis. Such studies assume added significance in leprosy, in which one of the main organs affected, namely skin, is readily available for investigation.

Clinically uninvolved nerves have been subjected to investigations by Weddell and co-workers (<sup>10</sup>), who reported changes, with the presence of acid-fast bacilli, in the apparently normal nerve of a patient with established leprosy lesions elsewhere. Dastur, *et al* (<sup>2</sup>), also observed changes by electron microscopy in clinically unaffected nerves of five patients of tuberculoid and borderline-tuberculoid types of leprosy who showed moderate thickening of some other larger nerves.

There is a paucity of histologic studies on unaffected skin in established cases of leprosy, though investigations of this nature have been carried out on the normal skin of asymptomatic contacts of leprosy patients by Khanolkar (<sup>6</sup>) and others with a view to understanding the evolution of early lesions. Histopathologic findings in biopsies of normal skin in a few cases of leprosy lead Bechelli, *et al* (<sup>1</sup>), to think, "that in the apparently healthy integument a certain condition exists which may be called potential, corresponding more or less to the type of the lesions present in other parts of the body."

A few references on the examination of

smears from normal skin are available. Periaswamy (<sup>7</sup>) observed that in borderline leprosy the bacilli were not only present in the lesions but also spread a little further into the periphery, though not invading the apparently normal skin. He advocated the use of smears from normal skin to differentiate between tuberculoid in reaction, borderline and infiltrative leprosy, especially when the lesions are extensive. Ramanujam and Ramu (<sup>8</sup>) found that the smears from apparently normal skin surrounding the borderline lesion within a distance of 0.5 cm to 2 cm was positive for bacilli in 5 of 28 cases (17.9%) studied. Fernandez (<sup>4</sup>) observed that the apparently normal skin near reacting lesions was always positive for acid-fast bacilli, the bacilli being granular, while distant areas were usually negative for bacilli.

Since leprosy lesions involve extensive areas of body surface, it was thought that a study of portions of skin from the neighborhood of established lesions and from sites far removed from the lesions would throw some light on the pathogenesis of the disease, because it may be postulated that apparently normal areas of skin are the probable sites of future extension of the pathologic process, and that the events taking place in these sites might represent the earliest stages of such an extension.

## MATERIALS AND METHODS

Patients admitted to the Central Leprosy Teaching and Research Institute Sanatorium, Chingleput, and those attending the outpatient clinic of the institute were the subjects of the investigation. One hundred patients, 94 males and 6 females ranging from 9 to 57 years of age were available. The selection was made after a thorough clinical examination. The disease typing was on the basis of the clinical and histopathologic features of the lesions. The histologic classification recommended by Ridley and Jopling (<sup>9</sup>) was generally adopted

<sup>1</sup> Received for publication 3 January 1972.

<sup>2</sup> This paper was read at the Silver Jubilee Annual Conference of the Indian Association of Dermatologists and Venereologists, Madras, India, January 1972.

<sup>3</sup> R. Ganapati, B.Sc., M.B.B.S., D.D.V., Research Officer, Acworth Leprosy Hospital, Wadala, Bombay-31, India; K. V. Desikan, M.D., Assistant Director of Pathology, Central Leprosy Teaching and Research Institute; C. G. S. Iyer, M.D., M.R.C. (Path), F.C.P.S., Director, Central Leprosy Teaching and Research Institute, Chingleput, Tamil Nadu, India.

for this purpose. In addition, a few intermediate groups like BB-BT, BL-BB, etc., were introduced.

For the purpose of this study, clinically normal skin was defined as skin that revealed normal color, texture and sensibility on physical examination. Patients were examined in direct as well as oblique light to ensure that the site chosen showed normal skin texture. Care was taken to avoid areas of skin where there was a suggestion of regressed lesions. Tests to elicit sensory changes were employed to ensure that the site of biopsy did not reveal any impairment of sensation.

In cases clinically diagnosed as BT, BT in reaction (BT-R), TT and MA (maculo-anesthetic [classification adopted by the Indian Association of Leprologists]), where the lesions were well demarcated, the normal skin was obtained fairly close to the edge of the lesion within a distance of 0.5 cm to 2 cm in most instances. In the types such as BB, BL or I, where the margin was less defined, that part of the skin adjoining the lesion which was thought to be clinically normal was chosen. In lepromatous leprosy, either the sites in the vicinity of plaques or macules or in cases of diffuse infiltration, sites far removed from the infiltrated skin, such as axilla, inguinal region or parts of skin near the midline of the back, etc., were selected.

Biopsies of the normal skin and the lesion were done and the tissues fixed in Zenker's solution before processing in the usual way. Sections of five micron thickness were examined after staining by hematoxylin and eosin stains, Mallory's aniline blue stain and Fite's modification of the Ziehl-Neelsen stain.

The sections of normal skin were examined with special reference to 1) alteration, if any, in the normal histologic features of the skin; 2) nature and distribution of inflammatory exudate; 3) pathologic changes, if any, in the cutaneous nerves; and 4) the distribution of acid-fast bacilli, when present, in the various components of the dermis.

The quantity of exudate in the dermis was graded as follows: "negligible" where stray inflammatory cells alone were to be found, such a finding being quite consistent

with the findings in normal skin of subjects in tropical countries; "minimum" where the cells were few, but found in small groups insufficient to constitute a lesion and by virtue of their location in certain special sites in the dermis, suggestive but not diagnostic of leprosy; and "moderate" where larger collections of inflammatory cells were found, usually as small foci consistent with the pattern obtained in early lesions of leprosy.

Though these gradings were done on an arbitrary basis, two observers examined all the biopsy specimens independently, and the final grading was done after a joint examination of the specimens by the observers.

## RESULTS

The quantity of exudate cells, their characteristics and distribution are shown in Table 1. All the 100 cases studied showed varying degrees of inflammatory cells in the dermis. Of 46 cases in the LL and BL groups, 12 showed moderate infiltration, whereas in the remaining groups only 4 cases of 54 showed this feature.

Lymphocytes were found in all cases. Histiocytes in addition to lymphocytes were found in 23 of 46 LL and BL cases, and 12 of 54 in the remaining groups. The superficial dermis was infiltrated in 98 cases and neurovascular bundles and cutaneous appendages were the sites of infiltration in 95 and 32 instances respectively.

Table 2 shows the degree of nerve infiltration. Infiltration of nerves was found in 61 cases. Sixteen cases showed moderate to heavy infiltration, of which 12 instances belonged to the LL & BL groups.

The morphological appearance of the nerves is tabulated in Table 3. Schwann cell hyperplasia was noticeable in 79 biopsies.

Table 4 presents a comparative analysis of the distribution of acid-fast bacilli in the apparently normal skin as well as the lesion.

Forty-eight biopsy specimens of normal skin and 66 lesion biopsies were positive for acid-fast bacilli. The ratio of positivity of normal skin to lesion positivity was 36 of 46 in the LL and BL groups and 12 of 20 in

TABLE 1. *The exudate cells in the dermis and their distribution.*

Bacilli location		LL & BL	BL-BB	BB-BT	BT	BT (R)	TT	MA	I & I-L	Total
No. of biopsies		46	6	7	22	4	9	2	4	100
Q U A N T I T Y	negligible	13	2	2	11	1	3	—	1	33
	minimum	21	4	4	11	1	5	2	3	51
	moderate	12	—	1	—	2	1	—	—	16
N A T U R E	lymphocytes	46	6	7	22	4	9	2	4	100
	lymphocytes & histiocytes	23	2	3	3	2	2	—	—	35
D I S T R I B U T I O N	superficial dermis	46	6	7	20	4	9	2	4	98
	neurovascular bundles	45	6	7	19	4	9	2	3	95
	appendages	15	2	3	5	2	4	1	—	32

TABLE 2. *Infiltration of the nerves.*

	LL & BL	BL-BB	BB-BT	BT	BT(R)	TT	MA	I & I-L	Total
Bacilli location									
No. of biopsies	46	6	7	22	4	9	2	4	100
No infiltration	11	2	3	12	1	4	1	3	37
Mild	22	4	2	10	1	4	1	1	45
Moderate	12	—	—	—	—	—	—	—	12
Heavy	—	—	1	—	2	1	—	—	4
No nerves seen in sec.	1	—	1	—	—	—	—	—	2

TABLE 3. *Morphology of the nerves.*

	LL & BL	BL-BB	BB-BT	BT	BT(R)	TT	MA	I & I-L	Total
Bacilli location									
No. of biopsies	46	6	7	22	4	9	2	2	100
Schwann cells hyperplasia	39	5	5	19	3	4	1	3	79
Normal	6	1	1	3	1	5	1	1	19
No nerves seen in sec.	1	—	1	—	—	—	—	—	2

TABLE 4. *Distribution of acid-fast bacilli in the normal skin and the lesion.*

Bacilli location	LL & BL		BL-BB		BB-BT		BT		BT(R)		TT		MA		I and I-L		Total	
	normal skin	lesion	normal skin	lesion	normal skin	lesion	normal skin	lesion	normal skin	lesion	normal skin	lesion	normal skin	lesion	normal skin	lesion	normal skin	lesion
No. of biopsies	46	46	6	6	7	7	22	22	4	4	9	9	2	2	4	4	100	100
No. of positive biopsies	36	46	4	6	3	7	—	17	2	4	—	—	—	2	3	3	48	85
Nerves	31	42	3	6	3	5	—	15	2	4	—	—	—	2	3	3	42	77
Macrophages	26	46	1	6	1	4	—	1	—	3	—	—	—	—	—	3	28	63
Blood vessels	18	32	1	2	—	—	—	—	—	—	—	—	—	—	—	—	19	34
Arrectores pilorum muscles	15	38	1	4	—	3	—	4	—	1	—	—	—	—	—	1	16	51
Hair follicle cells	2	9	—	—	—	—	—	—	—	—	—	—	—	—	—	—	2	9

the BL-BB, BB, BB-BT, BT(R), I and I-L types. In 24 cases diagnosed as BT and MA the normal skin was negative for acid-fast bacilli, though in 19 such instances the bacilli were seen in biopsies of the lesions. In none of the nine cases, typed as TT were acid-fast bacilli seen either in the lesion or in the normal skin.

It can also be seen from Table 4 that nerves and macrophages were the commonest sites for the bacilli to be located. In 18 of 36 positive biopsies obtained from normal skin of LL and BL types, acid-fast bacilli were found in the walls of the blood vessels. In lesion biopsies of these types blood vessels showed bacilli in 32 of 46 instances. In 19 cases of 34 in the LL, BL, BL-BB and BB types, the lesion as well as the normal skin showed bacilli in the blood vessels.

Table 5 shows the relation of the extent of infiltration to the occurrence of bacilli. Of 48 biopsies which were positive for acid-fast bacilli, 33 showed negligible or minimal infiltration and 15 showed moderate infiltration.

## DISCUSSION

**Infiltrative changes.** All the cases studied showed some extent of inflammatory exudate distributed in the dermis (Fig. 1), the number of cases showing infiltration graded as "moderate" being more in the LL and BL groups as compared to others. This shows that even in parts of skin considered to be normal on clinical grounds, appreciable infiltration may be found. Similar observations were made by Gougerot and Degos (5) in their study of the histo-bacteriology of "invisible lepromas" which were lesions without visible external change and were therefore unsuspected, but which after intravenous injections of methylene blue showed up as round or oval blue spots two to four centimeters in diameter, or as larger irregular areas. These authors observed that the exudate was composed chiefly of Virchow's foamy cells with numerous bacilli, though they were unable to explain why such lesions should remain so underdeveloped as not to be visible.

This is in contrast to the BT and TT types in which the overall infiltration was

TABLE 5. Acid-fast bacilli in relation to the extent of infiltration.

Bacilli location	LL & BL				I & I-L	Total
	BL-BB	BB-BT	BT(R)			
No. of positive biopsies	36	4	3	2	3	48
I negligible	7	1	—	—	—	8
N						
F						
I						
L						
T minimum	17	3	2	—	3	25
R						
A						
T						
I						
O						
N moderate	12	—	1	2	—	15

generally found to be minimal or negligible, though biopsies were performed close to the edge of the lesion.

Lymphocytes, to a varying extent were found in all the cases that were studied. The more specific infiltration with histiocytes was found in 50% of the LL and BL

cases, whereas the appearance of histiocytes in the normal skin was an inconstant feature in the other types.

Practically all the cases showed infiltration in the superficial dermis and around small blood vessels and notably in relation to the neurovascular bundles. (Fig. 2).

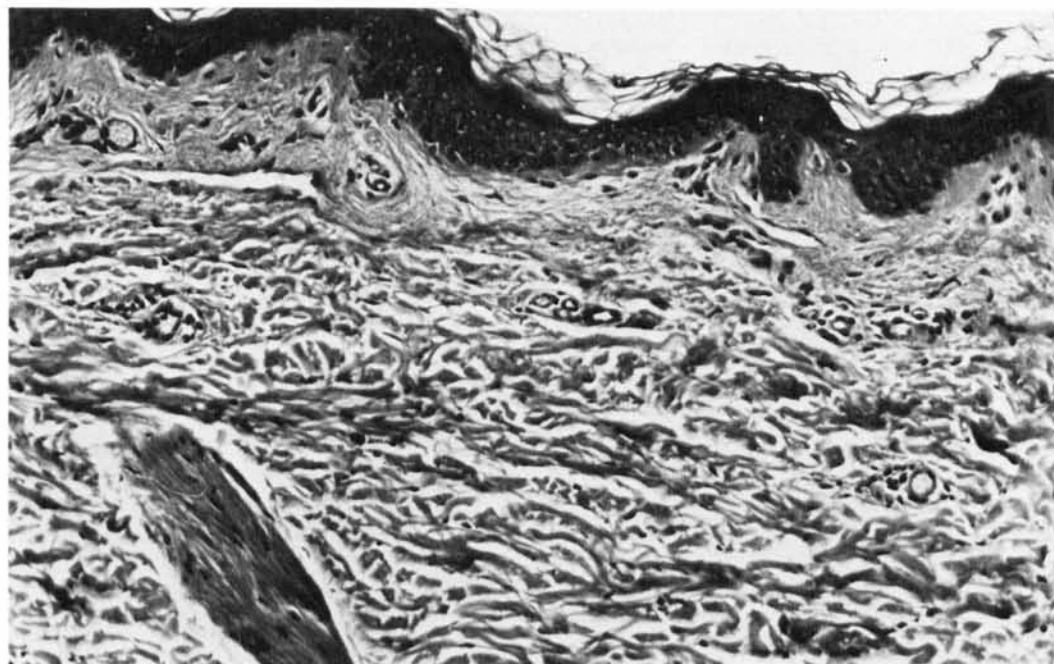


FIG. 1. Biopsy from the same patient shown in Figure 2 from apparently normal skin 1.5 cm away from the lesion shows negligible infiltration. H & E x 215.



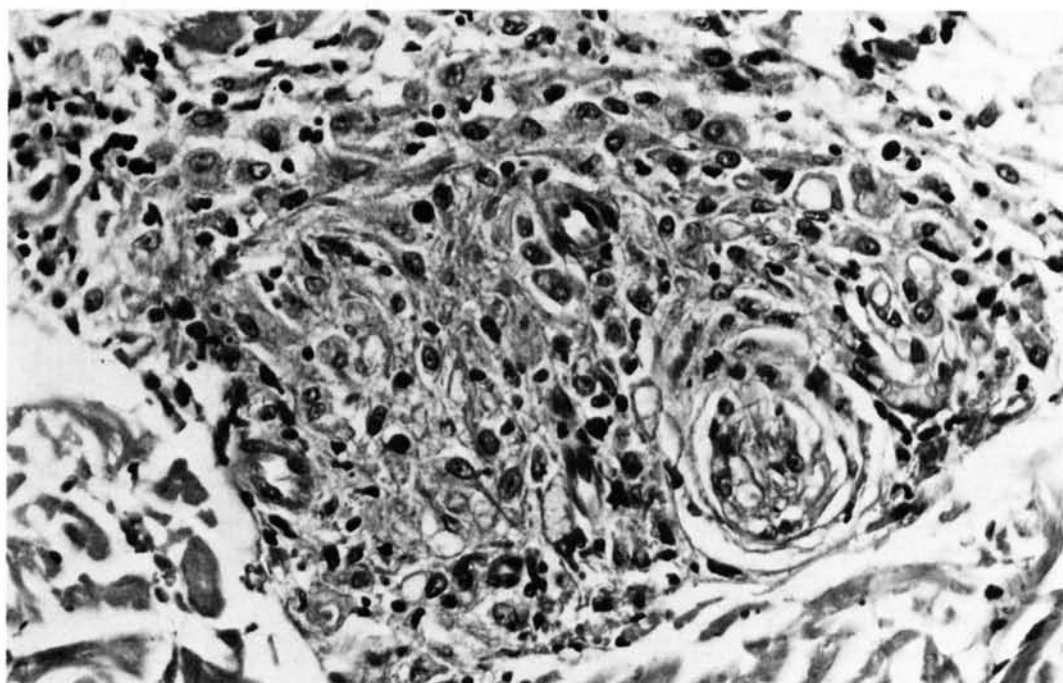


FIG. 2. Biopsy from a lepromatous lesion showing the inflammatory exudate in relation to a nerve. H & E x 430.

This feature is significant in view of the role played by the neurovascular channels in the pathogenesis of leprosy. Being found in patients known to be suffering from leprosy, this finding assumes added significance. However, it must be said that infiltration of neurovascular apparatus alone may not be indicative of leprosy unless accompanied by the presence of acid-fast bacilli in the nerves or elsewhere.

Infiltration of the cutaneous appendages was a feature noticeable to a variable extent in many of the cases. In this study, the paucity of exudate cells in most of the biopsies permitted a close observation of the exact relation of the infiltrating cells to the appendages. In most instances where the cells were found in the vicinity of the latter, the infiltration actually involved nerves which were in close proximity to the appendages, rather than the appendages themselves. This observation again points to the importance of the neurovascular components of the pilo-sebaceous units and sweat glands as the foci of early infiltration. The eventual fragmentation of these struc-

tures with a rich collection of inflammatory cells surrounding them, so constantly seen in established lesions of leprosy, is in fact the terminal stage of a process which is initiated by the gradual infiltration of the fine neurovascular elements in close proximity to the appendages.

**Neural changes.** Infiltration of nerves by lymphocytes in the normal skin was observed in the majority of the cases, and this feature was particularly noticeable in lepromatous cases. In rare instances, appreciable infiltration of nerve parenchyma classifiable as "heavy" was noticed. This shows that clinically normal looking skin is not incompatible with heavy infiltration of the underlying nerves, provided that such skin is in the neighborhood of a lesion.

The vast majority of the nerves showed Schwann cell hyperplasia. The nerves often appeared very prominent in the sections with intact, clearly delineated fibers and prominent Schwann cell nuclei whether or not they were involved in infiltration with exudate cells (Fig. 1). Only 61 cases showed infiltration with lymphocytes to

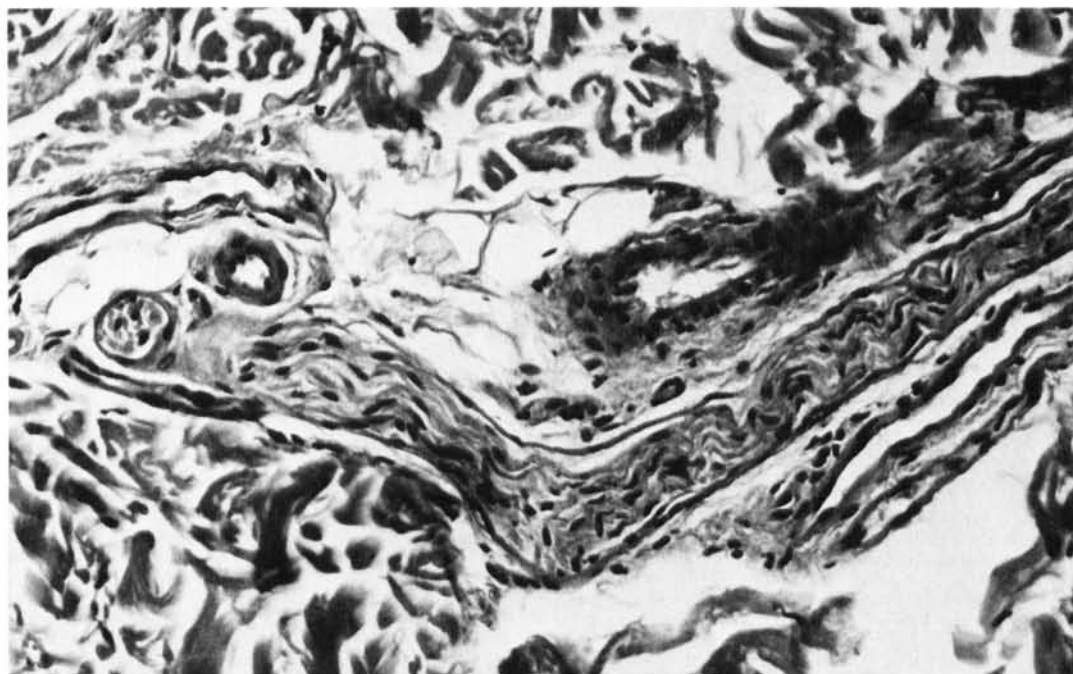


FIG. 3. Schwann cell hyperplasia. H & E x 340.

some extent, whereas the phenomenon of Schwann cell hyperplasia was noticed in 79 instances. This was particularly striking in BT types. Schwann cell hyperplasia would appear to be an early event in the extension of the disease process to uninvolved parts of skin in leprosy patients.

**Bacillary distribution.** It was striking that 36 of 46 normal skin biopsies in the LL and BL types were positive for acid-fast bacilli, nerves and macrophages being the commonest sites of location of bacilli. Types classified as BL-BB, BB, BB-BT, BT(R), I and I-L, also showed bacilli. However, in BT and MA, the normal skin failed to show any bacilli, though in most of such cases the adjoining lesions were positive. In none of the nine cases typed as TT could acid-fast bacilli be detected either in the skin or in the lesion.

A notable finding in this investigation is in relation to bacillema in leprosy. Of 36 positive biopsies obtained from "normal" skin of LL and BL types, acid-fast bacilli were found in the blood vessels in 18 instances. They were seen in the endothelial lining of blood vessel walls (Fig. 4) and rarely in the lumen of the vessels. In lesion

biopsies of 46 cases belonging to these types, evidence of bacillema was present in 32 instances. Cases typed as BL-BB and BB also showed similar findings though rarely. Of 34 cases of all types in which lesion biopsies revealed acid-fast bacilli in blood vessels, 19 cases showed this feature also in the apparently normal skin, providing support for bacillema phases in leprosy.

Another interesting observation in this study is the finding of acid-fast bacilli in a significant number of biopsies classified as showing "negligible" to "mild" infiltration. If Fite's stain had not been employed, the biopsies showing negligible infiltration might have been passed off as showing no abnormality.

## DISCUSSION

On the assumption that the histopathologic changes observed in the normal skin have a bearing on those seen in the established lesions in the same patient; the findings presented here may be useful in elucidating mechanisms of the pathogenesis of leprosy. A close observation of the morphologic states of the dermal nerve

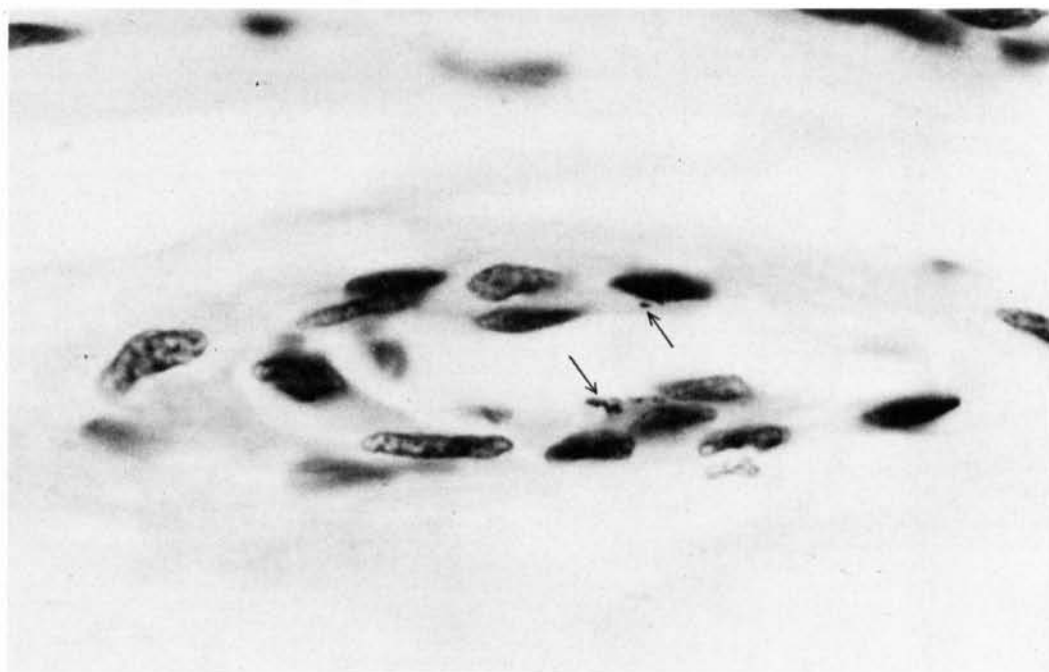


FIG. 4. Biopsy of apparently normal skin from a case typed as BL-BB, showing acid-fast bacilli in the endothelial lining of a blood vessel. This case showed negligible infiltration. F.F. x 1790.

twigs, their early infiltration and the distribution of bacilli in the dermis would appear to suggest that in all the types of leprosy, an early event in the extension of the disease process to the uninvolved parts of the skin is the Schwann cell hyperplasia as indicated by an extraordinary prominence of neural elements accompanied by nonspecific round cell collections in the superficial parts of the dermis.

In lepromatous leprosy, the appearance of bacilli in nerves, in some instances out of proportion to the quantity of exudate cells and histiocytes, is the next striking feature, with or without the presence of the bacilli in the endothelial cells lining the walls of blood vessels. The presence of bacilli in the blood vessels in biopsies of the lesion as well as normal skin in the same patient lends support to the concept of bacillemic phases in leprosy. According to Desikan and Job (3), during phases of bacteremia, bacilli may be phagocytosed by the endothelial cells of capillaries and small blood vessels. They multiply in these cells which may break down discharging bacilli into the blood stream.

In tuberculoid and borderline tuberculoid types of lesions the events would appear to be as follows. The location of bacilli in sites such as the nerve would initiate the development of cell-mediated immunity with its local expression of granulomatous reaction, even to small amounts of antigen. These changes are seen very well in the nerves in tuberculoid leprosy, where both the quantity of the cellular infiltration and also the character of the cells supports this contention.

The changes observed in this small series of cases warrant more enquiries of this nature in a larger number and variety of cases. It would also be important to study the histological appearance of cutaneous nerves in normal subjects and in various dermatological disorders, to assess the true significance of the nature of changes observed in the present investigation.

### SUMMARY

Since leprosy lesions involve extensive areas of the body surface, a study of portions of the apparently normal skin situated adjacent to and remote from the lesions



was made, with a view to understanding the mechanisms of extension of the disease process.

One hundred leprosy patients of all types were investigated. The results show that even in parts of skin considered to be normal on clinical grounds, significant infiltration with inflammatory cells such as lymphocytes and histiocytes may be found, especially in the lepromatous types and those near the lepromatous end of the spectrum.

A study of the distribution of acid-fast bacilli in the lesions as well as the apparently normal skin lends support to the occurrence of bacillemic phases in certain types of leprosy. A reference is made to the presence of bacilli in biopsy specimens showing negligible to mild infiltration of the dermis with inflammatory cells.

The significance of the morphologic and infiltrative changes seen in the nerves and the bacillary distribution in various types of leprosy are discussed.

The importance of such studies in understanding the pathogenesis of leprosy is stressed.

## RESUMEN

Debido a que las lesiones de lepra comprometen áreas extensas de la superficie corporal, se efectuó un estudio de zonas de piel aparentemente normal ubicadas en sitios adyacentes y alejados de las lesiones de lepra, con el fin de comprender los mecanismos que rigen el proceso por el cual se extiende la enfermedad.

Se investigaron cien pacientes de lepra de todos los tipos. Los resultados muestran que, aún en zonas de piel consideradas como normales desde el punto de vista clínico, puede encontrarse una infiltración significativa, con células inflamatorias tales como linfocitos e histiocitos, especialmente en los tipos lepromatosos y en aquellos pacientes cercanos al lado lepromatoso del espectro.

Un estudio de la distribución de bacilos ácido-resistentes en las lesiones, así como en la piel aparentemente normal, presta apoyo a la idea de la ocurrencia de fases bacilémicas en ciertos tipos de lepra. Se hace referencia a la presencia de bacilos en especímenes de biopsia que muestran una infiltración de discreta a moderada del dermis con células inflamatorias.

Se discuten el significado de los cambios morfológicos e infiltrativos observados en los

nervios y la distribución bacilar en los diversos tipos de lepra.

Se insiste sobre la importancia de tales estudios para la comprensión de la patogenia de la lepra.

## RÉSUMÉ

Les lésions de lèpre atteignent des surfaces étendues, du corps. Dès lors, l'étude des segments de peau apparemment saine, situés soit dans le voisinage, soit loin des lésions, s'indiquait, afin de mieux comprendre les mécanismes d'extension du processus morbide.

L'étude a porté sur 100 malades de la lèpre, atteints de tous les types de la maladie. Les résultats montrent que, même dans les portions de peau considérées comme normales du point de vue clinique, on peut observer, dans les cas lépromateux et dans les cas proches du type lépromateux dans le spectre de la classification, une infiltration significative à cellules inflammatoires telles que lymphocytes et histiocytes.

L'étude de la distribution des bacilles acido-résistants dans les lésions et dans la peau apparemment normale, semble indiquer que des phases bacillémiques surviennent dans certains types de lèpre. On mentionne la présence de bacilles dans les échantillons prélevés pour biopsie, qui montraient une infiltration négligeable ou très peu prononcée du derme, avec des cellules inflammatoires.

La signification des modifications morphologiques et de l'infiltration constatée dans les nerfs, ainsi que la distribution bacillaire dans les divers types de lèpre, est discutée.

On insiste sur l'importance de telles études en vue de comprendre la pathogénèse de la lèpre.

**Acknowledgement.** The authors are grateful to P. B. Nath, Research Assistant, Central Leprosy Teaching and Research Institute, Chingleput, Tamil Nadu, India, for the preparation of various biopsy specimens on which the study is based, and to R. V. Nerurkar of the Cancer Research Institute, Bombay, India, for the photomicrographs.

The investigation was undertaken during the tenure of a training fellowship offered to the first author by the Hind Kusht Nivaran Sangh, New Delhi, India.

## REFERENCES

1. BECHELLI, L. M., DA SILVA, E. and OLIVEIRA, A. B. On the histopathological findings in biopsies of apparently normal skin in cases of leprosy. *Internat. J. Leprosy* 13 (1945) 175. (Abstract)

2. DASTUR, D. K., RAMAMOCHAN, Y. and SHAH, J. S. Ultrastructure of the nerve in tuberculoid leprosy. Paper read at 9th Conference of Indian Association of Leprologists, Bhopal, India, 1971.
3. DESIKAN, K. V. and JOB, C. K. Visceral lesions caused by *M. leprae*; a histopathological study. Indian J. Path. Bact. 13 (1970) 100-108.
4. FERNANDEZ, J. M. M. Pathology of acute reaction in leprosy. Internat. J. Leprosy 4 (1936) 549-550. (Abstract)
5. GOUGEROT, H. and DEGOS, R. The histobacteriology of invisible lepromate revealed by methylene blue. Internat. J. Leprosy 7 (1939) 294-295.
6. KHANOLKAR, V. R. Perspectives in pathology of leprosy. Indian J. Med. Sci. 9 (Supplement 1) (1955) 13-23.
7. PERIASWAMY, V. Differentiation of tuberculoid reaction, borderline and lepromatous cases bacteriologically. Leprosy in India 31 (1959) 103-106.
8. RAMANUJAM, K. and RAMU, G. A study of borderline leprosy from the clinical, bacteriological and immunological aspects. Leprosy in India 37 (1965) 303-311.
9. RIDLEY, D. S. and JOPLING, W. H. Classification of leprosy according to immunity: a five group system. Internat. J. Leprosy 34 (1966) 255-273.
10. WEDDELL, G., PALMER, E., REES, R. J. W. and JAMISON, D. G. Experimental observations related to the histopathology of leprosy. In: Pathogenesis of Leprosy. Ciba Foundation Study Group, No. 15, p 3-15. J & A Churchill Ltd.: 104 Gloucester Place, London W1.