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Cross Reactivity of Lepromin with Other Mycobacterial Antigens[1]

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Lepromin is a peculiar intradermal diagnostic agent. It is one of the few in clinical use composed of whole organisms. It does not detect the presence or absence of the disease for which it is used. In fact, it induces positive reactions only in a "class" of leprosy patients, characteristically those with the tuberculoid type of the disease, while it fails to elicit responses in patients with the lepromatous type. This dichotomy is usually explained on the basis that the former are immunologically reactive, presumably to *Mycobacterium leprae*, while the latter are not; i.e., they are "anergic." This viewpoint is bolstered by the fact that bacilli are sparse in tuberculoid lesions, whereas lepromatous lesions teem with them.

An especially puzzling aspect of lepromin reactivity is the response of many apparently normal persons to it. One might ascribe this to a widespread prevalence of subclinical disease, but this seems improbable because such reactions occur in parts of the world where clinical leprosy is almost nonexistent ([1, 13]). Another suggestion is that lepromin may show cross reactivity in persons who have been sensitized by *Mycobacterium tuberculosis*, and indeed, vaccination with BCG may convert negative lepromin reactors to positivity ([3, 4, 7, 11]). This is also an insufficient explanation, since many reactors to lepromin show a negative tuberculin test.

The present study was intended to find whether sensitivity to lepromin may be induced by species of mycobacteria other than *M. leprae* or *M. tuberculosis*. For this purpose an animal (guinea-pig) system was used. These results also clarified to some extent the role of the bacillary versus the dermal component of lepromin in the sensitivity to this complex "antigen."

MATERIALS AND METHODS

Lepromin. A purified bacillary suspension was obtained by enzymatic digestion

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of ground, epidermis-free lepromata.[3] For the skin test we used 0.1 ml. of a suspension containing 1.42 x 10^8 bacilli per ml., as determined by a modification of Hanks' method ([5]). When tested in lepromatous and tuberculoid patients, in BCG-vaccinated persons and in contacts of cases of leprosy, it behaved like the usual crude lepromin.

Dermis suspension. This was made from ground normal human dermis. Its preparation and initial weight/volume relationship were identical to those used for lepromin. Dilutions were used as indicated.

Diluent. Phosphate-buffered saline, pH 7.4, was used, with 0.5 per cent w/v of phenol and 0.05 per cent v/v of Tween 80.

Animals. Albino random-bred guinea-pigs, with an initial weight of 350 to 550 gm. were used.

Microorganisms. *Candida albicans* from stock was grown in rotary culture in Sabouraud's fluid medium at room temperature for 48 hours, yielding the yeast phase. The organisms were harvested by centrifugation and then processed as described for the various mycobacteria. The mycobacteria used were *M. kansasii*, *M. phlei*, *M. butyricum*, BCG, and the Battey bacillus. These were grown on Trudeau or Loewenstein-Jensen solid media at 37°C until luxuriant growth was obtained. They were dislodged from the agar, washed twice with 0.05 per cent Tween 80 in water, heated at 80°C for 30 minutes, and washed twice more with Tween-water. Wet weights were determined by filtering suspensions through preweighed millipore filters of 0.45μ average pore diameter, and reweighing the filters. The weight of Tween-water retained was estimated by using this liquid alone in a preweighed filter. The bacilli were resuspended in an appropriate volume of diluent and kept frozen until used. Acid-fast staining and cultures in appropriate media were carried out to assure the exclusive presence of killed mycobacteria. In the case of *Candida*, wet mounts showed only yeast-like organisms, and inoculation of Sabouraud slants resulted in no growth.

Old Tuberculin (OT) was purchased from Wyeth Laboratories, and *Freund's incomplete adjuvant* from Difco Laboratories.

With these reagents and organisms two experiments were performed. In the first, groups of five animals were used. The first group received 0.2 ml. of 1:1 diluent in incomplete Freund's adjuvant in each of the four foot pads and subcutaneously in the neck, for a total of 1 ml. The other five groups each received one of the mycobacteria, 1 mgm. emulsified in incomplete Freund's adjuvant in each injection site for a total of 5 mgm. of bacilli. Five weeks later the animals were tested intradermally with 0.1 ml. of each of the following: lepromin, lepromin 1:10, dermis suspension, dermis suspension 1:10, and OT 1:10. Reactions were read at 4 hours and at 1, 2, 5, 10, 16, and 21 days after injection. After the last reading, skin sites were biopsied and specimens were fixed in buffered formalin, pH 7.4, and stained with hematoxylin-eosin, Giemsa, Ziehl-Neelsen, and methyl green pyronin (modified for formalin fixation ([12]).

In the second experiment, six groups of five animals each were used. The first group had no treatment; the second received diluent in Freund's incomplete adjuvant in the four foot pads; the third received a total of 4 mgm. wet weight of *Candida albicans* in adjuvant and divided among the four foot pads. The remaining three groups received, respectively, *M. kansasii*, *M. butyricum*, and BCG, 4 mgm. wet weight per animal divided among the four foot pads. After five weeks the animals were tested with lepromin, lepromin 1:10, dermis suspension, and dermis suspension 1:10. The reactions were read at the same time intervals as in the first experiment, and here also skin sites were biopsied and specimens stained as described.

RESULTS

Experiment I. Table 1 and Figure 1 show that all the groups sensitized with myco-

[3] Lepromatous tissue kindly supplied by Dr. Jacinto Convit, Medical Chief, División de Dermatología Sanitaria, Ministerio de Sanidad y Asistencia Social, Caracas, Venezuela.

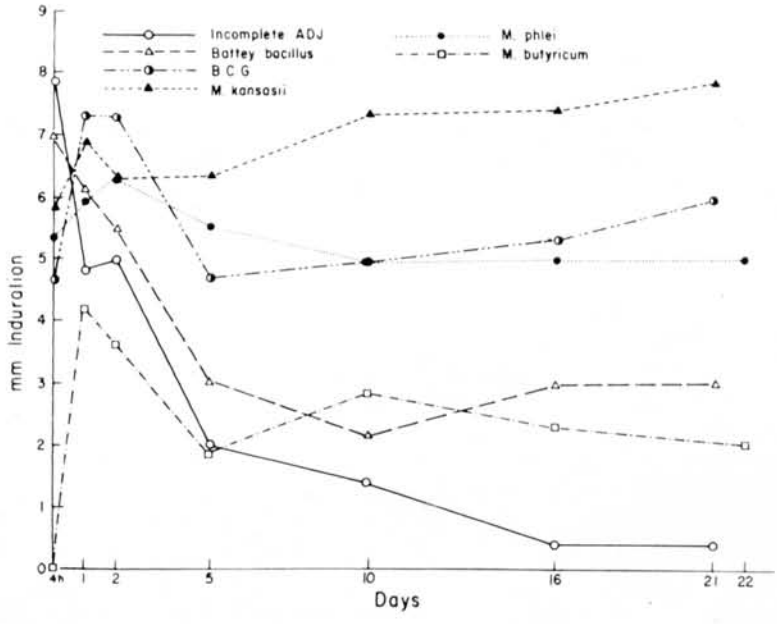

FIG. 1. Reactions to full strength lepromin. Direct readings (Experiment 1). Values shown for the different groups are the arithmetic means of millimeters of induration.

bacteria gave stronger inflammatory responses to lepromin than did the controls. The difference was manifest at five days, but was most marked by ten days and later. This was not a nonspecific response to an inflammatory stimulus, since the reactions to the dermis suspension were, if anything, less marked in the sensitized animals than in controls.

In Figure 1, the reactions to full strength lepromin show a peak at 24 to 48 hours, a depression at five days, and then a higher plateau beginning at ten days and sloping gently upward or downward depending on the sensitizing mycobacterium. In contrast, the readings in the control animals continued downward after the initial higher levels. Figure 2 shows "corrected" readings, i.e., the differences between lepromin and dermis suspensions both at full strength. These values tended to increase as the experiment progressed, in contrast to those in the unsensitized controls. Similar results were seen when, to stress the element of specificity, "corrected" readings were plotted for lepromin, 1:10, minus dermis suspension full strength. This curve was biphasic with a low point at five days and increasing values thereafter. It appeared also that there were differences in intensity of response to lepromin induced by the mycobacteria used, with *M. kansasii*> *BCG*> *M. phlei*> Battey bacillus> *M. butyricum*. The difference between the last two was very small.

The large sensitizing doses of mycobacteria used produced considerable local inflammation, and there was appreciable loss of weight and some mortality from pneumonia (Table 1). Beta hemolytic streptococci were isolated from the lungs and pleural fluids of these animals, but no acid-fast bacilli were found. While most of the animals in the *M. butyricum* group survived, their general state was poor, and their reactions to the dermis suspension were the lowest of all the groups. Their reactions to OT were also much smaller than those observed in the other experimental groups, and it might be questioned whether the poor response to lepromin after *M. butyricum* sensitization was due to lack of cross reactivity or to nonspecific lowering of capacity of response.

The histologic study of skin sites at 21 days correlated well with gross observations. In the lepromin site, normal animals

TABLE 1. *Readings (mm. induration) after injection of lepromin and dermis suspension (Experiment I)*

Sensitizing agent	Guinea-pig Nos.	Time of readings													
		4 hours		1 day		2 days		5 days		10 days		16 days		21 days	
		Lep.[a]	D.[b]	Lep.	D.	Lep.	D.	Lep.	D.	Lep.	D.	Lep.	D.	Lep.	D.
Incomplete Freund's adjuvant	1	6	7	0	5	3	4	0	0	0	0	0	0	0	0
	2	7	6	5	5	5	4	0	0	0	0	0	0	0	0
	3	8	7	6	6	6	6	4	0	1	0	0	0	0	0
	4	10	7	7	5	7	4	4	2	3	0	2	0	2	0
	5	8	7	6	5	4	4	2	0	3	0	0	0	0	0
	Means	7.8	6.8	4.8	5.2	5.0	4.4	2.0	0.4	1.4	0	0.4	0	0.4	0
Battey bacillus	6	5	5	6	4	5	3	4	0	4	1	5	0	4	0
	7	7	7	7	6	6	3	2	0	1	0	2	0	3	0
	8	8	6	5	3	5	4	2	0	0	0	0	0	0	0
	9	8	5	7	3	6	3	4	0	4	0	5	0	5	0
	10	- - -	- - -	- - -	- - -	Dead	- - -	- - -	- - -	- - -	- - -	- - -	- - -	- - -	- - -
	Means	7.0	5.8	6.25	4.0	5.5	3.3	3.0	0	2.25	0.25	3.0	0	3.0	0
BCG	11	- - -	- - -	- - -	- - -	Dead	- - -	- - -	- - -	- - -	- - -	- - -	- - -	- - -	- - -
	12	5	6	8	6	8	6	6	0	6	0	5	0	6	0
	13	- - -	- - -	- - -	- - -	Dead	- - -	- - -	- - -	- - -	- - -	- - -	- - -	- - -	- - -
	14	4	5	7	6	8	0	2	0	4	0	5	0	5	0
	15	5	0	7	4	6	0	6	0	5	0	6	0	7	0
	Means	4.7	3.7	7.3	4.7	7.3	2.0	4.7	0	5.0	0	5.3	0	6.0	0

[a] **Lepromin, full strength.** Mm. induration (diameter).
[b] **Dermis suspension, full strength.** Mm. induration (diameter).

TABLE 1.—*Continued*

Sensitizing agent	Guinea-pig Nos.	Time of readings													
		4 hours		1 day		2 days		5 days		10 days		16 days		21 days	
		Lep.[a]	D.[b]	Lep.	D.	Lep.	D.	Lep.	D.	Lep.	D.	Lep.	D.	Lep.	D.
M. kansasii	16	6	0	8	5	8	5	8	4	8	4	9	2	8	0
	17	6	0	6	3	7	0	8	0	9	0	8	0	9	0
	18	3	0	7	3	5	3	4	2	7	1	6	0	7	0
	19	8	5	6	3	5	3	5	0	5	0	6	0	7	0
	20	----	----	----	----	Dead	----	----	----	----	----	----	----	----	----
	Means	5.8	1.3	6.8	3.5	6.3	2.8	6.3	1.5	7.3	1.3	7.3	0.5	7.8	0
M. phlei	21	4	0	5	4	5	2	----	----	----	----	Dead	----	----	----
	22	----	----	----	----	Dead	----	----	----	----	----	----	----	----	----
	23	----	----	----	----	Dead	----	----	----	----	----	----	----	----	----
	24	6	0	6	3	8	3	5	0	5	0	5	0	4	0
	25	6	2	7	3	6	2	6	0	5	0	5	0	6	0
	Means	5.3	0.7	6.0	3.3	6.3	2.3	5.5	0	5.0	0	5.0	0	5.0	0
M. butyricum	26	0	0	4	2	3	2	0	0	2	0	2	0	2	0
	27	0	0	4	0	2	0	0	0	4	0	4	0	4	0
	28	0	0	4	3	6	2	4	0	3	0	2	0	0	0
	29	0	0	4	0	2	0	3	0	4	0	----	Dead	----	----
	30	0	0	5	3	5	2	2	0	1	0	1	0	2	0
	Means	0	0	4.2	1.6	3.6	1.2	1.8	0	2.8	0	2.3	0	2.0	0

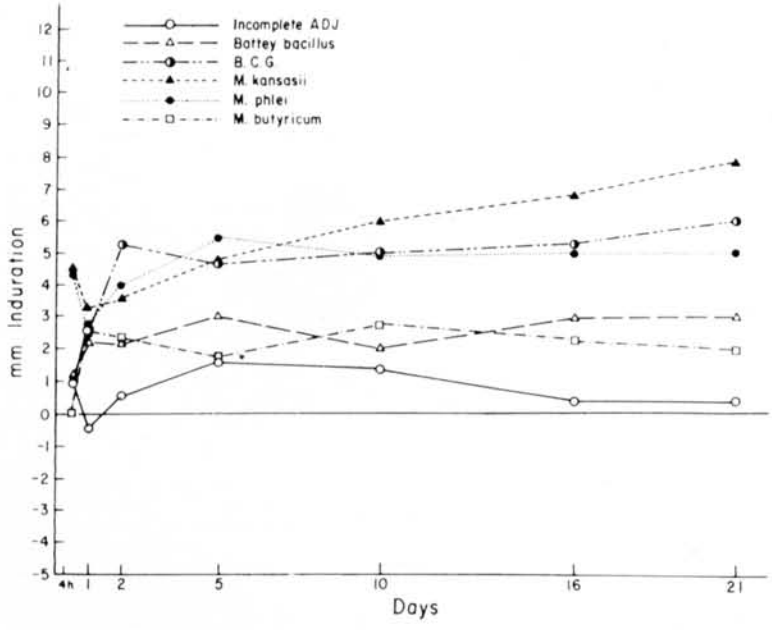

FIG. 2. Reactions to full strength lepromin. Corrected readings (Experiment I). Values shown are the arithmetic means of the difference between millimeters of induration at the lepromin site and the site injected with dermis suspension.

showed only a slight inflammatory infiltrate, with some histiocytes and some large fusiform cells dispersed in the dermis. Bacilli were present, free or inside the fusiform cells. The sites injected with dermis suspension were practically normal except for some increase in the number of fibroblasts and very slight capillary dilatation. In the sensitized animals the reaction to dermis suspension was similar to that seen in the controls, while the sites injected with lepromin ran the gamut of inflammatory reaction from a moderate granulomatous infiltrate in the deep dermis and subcutaneous tissue, to a tuberculoid granuloma with epithelioid islands and giant cells, to necrosis with an intense inflammatory infiltrate, granulomatous in nature but heavily contaminated with polymorphonuclears around the necrotic focus formed by pale, ghost-like histiocytes filled with ingested bacilli. In many instances in the lepromin sites of sensitized animals, small islands of plasma cells were seen. In general, the intensity of the infiltrate paralleled the magnitude of the gross changes.

Experiment II. The animals in this experiment were isolated from the rest of the colony and given Sulfaquinoxaline *per os* for 10 days at the beginning of the experiment, and again whenever it was required. Ulcers of the foot pads were treated with hydrogen peroxide and a topical antibiotic ointment (Neo-Polycin). The general condition of the animals was much better than in the first experiment, and no deaths occurred.

Readings in the sites injected with lepromin and dermis suspension are shown in Table 2; lepromin reactions are recorded in Figure 3. Again the animals sensitized with mycobacteria showed higher readings than did the three control groups. These reactions did not show the dip at five days seen in the first experiments; there was a fall at 24-48 hours, and then a slow rise that reached its maximum at 10 days and remained high thereafter. The control groups showed a progressive decrease of the reactions which approached zero. The corrected readings (Fig. 4) emphasize the differences between sensitized and control groups.

The reactions in this experiment were larger than those in Experiment I, particularly in the *M. butyricum* group. However,

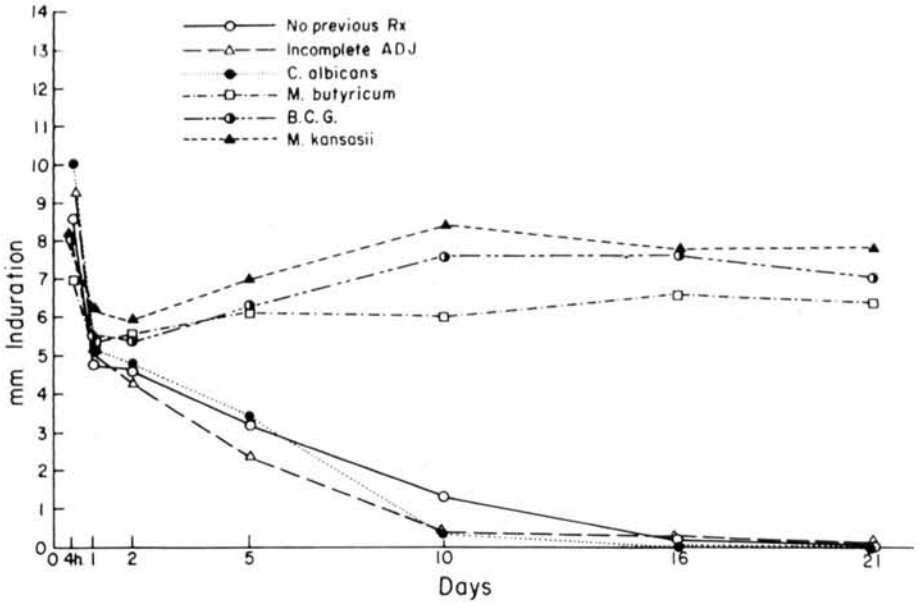

FIG. 3. Reactions to full strength lepromin. Direct readings (Experiment II). (See legend, Fig. 1)

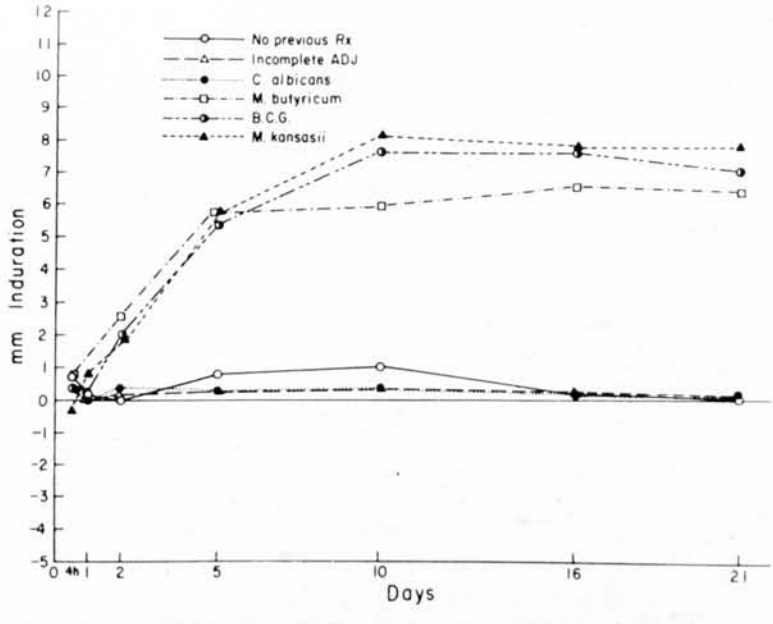

FIG. 4. Reactions to full strength lepromin. Corrected readings (Experiment II). (See legend, Fig. 2)

TABLE 2. *Readings (mm. induration) after injection of lepromin and dermis suspension (Experiment II)*

Sensitizing agent	Guinea-pig Nos.	Time of readings													
		4 hours		1 day		2 days		5 days		10 days		16 days		21 days	
		Lep.[a]	D.[b]	Lep.	D.	Lep.	D.	Lep.	D.	Lep.	D.	Lep.	D.	Lep.	D.
None	43	8	8	4	4	4	4	4	4	3	0	0	0	0	0
	44	9	8	5	5	4	4	3	3	0	0	0	0	0	0
	45	7	7	5	4	5	5	3	3	0	0	0	0	0	0
	46	9	8	5	5	5	5	3	0	1	0	0	0	0	0
	47	10	8	5	5	5	5	3	2	3	2	1	0	0	0
	Means	8.6	7.8	4.8	4.6	4.6	4.6	3.2	2.4	1.4	0.4	0.2	0	0	0
Incomplete Freund's adjuvant	53	10	10	5	5	4	4	0	0	0	0	0	0	0	0
	54	9	9	5	4	4	4	2	3	0	0	0	9	0	0
	55	10	9	6	6	5	4	3	3	0	0	0	0	0	0
	57	9	8	4	5	4	4	3	2	0	0	0	0	0	0
	58	8	8	5	5	4	4	4	3	2	0	1	0	1	0
	Means	9.2	8.8	5.0	5.0	4.2	4.0	2.4	2.2	0.4	0	0.2	0	0.2	0
C. albicans	60	10	9	4	4	4	4	3	2	1	0	0	0	0	0
	61	12	11	6	6	5	4	3	4	0	0	0	0	0	0
	62	9	8	5	5	4	4	3	3	0	0	0	0	0	0
	63	10	11	6	5	5	5	4	4	0	0	0	0	0	0
	64	10	10	5	5	5	4	4	3	0	0	0	0	0	0
	Means	10.2	9.8	5.2	5.0	4.6	4.2	3.4	3.2	0.2	0	0	0	0	0

[a] Lepromin, full strength. Mm. induration (diameter).
[b] Dermis suspension, full strength. Mm. induration (diameter).

TABLE 2.—*Continued*

Sensitizing agent	Guinea-pig Nos.	Time of readings													
		4 hours		1 day		2 days		5 days		10 days		16 days		21 days	
		Lep.[a]	D.[b]	Lep.	D.	Lep.	D.	Lep.	D.	Lep.	D.	Lep.	D.	Lep.	D.
M. butyricum	69	6	5	4	3	4	3	6	0	5	0	7	0	7	0
	70	8	7	6	5	6	3	6	0	5	0	4	0	4	0
	71	10	8	6	5	5	3	5	0	5	0	6	0	6	0
	72	6	5	5	3	6	2	7	1	8	0	9	0	8	0
	74	5	6	6	4	7	4	7	1	7	0	7	0	7	0
	Means	7.0	6.2	5.4	4.0	5.6	3.0	6.2	0.4	6.0	0	6.6	0	6.4	0
BCG	76	9	9	6	6	5	4	7	1	8	0	7	0	7	0
	77	9	7	6	6	6	3	7	0	8	0	8	0	8	0
	79	8	7	6	5	7	3	6	0	9	0	8	0	6	0
	80	9	8	5	5	5	4	6	2	7	0	7	0	6	0
	81	5	7	5	7	4	3	5	1	6	0	8	0	8	0
	Means	8.0	7.6	5.6	5.8	5.4	3.4	6.2	0.8	7.6	0	7.6	0	7.0	0
M. kansasii	84	9	9	8	5	7	5	8	2	9	0	8	0	8	0
	85	7	8	5	6	6	4	7	2	9	0	8	0	9	0
	86	9	9	7	6	6	5	7	1	9	1	7	0	7	0
	88	7	7	5	4	6	3	7	0	9	0	10	0	9	0
	92	9	10	6	6	5	4	6	1	6	0	6	0	6	0
	Means	8.2	8.6	6.2	5.4	6.0	4.2	7.0	1.2	8.4	0.2	7.8	0	7.8	0

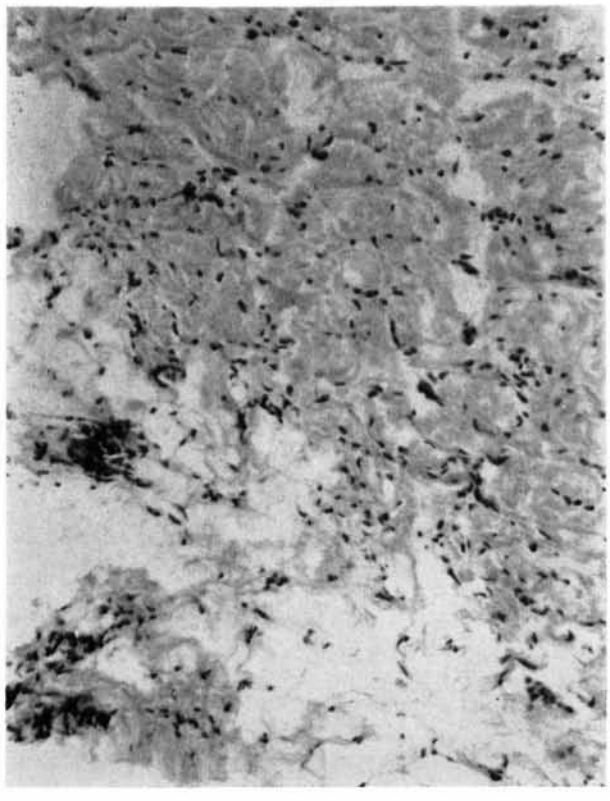

FIG. 5. Lepromin reaction in an animal sensitized with incomplete adjuvant alone; deep dermis and subcutaneous tissue. (Note lack of granulomatous inflammatory infiltrate.) Hematoxylin-eosin, approximately 40X.

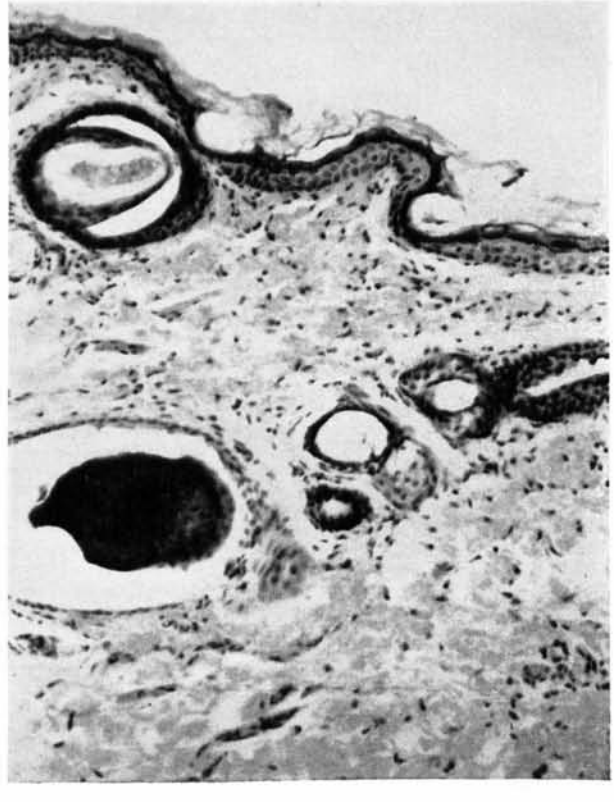

FIG. 6. Dermis suspension in an animal sensitized with *M. butyricum*. (The tissue is practically normal, with some increase of fibroblasts.) Hematoxylin-eosin, approximately 40X.

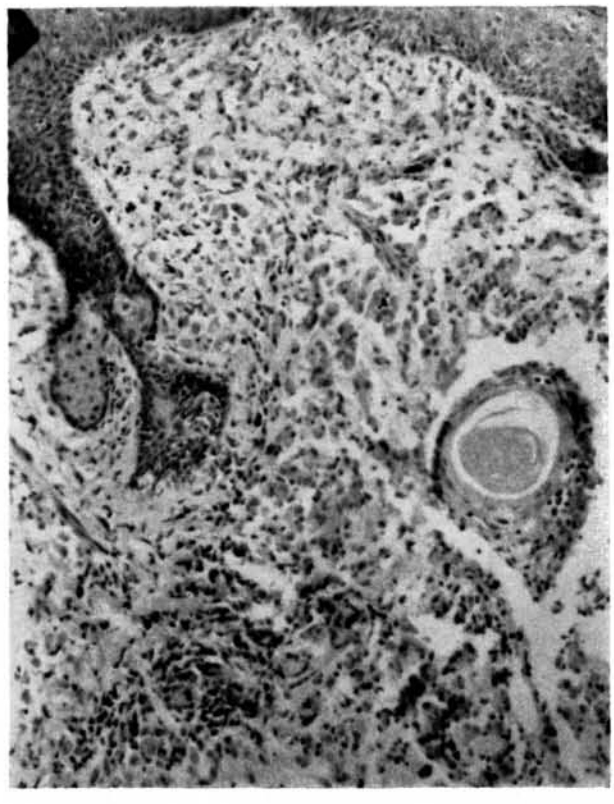

FIG. 7. Lepromin in an animal sensitized with *M. butyricum;* superficial level. (While the infiltrate is not massive, as in the deep dermis, there is a marked monocytic infiltrate.) Hematoxylin-eosin, approximately 40X.

this still showed the smallest reactions of any experimental group, although they were significantly higher (t test) than those in the control groups ($P < 0.001$ at 5, 10, 16 and 21 days). The results in all control groups were similar.

Histologic examination of test sites (Figs. 5-10) showed results comparable to those of the first experiment except that the granulomatous inflammatory infiltrate was heavy in all cases, starting deep in the subcutaneous tissue just above the muscular layer and extending into the superficial dermis.

DISCUSSION

In the guinea-pig there is cross reactivity between lepromin and various mycobacteria, as measured by the intradermal test. This cross reactivity depends upon the bacillary component of lepromin, and manifests itself by a granulomatous response that resembles accelerated tubercle formation. These results were not unexpected; Larson *et al.* [8, 9, 10] and Ribi *et al.* [16] have shown that mycobacterial cell walls may induce sensitivity to themselves and to the cell walls of a wide range of other mycobacteria, whereas sensitivity to protoplasmic fractions is more restricted in range and tends to parallel what is seen with purified tuberculins. The inflammatory response produced by protoplasmic constituents is of the tuberculin type, while the sensitivity to cell walls is more apparent in the formation of a granuloma. In the human population of certain regions of the United States infestation with *M. kansasii* and other mycobacteria is prevalent [2, 6, 14]; yet this cannot always be detected by the use of purified "*M. tuberculosis* tuberculin"; "purified tuberculins" prepared from cultures of appropriate mycobacteria are required. Since the lepromin test employs whole bacilli, positive granulomatous responses may occur whenever sensitization to any of a variety of other mycobacteria is present, and it is perfectly possible that a person with a negative test to "*M. tuberculosis* tuberculin" may nonetheless have been sensitized by other mycobacteria, and

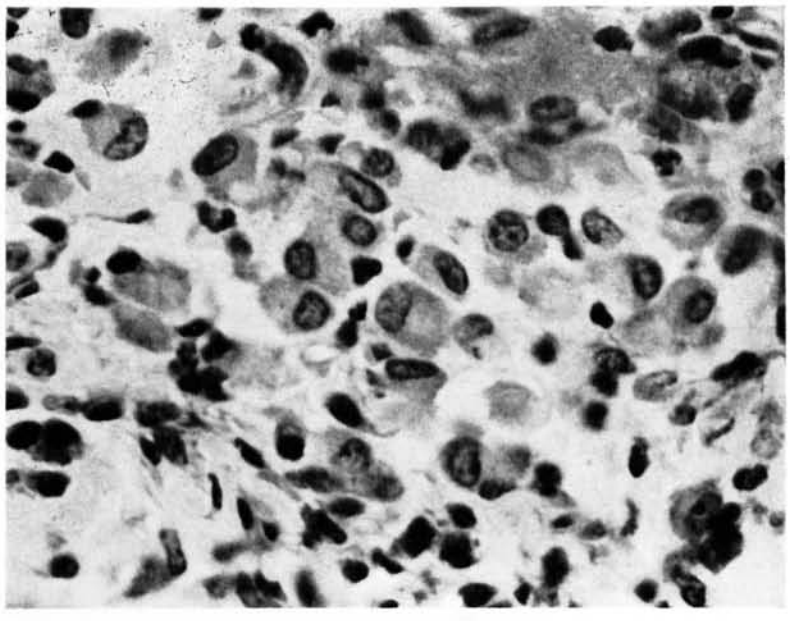

FIG. 8. Same as Figure 7, higher magnification. (Note the typical monocytic morphology of the cells, some of which are fused.) Hematoxylin-eosin, approximately 200X.

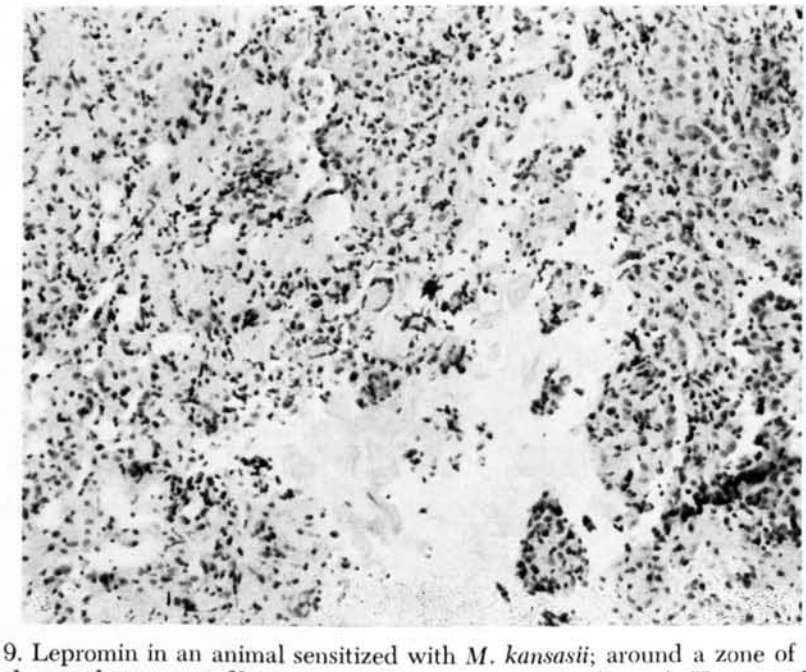

FIG. 9. Lepromin in an animal sensitized with *M. kansasii*; around a zone of necrosis. (Marked granulomatous infiltrate in which a giant cell is shown.) Hematoxylin-eosin, approximately 40X.

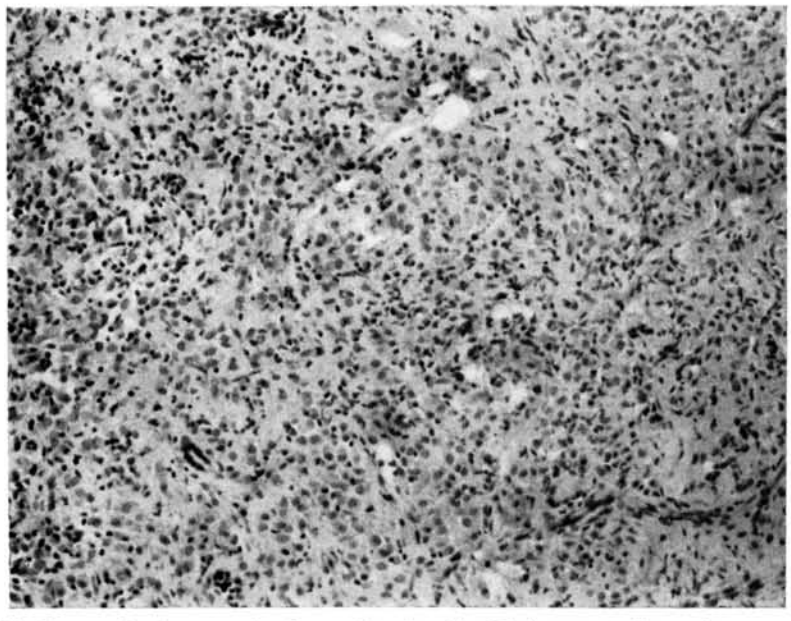

FIG. 10. Lepromin in an animal sensitized with *M. kansasii*, deep dermis. (Massive and solid granulomatous infiltrate, formed mainly by fused histiocytes.) Hematoxylin-eosin, approximately 40X.

could show a positive reaction to lepromin. Thus, the exposure of different populations to various mycobacteria may explain in part the differences in lepromin reactivity among different geographic groups.

Interesting recent studies ([15]) in guinea-pigs show that different mycobacteria can produce resistance to experimental tuberculosis, and that, while BCG is best for this purpose, other organisms, e.g., *M. kansasii*, are quite effective. In this experimental system, acquired resistance was found to have a ceiling, and combined immunizing infections were additive only up to that ceiling; i.e., in an animal immunized by *M. kansasii*, additional immunization with BCG provided very little extra protection. Whether an analogous situation exists in respect to the protective effect of BCG in leprosy is an interesting question. The fact that a mycobacterium may induce a sensitivity that can be detected by cross reactivity with lepromin does not necessarily mean that this mycobacterium is able to induce resistance to leprosy. But it may, and it is possible that the resistance-inducing capacity of the mycobacteria prevalent in various regions may help to explain geographic differences in incidence and clinical forms of leprosy as well as the observed value of BCG or other mycobacterial vaccinations.

We would suggest that field studies with tuberculin in connection with leprosy be preceded by studies of mycobacterial ecology by the use of specific tuberculins. Such studies might explain the observed incidence of lepromin positivity, and might also throw light on the putative immune status of a population.

SUMMARY

Groups of guinea-pigs were injected with different mycobacteria and *Candida albicans* emulsified in incomplete Freund's adjuvant, as well as with this adjuvant alone.

The animals sensitized to mycobacteria reacted to the intradermal injection of a purified lepromin with the formation of granulomata. This was a specific phenomenon, since no increased reaction to a suspension of human dermis was observed, and only animals that had received mycobacteria reacted in this fashion.

The chosen mycobacteria were representative of a wide spectrum within the genus and included potentially pathogenic and saprophytic species.

These results suggest that the positivity of the Mitsuda test in persons free of contact with leprosy and of infestation with *Mycobacterium tuberculosis* may be due to contact with any of a variety of mycobacteria. The interest of the possible relation between mycobacterial ecology and the prevalence of positive lepromin reactions and of leprosy and its clinical forms is emphasized.

RESUMEN

Grupos de cobayos fueron inyectados con diferentes micobacterias y *Candida albicans* emulsificada en un medio incompleto y complementario de Freund, como también con este medio complementario solo.

Los animales sensibilizados a las micobacterias reaccionaron a la inyección intradérmica de lepromina purificada con la formación de granulomas. Este fué un fenómeno específico, pues no se observó aumento en la reacción a una suspensión de piel humana, y solo los animales que recibieron micobacterias reaccionaron de esta manera.

Las micobacterias escogidas fueron representativas de una gran variedad dentro del genus e incluyó especies potencialmente patógenas y saprófitas.

Estos resultados sugieren que la positividad del test de Mitsuda en personas libres de contacto con lepra y de infección con *M. tuberculosis* puede ser debido al contacto con cualquier otro tipo de variedad de micobacterias. Se hace notar el interés de una posible relación entre ecología micobacteriana y la prevalencia de reacciones de lepromina postivas como también de lepra y de sus formas clínicas.

RÉSUMÉ

Des groupes de cobayes ont été injectés, soit avec différentes mycobactéries et avec *Candida albicans*, ces préparations ayant été émulsifiées dans de l'adjuvant incomplet de Freund, soit avec cet adjuvant seul.

Les animaux sensibilisés aux mycobactéries ont réagi à l'injection intradermique d'une lépromine purifiée, par la formation de granulomes. Ceci constituait un phénomène spécifique, car aucune augmentation de la réaction n'a été observée avec une suspension de derme humain, et seuls les animaux auxquels des mycobactéries avaient été administrées, ont réagi de cette manière.

Les mycobactéries sélectionnées étaient représentatives d'un large spectre dans ce genre, et comprenaient des espèces potentiellement pathogénes et des espèces saprophytes.

Ces résultats suggèrent que la positivité de l'épreuve de Mitsuda chez des personnes n'ayant pas eu de contact avec la lèpre, et n'étant pas non plus infectées par *M. tuberculosis*, peut être due au contact avec n'importe laquelle parmi de nombreuses mycobactéries. On souligne l'intérêt d'une relation possible entre l'écologie mycobactérienne et la prévalence des réactions positives à la lépromine, de la lèpre et de ses différentes formes cliniques.

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REFERENCES

1. Azulay, R. and Convit, J. The Mitsuda test in non-leprous persons in a non-endemic country. Internat. J. Leprosy **15** (1947) 264-266.
2. Carpenter, R. L., Patnode, R. A. and Goldsmith, J. B. Comparative study of skin-test reactions to various mycobacterial antigens in Choctaw County, Oklahoma. American Rev. Resp. Dis. **95** (1967) 6-11.
3. Chambon, L., Destombes, C. and Nette, R. Etude de l'allergie a la lépromine chez des lépreux vaccinés par B.C.G. Bull. Soc. Path. exot. **50** (1957) 631-633.
4. Convit, J. An investigation of leprosy in the German ethnic group of Colonia Tovar in Venezuela. IV. Clinical findings and variations in the Mantoux and Mitsuda reactions observed during five years after BCG vaccination of Mitsuda-negative contacts. Internat. J. Leprosy **24** (1956) 38-44.

5. Hanks, J. H., Chatterjee, B. R. and Lechat, M. F. A guide to the counting of mycobacteria in clinical and experimental materials. Internat. J. Leprosy **32** (1964) 156-167.

6. Klare, K. C., Abeles, H., Aronsohn, M. H., Chaves, A. D., Glass, R. and Robins, A. B. The prevalence of atypical mycobacterial tuberculin sensitivity in a selected population in New York City. American Rev. Resp. Dis. **95** (1967) 103-106.

7. Kooij, R. and Rutgers, A. W. F. Leprosy and tuberculosis. A comparative study with the aid of skin tests with tuberculin, killed BCG, and the Dharmendra lepromin in South African Bantus. Internat. J. Leprosy **26** (1958) 24-40.

8. Larson, C. L., Ribi, E., Wicht, W. C. and List, R. Skin reactions produced in rabbits by cell walls and protoplasm of *Mycobacterium tuberculosis* and *M. butyricum*. American Rev. Resp. Dis. **83** (1961) 184-193.

9. Larson, C. L., Bell, J. F., List, R. H., Ribi, E. and Wicht, W. C. Symposium on relations of structure of microorganisms to their immunobiological properties. II. Host-reactive properties of cell walls and protoplasm of mycobacteria. Bact. Rev. **27** (1963) 341-351.

10. Larson, C. L. and Ribi, E. Mycobacterial cellular antigen fractions. American Rev. Resp. Dis. **92** (1965) 49-53. (Part 2)

11. Leiker, D. L. Studies on the lepromin test. III. Influence of tuberculosis contact and other factors on the size of the lepromin reaction. Internat. J. Leprosy **29** (1961) 488-495.

12. Martins, A. B. Personal communication.

13. Montestruc, E. and Despierres, G. La réaction de Mitsuda et le rôle du BCG dans la positivité de cette réaction en milieu indemme de lèpre. Bull. Soc. Path. exot. **53** (1960) 630-634.

14. Palmer, C. E. and Edwards, L. B. Sensitivity to mycobacterial PPD antigens with some laboratory evidence of its significance. Tuberkuloza **18** (1966) 193-200.

15. Palmer, C. E. and Long, M. W. Effects of infections with atypical mycobacteria on BCG vaccination and tuberculosis. American Rev. Resp. Dis. **94** (1966) 553-568.

16. Ribi, E., Larson, C. L., List, R. and Wicht, W. C. Immunologic significance of the cell wall of mycobacteria. Proc. Soc. Exper. Biol. & Med. **98** (1958) 263-265.