J. Pharmacol. Exptl. Therap. **127**, 182 (1959).

13. Cohen, B. and Smith, A. H., J. Biol. Chem. **39**, 489 (1919).

14. Michie, D. D., Goldsmith, R. S., Mason, A. D., and Moncrief, J. A., J. Trauma **3**, 111 (1963).

15. Moncrief, J. A., Ann. Surg. **164**, 723 (1966).

16. Aviado, D. M. Pharmacology of the Bronchial Circulation in Pharmacology of the Lung, Part 2, Ed. D. M. Aviado from Proceedings of the First International Pharmacological Meeting, General Editor B. Uvnäs. Macmillan, New York (1963).

17. Woodbury, R. A. and Hamilton, W. F., J. Pharmacol. Exptl. Therap. **71**, 293 (1941).

18. Luisada, A. A., Liu, C. K., Jona, E., and Polli, J. F., Angiology **6**, 503 (1955).

19. Feeley, J. W., Lee, T. D., and Milnor, W. R., Am. J. Physiol. **205**, 1193 (1963).

20. Moon, V. H. and Morgan, D. R., Proc. Soc. Exptl. Biol. Med. **33**, 560 (1936).

21. Di Mattei, P., Arch. Intern. Pharmacodyn. **140**, 368 (1962).

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Passive Transfer of Hypersensitivity to Lepromin (33562)

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Reactions to lepromin are generally conceded to have importance as an aid to clinical judgment, but there is question about which main component of this complex "antigen," bacilli or dermal tissue, is responsible for the granulomatous inflammatory response (1–3). It is also uncertain whether this phenomenon is immunological in nature, and if so, to which of the recognized types of hypersensitivity it belongs.

In respect to the first question, we had earlier found (4) the bacillary component of a purified lepromin (see below) to be mainly responsible for the tissue response observed following its injection into the foot pads of normal guinea pigs. This was marked by a continuing hypertrophy of the draining lymph node, and increasing numbers of contained pyroninophilic cells. In regard to the second question, it was observed that in guinea pigs sensitized to any of a variety of mycobacteria, the intradermal injection of test lepromin produced granulomas which were grossly and histologically markedly different from reactions observed in control animals, or in the sensitized ones injected with dermis suspension (5, 6). These responses suggested that immunologically specific sensitivity had been induced to lepromin by the mycobacteria. We wished to confirm this point further and to determine the nature of this sensitivity. The work reported here describes efforts to find whether specific hypersensitivity is induced by purified lepromin, and if so, whether this is transferable to normal animals by serum or lymphoid cells.

Methods. For work with lepromin in animals it was requisite to eliminate, as far as possible, the antigenic and phlogistic properties of heated human tissue which is a constituent of this test substance. A purified lepromin (i.e., a suspension of *M. leprae*) was prepared by sequentially digesting heated, ground, epidermis-free lepromata with DNAase, RNAase, and Pronase. This was done in barbitone–barbitone sodium buffer, pH 7.4, with adjustment to the optimal pH of the enzyme being used with NaOH or HCl, and the addition of $CaCl_2$, and $MgCl_2$ as required. Residual particulate matter was washed with phosphate buffered saline, pH 7.4, with 0.05% Tween-80 (PBS-T) and recovered by filtration through Millipore membranes. This consisted essentially of bacillary bodies, as shown by the Ziehl–Neelsen, Wright–Giemsa and acid orcein stains. The final suspension was prepared in phosphate buffered saline, pH 7.4, with 0.5% phenol and 0.05% Tween-80 added.

Two concentrations were prepared: "strong lepromin" and "test lepromin," containing approximately 430×10^6 and 140×10^6

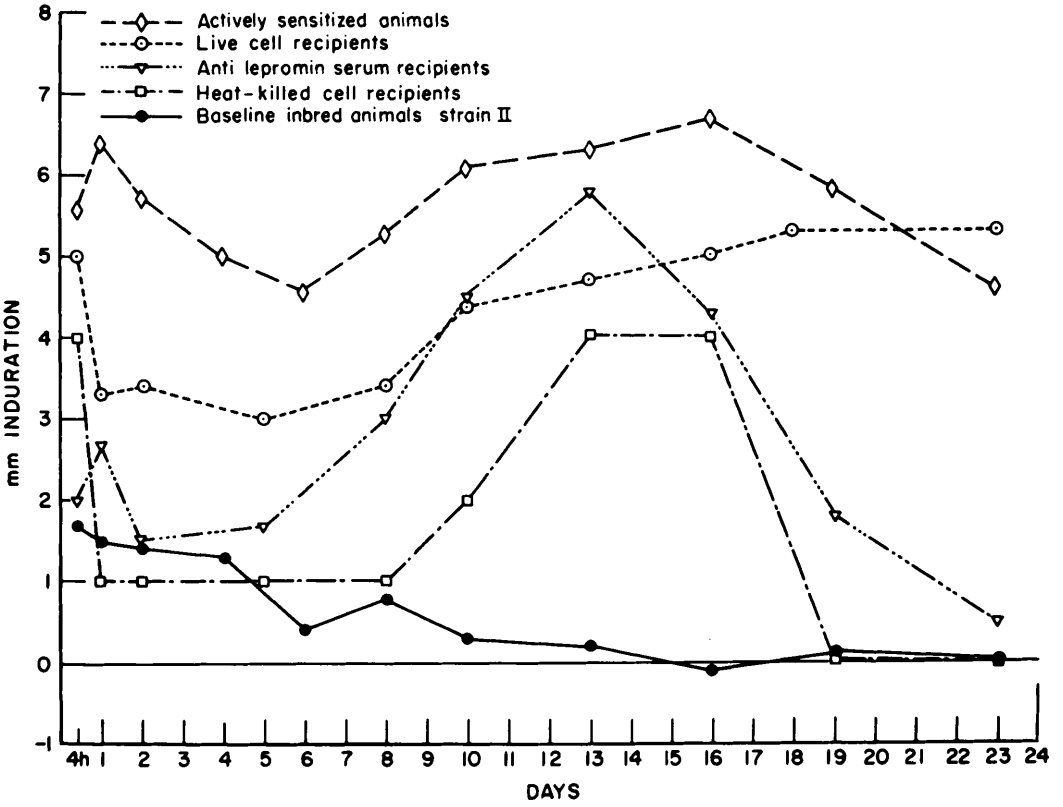

FIG. 1. Reactions to "test" lepromin, corrected readings (Expt. 1). Values shown are the arithmetic means of the difference between diameter of induration (mm) of the site injected with test lepromin and the site injected with equivalent dermis suspension.

bacilli/ml, respectively. Suspensions of normal human dermis were prepared in exactly the same fashion.

Preliminary to these experiments we had determined that pronase-treated heat-killed suspensions of BCG remained acid fast, and successfully induced tuberculin hypersensitivity in guinea pigs. Further, Pronase at a 1:100 enzyme:substrate concentration digested about 70% of the proteins of ground heated human dermis in 48 hr, as determined by Satake's method (7). In this procedure the concentrations of amino acids and peptides in the supernatant of 10% cold TCA precipitates of Pronase-treated dermal digests are compared with those found after digestion with 6 *N* HCl for 24 hr at 110° *in vacuo* (8).

"Test" lepromin behaved like whole (crude) lepromin in several trials in patients with lepromatous and tuberculoid leprosy and in clinically healthy relatives of these patients, as well as in BCG vaccinated individuals.

Experimental. Methods. Two transfer experiments were performed. In the first, 10 guinea pigs of inbred strain 2 were sensitized to lepromin as follows: "strong" lepromin emulsified in incomplete Freund's adjuvant was injected into the four foot pads and subcutaneously in the neck for a total of 0.5 ml of lepromin/animal. Four weeks later the animals received two intradermal injections of 0.1 ml each of "test" lepromin. After 4 weeks, these animals were tested by injecting intradermally 0.1 ml each of "test" lepromin, "test" lepromin 1:10 and equivalent human dermis suspension. At the same time these tests were made in 10 control animals of the same strain and of similar weight

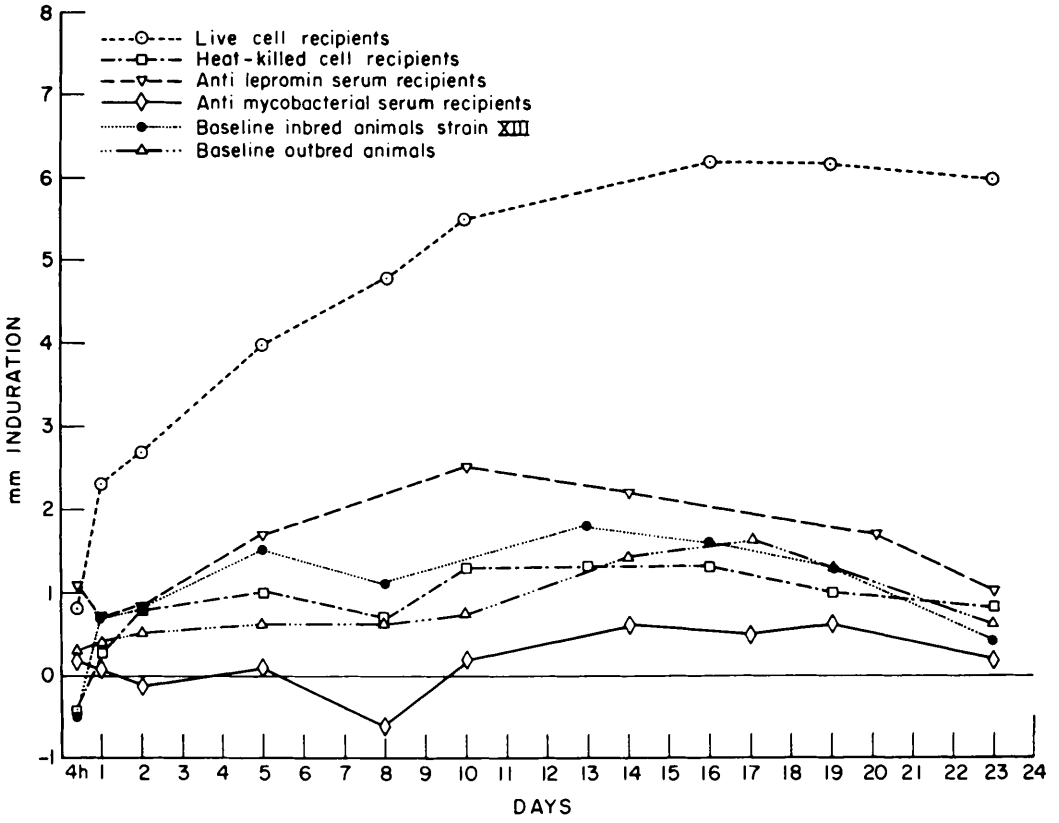

FIG. 2. Reactions to "test" lepromin corrected readings (Expt. 2) (see legend of Fig. 1).

which had received incomplete Freund's adjuvant in buffered diluent, or intradermal diluent alone, at the times when the experimental animals were receiving lepromin.

Direct and "corrected" readings were recorded. The latter represent the difference between diameters of induration in mm, of sites injected with equivalent concentrations of lepromin and of dermis suspension (Fig. 1).

The existence of sensitivity to the bacillary component of lepromin in the sensitized animals was evidenced by increased inflammatory responses which led to granuloma formation by 23 days after injection (Fig. 1). On the other hand there was no difference between the experimental and control groups in the sites tested with dermis suspension. Five weeks after the previous test, the experimental group was boosted by two simultaneous intradermal injections of 0.1 ml each of test lepromin. Twenty-one days later these animals were bled, and spleens, lymph nodes, and mineral oil-induced peritoneal exudate cells were harvested and washed three times. The peritoneal cells were injected intravenously and the spleen and lymph node cells intraperitoneally into three isologous recipients in a donor: recipient ratio of 2.5:1. A fourth recipient was given the cell suspension treated by heat. Four other guinea pigs received 10 ml each of serum in divided doses intraperitoneally; two animals each had 5 ml coinciding with the intradermal injections of antigens and 5 ml the next day. The other two received 2 ml at the time of intradermal injections and 1 ml every 72 hr. The recipients were tested for reactivity with the same antigens as had been used in the donors.

Figure 1 shows the results of corrected readings in actively sensitized and recipient animals. It is clear that sensitivity conducive to granuloma formation and directed against the bacillary component of lepromin was

transmitted by live cells. Heat killed cells and serum (given by either schedule) did not transfer this, but there was an indication of an increase in earlier inflammatory reactions in these recipients.

The second experiment was carried out to extend these results. Seventeen guinea pigs of inbred strain 13 were sensitized along with 11 outbred albino animals by a scheme identical to the one used in the previous experiment except that they were given an additional booster consisting of two simultaneous intradermal injections of 0.1 ml each of "test" lepromin. Control animals for levels of reactivity were provided by 10 strain 13 and 10 outbred normal albino guinea pigs.

Serum was obtained from outbred and inbred sensitized animals and administered intraperitoneally to six normal outbred recipients. Each animal received a total of 30 ml distributed as follows: 3 ml the day before injection of test antigens, and then 3 ml three times weekly for 3 weeks. Pooled antimycobacterial guinea pig serum (anti-BCG, anti-*M. kansasii*, and anti-*M. butyricum*) with a Middlebrook-Dubos hemagglutinin titer in excess of 1:1024 was given to nine additional outbred guinea pigs in the same amounts and intervals as above.

Heat-killed cells were provided by the sensitized outbred animals and given to four albino outbred receptors in a donor recipient ratio of 3:1. Live cells were provided by the sensitized inbred animals and injected into five isologous receptors (one died shortly after injection).

The results summarized in Fig. 2 confirm the findings of the first experiment and establish the fact that heat-killed cells and serum failed to transfer the capacity to react with granulomas to lepromin.

Conclusions. These experiments indicate that the capacity to form a granulomatous response to lepromin can be induced in guinea pigs as a reaction of hypersensitivity directed toward *M. leprae*, and that this reactivity is transferable by viable lymphoid cells but not by heat-killed cells or by serum. On this basis, this responsiveness qualifies for inclusion in the category of the delayed hypersensitive states.

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1. Beasley, W. B. R., Trans. Roy. Soc. Trop. Med. Hyg. **54**, 459 (1960).

2. Kooij, R. and Gerritsen, T. H., Intern. J. Leprosy **24**, 171 (1956).

3. Leiker, D. L., Intern. J. Leprosy **29**, 157 (1961).

4. Goihman-Yahr, M., Raffel, S., and Ferraresi, R. W., J. Invest. Dermatol. **51**, 19 (1968).

5. Goihman-Yahr, M., Raffel, S., and Ferraresi, R. W., Bacteriol. Proc. 97, (1968.)

6. Goihman-Yahr, M., Raffel, S., and Ferraresi, R. W., Intern. J. Leprosy **36**, 129 (1968).

7. Satake, K., Okuyama, T., Ohashi, M., and Shinoda, T., J. Biochem. (Tokyo) **47**, 654 (1960).

8. Hirs, C. H. W., Stein, W. H., and Moore, S., J. Biol. Chem. **211**, 941 (1954).

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