SPECIFIC TISSUE ALTERATION IN LEPROUS SKIN

III. Specific Reaction Due to Various Agents*

F. SAGHER, M.D., E. LIBAN, M.D. and E. KOCSARD, M.D.†

It was originally observed that the histology of a positive tuberculin test in leprous patients simulated a leproma-like lesion (1, 2). Biopsies of a positive tuberculin skin test in these patients from two to twenty-eight days after the injection of O.T. or PPD revealed a specific reaction. This reaction varied from small nests of foam cells to large granulomatous structures identical to those found in active lepromatous leprosy.

To determine whether this specific reaction of leprous skin was the result of altered tissue reactivity in the leprous host or whether it was due to the nature of the O.T. or PPD, the following investigation was undertaken. Leishmanin, milk and peptone were administered intradermally and the skin reaction was studied histologically two to four days after injection. In addition, the skin lesions resulting from insect bites and erythema nodosum-like lesions appearing during sulfone or thiosemicarbazone therapy were also examined.

MATERIAL AND METHODS

Leishmanin, milk and peptone were injected intradermally into the upper arm or forearm in areas clinically free of leprous lesions. The test subjects were in different stages of lepromatous leprosy. Their clinical and laboratory data are presented in Table I.

1. Leishmanin. 0.1 ml of a vaccine containing 100,000 killed flagellates of Leishmania tropica was used. This vaccine has a high specificity being positive in 91.6 per cent of cutaneous leishmaniasis, as shown by Dostrovsky and Sagher (3).

a. In leprous patients with leishmaniasis. Five leprous patients developed a positive skin reaction to the vaccine. Four of these gave a history of cutaneous leishmaniasis and had scars which remained after cure of the disease. No history of leishmaniasis was obtained from the fifth, nor did he show any evidence of this disease.

In 23 leprous patients without a history or scars of leishmaniasis, no reaction was elicited by the injection of the vaccine. Biopsies were not taken from these subjects.

b. In non-leprous patients with cutaneous leishmaniasis. For control, 21 biopsies of positive leishmanin tests were studied histologically from 13 subjects with active or inactive cutaneous leishmaniasis. The biopsies were performed from 2 to 22 days following injection.

* From the Department of Dermatology and Venereology (Dr. A. Dostrovsky, Director), the Department of Pathology (Dr. H. Ungar, Director) of the Hebrew University Hadassah Medical School, Jerusalem, Israel, and the affiliated Hospital for Hansen's Disease (Dr. F. Sagher, Physician in Charge) of the Ministry of Health, Government of Israel.

† At present in Sydney, Australia.

Received for publication November 6, 1952.

TABLE I

Specific reactions due to various agents in leprous patients

CLINICAL AND LABORATORY DATA									HISTOLOGICAL AND BACTERIOLOGICAL RESULTS OF THE TESTS		
						Smears from				Acid-fast bacilli in	
Material	Name	Sex	Age	Duration of leprosy in years	Clinical type of leprosy	Nasal mucous membrane	Ear-lobe	Lepromin test	Biopsy grade	Smears from biopsy ground	Histolog. sections
Leishmanin test	R. S.	M	31	17	a.l.l.	+++	+++	−	++	−	−
	R. R.	F	35	15	a.l.l.		+++	−	+++	−	−
	C. R.	M	45	21	a.l.l.	+	++++	−	+++	−	−
	K. N.	M	42	30	i.l.l.	−	++	−	++	−	−
	A. L.	F	23	10	a.l.l.	−	++++	−	++	+	−
Milk	G. S.	M	24	5	a.l.l.	+++	+++	−	+	−	−
	I. J.	M	22	6	i.l.l.	−	−	−	+++	+	G
	F. R.	F	30	7	a.l.l.	++	+++	−	++	G	+
	C. N.	M	46	12	a.l.l.	+++	+++	−	+++	++	+
	G. M.	F	32	4	i.l.l.	−	−	−	+	−	−
Peptone	I. B.	F	75	6	undetermined	−	−	+	++	−	G
Insect-bite	F. R.	F	30	7	a.l.l.	++	+++	−	++	+	G
									++*	G	−
Erythema nodosum-like eruptions appearing during chemotherapy	M. R.	M	26	8	a.l.l.	+++	+++	−	+++	++	+++
	S. M.	F	19	7	a.l.l.	+++	+++	−	+++	G	G
	F. C.	M	23	6	a.l.l.	+++	+++	−	+++	−	−
									+++*	++	+++
									+++*	−	−
	C. N.	M	46	12	a.l.l.	+++	+++	−	+++	+	−
	J. M.	M	32	22	a.l.l.	+++	++++	−	+++	−	G

Explanations: Clinical type of leprosy: a.l.l.—active leprom. leprosy.
i.l.l.—inactive leprom. leprosy.

Biopsy results: + foam cell nests.
++ pre-lepromatous reaction.
+++ leproma-like reaction.

Bacillary findings: G-acid-fast granules.

* repeated biopsies and smears.

2. Milk. 0.1 ml of fresh boiled cow's milk was injected into five leprous patients. In all, a papule with marked erythema appeared following the injection. In three of the patients active leprous skin lesions were present; two patients showed no active signs of the disease and no bacilli could be found in smears taken from their nasal mucous membrane and from the ear.

3. Peptone. 0.1 ml of sterile 1.0 per cent peptone solution was given to five patients. Only one, with an undetermined type of leprosy, developed an erythematous papule which was biopsied.

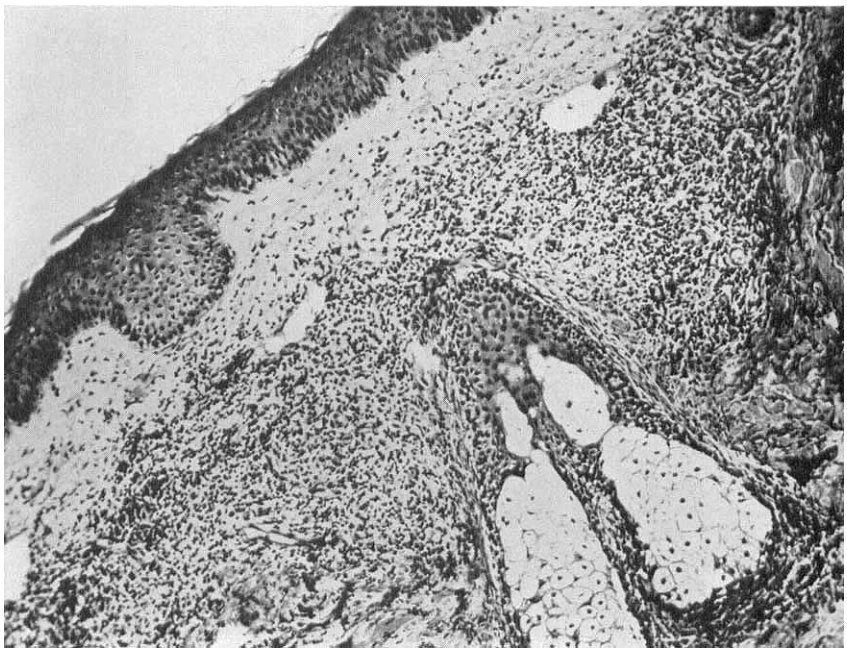

FIG. 1. Positive leishmanin-test in a leprous patient. Leproma-like reaction (3+) in the dermis, surrounding a sebaceous gland. A typical border-zone between the granulomatous structure in the dermis and the basal layer of the epidermis is left, free of infiltrations. (Hematoxylin-eosin, 95×.)

4. Insect-bites. A single patient, with active lepromatous leprosy, was bitten accidentally by *Phlebotomus papatasii* (sand fly). She developed papular lesions on her extremities which persisted for more than a week. This same event occurred one year later. On both occasions papules were excised one week after their appearance.

5. Treatment reactions. Five patients with active lepromatous skin lesions developed erythema nodosum-like eruptions during treatment with sulfones or thiosemicarbazones. Seven biopsies were excised from these subjects.

6. Controls. Forty-one biopsies from 34 leprous patients were taken from areas of normal looking skin for histological study. They were in various stages of their disease.

All biopsy material was fixed in 10 per cent formalin or Zenker's fluid. Paraffin sections were stained with hematoxylin-eosin, Van Gieson's and Ziehl-Neelsen's method as modified by Putt (4). Frozen sections were stained with Sudan IV. Thirty-one smears were taken from the floor of the biopsy wounds and stained with the Ziehl-Neelsen technic.

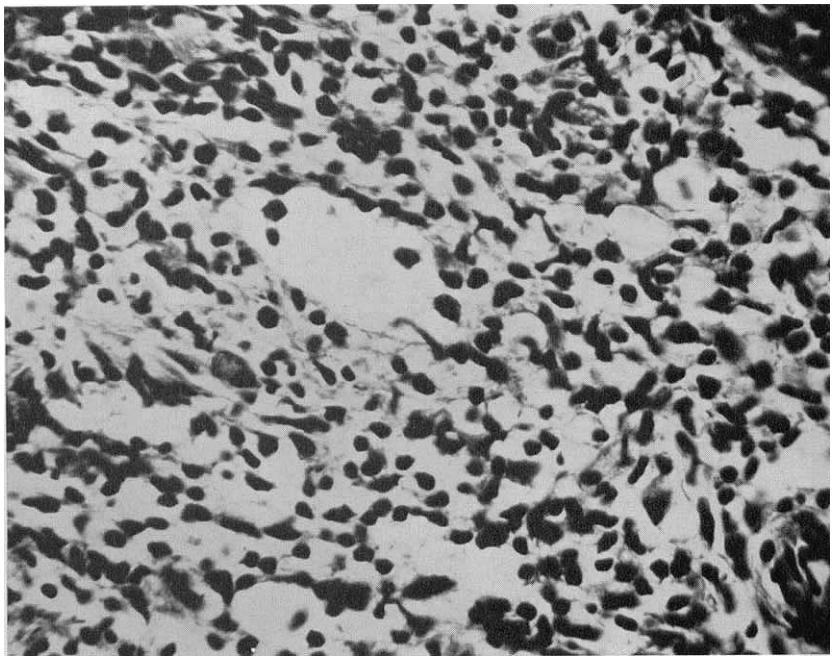

FIG. 2. The same section as in fig. 1, at higher magnification (560×). Histiocytes with large vacuoles predominate, intermingled with lymphocytes and fibrocytes.

RESULTS

The results of the present investigation are summarized in Tables I and II. For the grading of the results the same method was used as in the previous communication (2).

The reactions ranged from small foam cell nests in the subpapillary layer of the dermis (grade 1+) to large granulomatous structures occupying extensive areas of the dermis (grade 3+) and labelled leproma-like reaction. Grade 2+ was a reaction between these extremes and was called pre-lepromatous reaction.

1. Leishmanin test.

a) In leprous patients with cutaneous leishmaniasis. Three pre-lepromatous (2+) and two leproma-like reactions (3+), (Figs. 1 and 2) were seen in the skins of the five positive reactors. Acid-fast bacilli were not demonstrated in the histological preparations. One smear from the biopsy floor of one patient showed a few acid-fast bacilli.

b) In non-leprous patients with cutaneous leishmaniasis. No foam cells were

found in any instance from the 21 biopsies. In all, there was a non-specific lymphocytic infiltration scattered throughout the dermis (Figs. 3 and 4). In one of these there was in addition a granulomatous lesion and in another case there were small foci of histiocytes and epithelioid cells.

2. Milk: The biopsies revealed two leproma-like reactions (3+), (Fig. 5), one pre-lepromatous lesion (2+), (Fig. 6) and two small foci of foam cells in the subpapillary layer of the dermis (1+), (Fig. 7). In two cases acid-fast bacilli

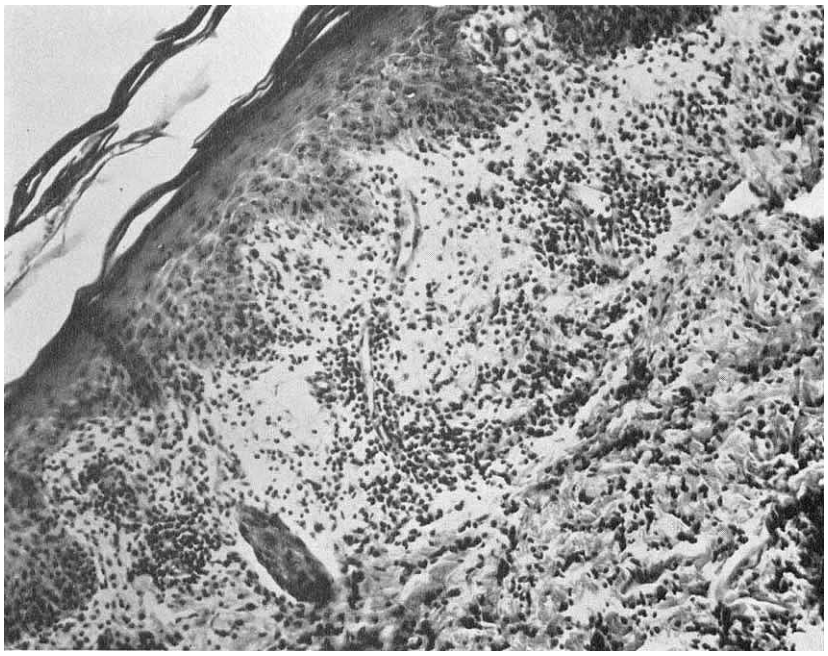

FIG. 3. Positive Leishmanin-test in a non-leprous patient. The subpapillary layer of the dermis is edematous and contains small perivascular infiltrations of lymphocytes. (Hematoxylin-eosin, 140×.)

were demonstrated both in the histological preparations and in the smears from the biopsy floor.

3. Peptone: The skin reaction was histologically 2+.

4. Insect-bites: The papules revealed in both instances one year apart a 2+ reaction.

5. Treatment reaction: The 7 biopsies from 5 patients were histologically 3+ lesions (Fig. 8). In two instances acid-fast bacilli were found in sections and in three, a moderate number of acid-fast bacilli were seen in the smears taken from the biopsy floor.

6. Controls: In 27 cases there was no reaction histologically. In ten the lesions were graded 1+, in three 2+, and in one 3+. Acid-fast bacilli were seen in five

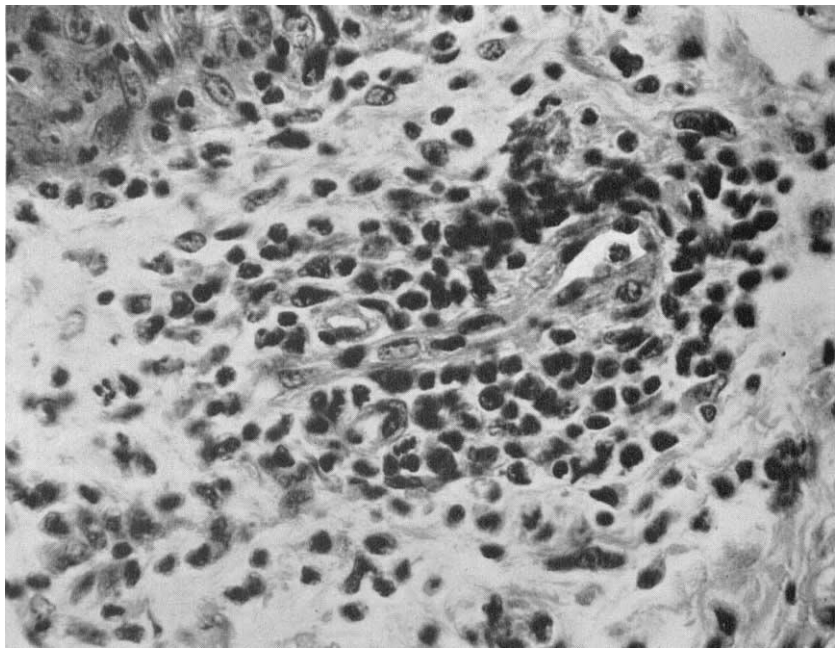

FIG. 4. The same section as in fig. 3., at higher magnification (560×). The perivascular infiltrations are composed mainly of lymphocytes and a small number of neutrophile leucocytes and non-vacuolated histiocytes.

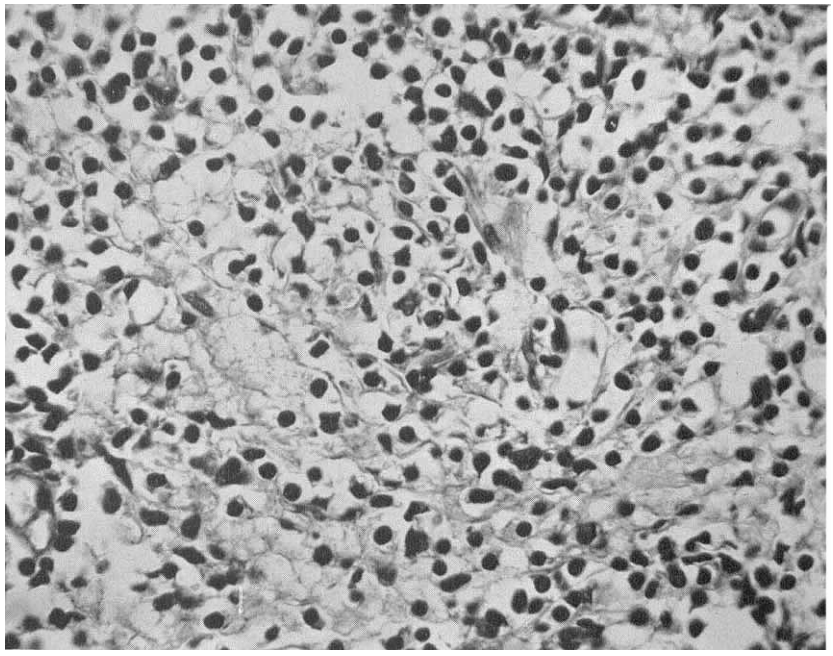

FIG. 5. Milk injection in a leprous patient. A leproma-like reaction, (3+), at high magnification (480×). The granulomatous structure shows almost only histiocytes with a few large or many small vacuoles. (Hematoxylin-eosin.)

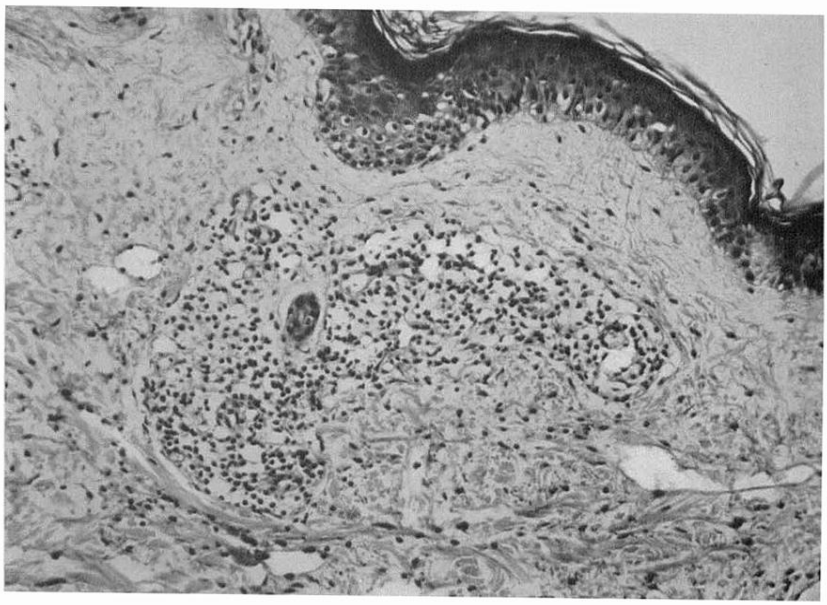

FIG. 6. Milk injection in a leprous patient. Pre-lepromatous reaction (2+). In the subpapillary layer of the dermis a large infiltration is seen, consisting mostly of foam cells with large vacuoles. (Hematoxylin-eosin, 160×.)

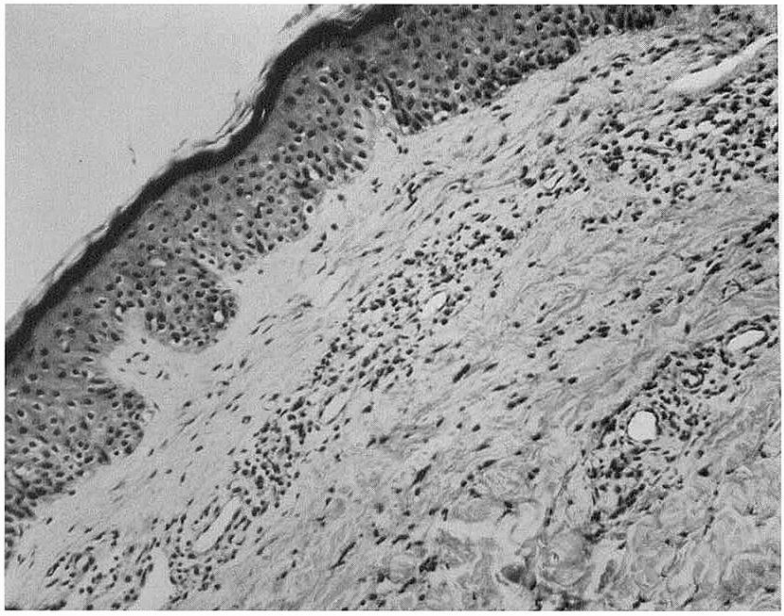

FIG. 7. Milk injection in a leprous patient. Foam cell nests (1+). Narrow strands of foam cell nests around the blood vessels in the subpapillary layer of the dermis. (Hematoxylin-eosin, 160×.)

biopsies and acid-fast granules in four. Acid-fast bacilli were found in smears from the biopsy floor in thirteen cases and acid-fast granules in two cases.

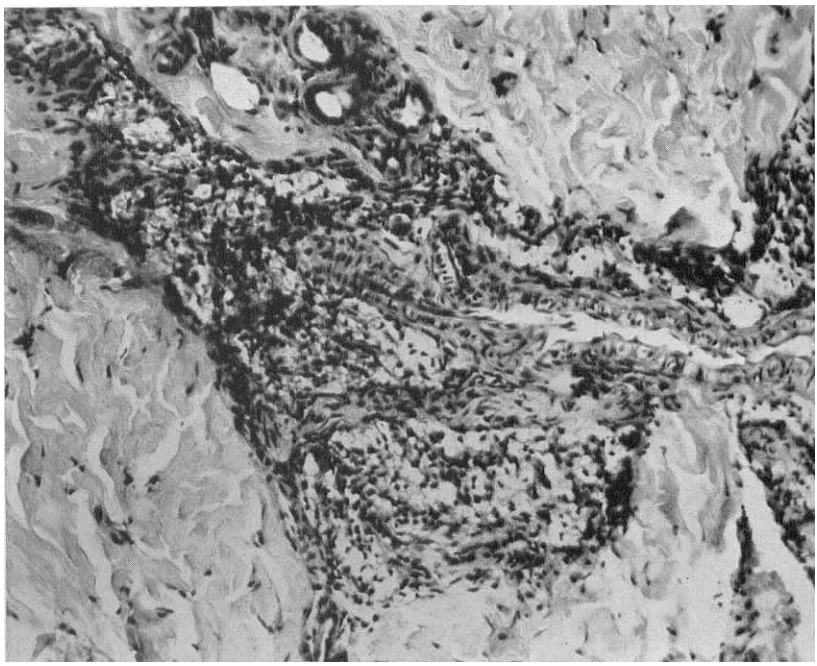

FIG. 8. Erythema nodosum-like eruption in a leprous patient. A leproma-like lesion in the depth of the dermis, composed of vacuolated histiocytes and fibrocytes. (Hematoxylin-eosin, 160×.)

TABLE II

Comparison of the histological and bacterial findings in the tests and control leprous subjects

	NUMBER OF		BIOPSY RESULTS				ACID-FAST BACILLI IN									
							Smears from biopsy ground					Histolog. sections				
	Patients	Biopsies	−	+	++	+++	−	G	+	++	+++	−	G	+	++	+++
Test	17	20	0	2	7	11	10	3	4	3	0	11	5	2		2
Control†	34	41	27	10	3	1	15	2	9		4*	32	4	2	1	2

* Smears from the biopsy floor were not done in 11 instances.

† From normal-looking skin of leprous patients.

DISCUSSION

The skin of leprous patients appears to have acquired a specific altered reactivity and to react to a variety of stimuli with a characteristic response. This was suggested by the previous investigation in which positive tuberculin skin tests, on microscopic examination, consisted of lesions resembling leprosy. This concept

was further strengthened by the result of the present investigation. The reaction of leprous skin to injury appears to be a specific altered response.

It might be possible that the specific altered response of leprous skin is merely the exaggeration of pre-existing lesions which were not clinically apparent. Wade (5), Büngeler and Martins de Castro (6), Maurano (7), and Manalang (8) have shown that lepromatous or pre-lepromatous lesions were occasionally present in areas of leprous skin which were clinically free of the disease. In the present investigation, 41 biopsies were taken from regions of leprous skin which were clinically normal in appearance. In 27 (66 per cent) no foam cells or lepromatous lesions were found. In the remaining 14, foam cell infiltrations were present ranging in severity from 1+ to 3+. In contrast to these findings, foam cells or leproma-like structures were found in all 20 skin biopsies taken from 17 leprous patients that had developed a lesion as a result of injury from five unrelated materials. In short, whenever reaction to injury occurred in leprous skin, foam cell lesions typical of leprosy were found.

It is also significant that the intradermal injection of leishmanin into five leprous patients with leishmaniasis evoked the characteristic foam cell response. In 21 examinations of leishmaniasis without leprosy the leishmanin test resulted in a skin reaction which was totally different and quite non-specific.

Further evidence in favor of the above concept was derived from the bacteriological studies. The foam cell of lepromatous skin lesions is usually filled with lepra bacilli. In the foam cell reaction elicited by the different materials employed here acid-fast bacilli were relatively rarely seen. Further, in most of the smears from the biopsy floor no acid-fast bacilli or granules were detected. It seems, therefore, that the induced skin reactions described above were not due to an increase or local accumulation of acid-fast organisms. The fact that acid-fast organisms were occasionally found in the induced skin lesions is of no etiological significance since they may be found in the leprous skin free of clinical signs (Köbner (9), Stein and Steperin (10), our controls).

The identical histologic reaction in the leprous patients to five unrelated materials indicates that the altered reactivity of the tissue is the predominant factor determining the specificity of the reaction. The nature of the injected material is apparently of little consequence. Organisms with and without a lipid component (killed tubercle bacilli and leishmania flagellates), protein (peptone), lipid (milk), insect-bite and sulfone therapy, all elicited histologically identical reactions, when the test material provoked a clinical response.

Lastly, the stage of the disease in leprous skin apparently plays no role in the reactions evoked. Foam cell aggregates were provoked in patients with active skin lesions as well as in patients in whom all clinical signs of the disease had disappeared.

SUMMARY

Intradermal injection of leishmania vaccine, milk or peptone into leprous patients, with or without active lepromatous leprosy, elicited a specific foam cell reaction typical for leprosy. A similar reaction was seen in the skins of leprous

patients bitten by sand fly and with eruptions appearing during chemotherapy.

In the normal appearing skin of a control series of leprous patients, foam cells were not found in two thirds of the cases and only minimal infiltrations in the remaining third. In a second control series of non-leprous patients with cutaneous leishmaniasis, the injection of leishmania vaccine evoked a non-specific inflammatory reaction in the dermis.

It is concluded that leprosy causes a specific altered reactivity of the skin which reacts to injury with a histological picture characteristic of the host's tissue response.

REFERENCES

1. SAGHER, F., KOCSARD, E. AND LIBAN, E.: Specific tissue alteration in leprous skin. I. The transformation of the tuberculin reaction in leprous patients into leproma-like lesions. Internat. J. Leprosy (in press).
2. SAGHER, F., KOCSARD, E. AND LIBAN, E.: Specific tissue alteration in leprous skin. II. The histology of the tuberculin reaction in leprosy. J. Invest. Dermat. **19:** 499–508, 1952.
3. DOSTROVSKY, A. AND SAGHER, F.: The intracutaneous test in cutaneous leishmaniasis. Ann. Trop. Med. **40:** 265–269, 1946.
4. PUTT, F. A.: A modified Ziehl-Neelsen method for demonstration of leprosy bacilli and other acid-fast organisms. Am. J. Clin. Path., **21:** 92–95, 1951.
5. WADE, H. W.: Tuberculoid changes in leprosy; lepra-reaction in tuberculoid leprosy. Internat. J. Leprosy, **2:** 279–292, 1934.
6. BÜNGELER, W. AND MARTINS DE CASTRO, A. F.: Untersuchungen über den klinischen Verlauf der histologischen Veränderungen allergischer Reaktionen bei der Lepra. IV. Mitteilung: Klinische und histologische Untersuchung über die spontane Reaktion beim lepromatösen Aussatz. Virchows. Arch. **306:** 404–426, 1940.
7. MAURANO: cit by BÜNGELER, W. AND MARTINS DE CASTRO, A. F. (6).
8. MANALANG, C.: Significance of pathological findings in biopsy materials from lepers. Part. V. Monthly Bull. Bureau of Health. Manila. **23:** 85–91, 1947.
9. KÖBNER: cit. by BÜNGELER, W. AND MARTINS DE CASTRO, A. F. (6).
10. STEIN, A. A. AND STEPERIN, M.: Excretion of lepra bacilli by lepers. Urol. & Cutan. Rev. **38:** 776–778, 1934.