Comparison of Reactions to Human and Armadillo Lepromins in Leprosy [1, 2, 4, 5]

W. M. Meyers, S. Kvernes and C. H. Binford [3]

The ancient chronic disease of leprosy is still a major health problem in many endemic areas. The World Health Organization estimates that there are more than ten million leprosy patients in the world today. While there have been remarkable advances in the basic understanding of leprosy in the past two decades, there remain several significant obstructions to progress in medical management and epidemiology. One of these barriers is the lack of adequate supplies of a standardized reagent (lepromin) for skin testing. *Mycobacterium leprae*, the causative agent of leprosy, has not been cultured *in vitro* and infected human tissue remains the sole source of lepromin. Only a small percentage of leprosy treatment centers utilize the lepromin reaction routinely, and in many of these centers the antigenic strength of the lepromin employed varies widely from acceptable standards.

The classic lepromin reaction, first studied by Hayashi (10) and Mitsuda (15), is the cutaneous response to the intradermal injection of autoclaved suspensions of human tissue naturally infected with *M. leprae*.[6] This suspension contains whole *M. leprae* and variable amounts of soluble tissues and bacillary extract and debris. The lepromin reaction has two components: the early, or Fernandez (6) reaction (48 hours), and the late or Mitsuda reaction (four weeks). Early reactions are equated generally with a pre-existing delayed hypersensitivity to *M. leprae* and late reactions to either a pre-existing or a stimulated delayed hypersensitivity to injected antigen. Due to a marked variability in the Fernandez reaction and poor correlation with clinical forms of leprosy, most leprologists are reluctant to attach specific clinical significance to this response. An important exception to this is the reaction to Dharmendra's lepromin (4), a purified chloroform-ether-extracted suspension of *M. leprae*, which regularly produces only the 48-hour reaction. Such reactions generally carry the same significance as the Mitsuda reaction to integral (Mitsuda-Hayashi-Wade) lepromin. The Mitsuda reaction is uniformly recognized to have particular usefulness in the clinical classification of leprosy patients and hence is of substantial prognostic value. Considerable epidemiologic data have also been derived from studies of the lepromin reaction in whole populations and contacts.

There is growing evidence that the clinical spectrum of leprosy correlates well with the level of cell-mediated immunity (CMI) to *M. leprae* (2, 19). Thus lepromatous patients demonstrate a marked depression of CMI while tuberculoid patients react strongly, and borderline patients are intermediate. Since Mitsuda reactions are probably a clinical manifestation of CMI (7), they correlate closely with the clinical forms of leprosy: lepromatous patients give no or only weak reactions; tuberculoid patients, strong reac-

[1] Received for publication 27 January 1975.

[2] Supported by American Leprosy Missions, Inc., 297 Park Ave. South, New York, N.Y. 10010, and by Grant AI-11-620-01 from the National Institutes of Allergy and Infectious Diseases, Bethesda, Maryland 20014.

[3] W. M. Meyers, M.D., Ph.D., Professor of Pathology, ALM Leprosy Atelier, Department of Pathology, University of Hawaii School of Medicine, Honolulu, Hawaii 96816; S. Kvernes, M.D., Director, Kivuvu Leprosarium, Institut Médical Evangélique, Kimpese, Republic of Zaire; C. H. Binford, M.D., Chief, Special Mycobacterial Diseases Branch, Armed Forces Institute of Pathology, Washington, D.C. 20306. Reprint requests should be addressed to Dr. Meyers, Dept. of Infectious and Parasitic Disease Pathology, Armed Forces Institute of Pathology, Washington, D.C. 20306.

[4] The opinions or assertions contained herein are the private views of the authors and are not to be construed as official or as reflecting the views of the Department of the Army or the Department of Defense.

[5] In conducting the research described in this report, the investigators adhered to the "Guide for Laboratory Animal Facilities and Care," as promulgated by the committee on the Guide for Laboratory Animal Facilities and Care of the Institute of Laboratory Animal Resources, National Academy of Sciences-National Research Council.

[6] A standard nomenclature for lepromins of various origins has not been formulated. In this paper we refer to the two lepromins we employed as follows: lepromin derived from infected human tissue as lepromin-H; and lepromin derived from infected armadillo tissue as lepromin-A.

tions; and borderline patients, intermediate reactions. A precise immunologic classification of each leprosy patient is perhaps the most important concern of the clinician in developing a rational program of treatment for the individual patient.

Lepromins prepared from *M. leprae* infected mouse ([5]) and chipmunk ([14]) tissues produce reactions in leprosy patients comparable to those obtained with lepromin-H. However, adequate supplies of such tissues for the manufacture of large quantities of lepromin for general clinical use are not expected to be forthcoming. The development of *M. leprae* infections in armadillos ([11, 12]) has made available for the first time large volumes of infected tissues for lepromin production. We report here the results of our study of reactions to lepromin-A and lepromin-H in leprosy patients, performed primarily to assess the usefulness of lepromin-A as a substitute for lepromin-H.

MATERIALS AND METHODS

Leprosy patients. Between November 1973 and October 1974 we lepromin-tested 115 leprosy patients registered at Kivuvu, the leprosy care facility of the Institut Médical Evangélique at Kimpese, Republic of Zaire. All patients were Bantu: 113 of the Kongo tribal group and 2 Mbundu. Fifty-three were Zairian and 62 Angolan. Age and sex distributions are shown in Table 1. Where age could not be established, estimates were made to the nearest five years. Admission diagnoses and classification of leprosy patients (Table 2) were based on the criteria summarized by Ridley and Jopling ([17]). These criteria are: clinical features, bacteriologic density of skin smears, lepromin reaction (lepromin-H), and histologic features. We employed the designations of Ridley and Jopling in classifying patients: LL, lepromatous; BL, borderline-lepromatous; BB, borderline; BT, borderline-tuberculoid; and TT, tuberculoid. Fifty-three patients had never received antileprosy therapy, sixty had received sulfone therapy for one month to nine years, and one had been on clofazimine for one month. One patient was classified as healed and had been off therapy for 15 years. Five patients (four LL and one BB) were receiving prednisolone on an alternate day program during the lepromin testing. Thirty-nine patients had had one previous lepromin test (lepromin-H) six months to eight years prior to the tests reported here. We are uncertain of the BCG vaccination history of each patient. None of the patients had clinical evidence or a known history of tuberculosis or other mycobacterial disease except for leprosy.

TABLE 1. *Age and sex distribution of leprosy patients.*

Age (years)[a]	Male	Female
11-14	2	1
15-24	4	7
25-34	11	8
35-44	20	18
45-54	15	7
55-64	9	7
65-70	2	4
Total	63	52

[a]Average age 40.1 years. Age range 11 to 70 years.

TABLE 2. *Distribution of patients by class of leprosy.*

Class[a]	No. patients	Percent
LL	39	34
BL	8	7
BB	21	18
BT	19	17
TT	28	24
Total	115	100

[a]See text for class notations.

The armadillo is not native to Zaire or Angola and none of the patients studied had had contact with armadillos or products of the armadillo prior to this study.

Preparation of lepromins. Lepromin-H was prepared from pooled lepromas excised from three untreated leprosy patients recently admitted to Kivuvu. Lepromin-A was produced from heavily infected tissue of a nine-banded armadillo *(Dasypus novemcinctus)* which had been injected with an homogenate of human lepromas from a patient in Surinam. The human lepromas used to prepare the inoculum for this armadillo were provided by Dr. S. J. Bueno de Mesquita, Paramaribo, Surinam. The infected armadillo tissue (Animal #L-5), and normal armadillo tissues were kindly supplied by Dr. G. P. Walsh and Dr. E. E. Storrs of Gulf South Research Institute, New Iberia, Louisiana.

We employed the Mitsuda-Hayashi-Wade technic ([18]) to prepare both lepromins. Phenol (0.5%) was used as a preservative and both preparations were stored at 0-4°C throughout the study. Acid-fast bacillary counts were kindly performed by Dr. John Hanks, John Hopkins University School of Medicine, Baltimore. The relative proportions of soluble protein of tissue and bacterial origin in this preparation are not known.

A control normal armadillo extract was prepared from autoclaved homogenates or subcutaneous tissue and muscle and preserved with phenol (0.5%).

Lepromin testing. The lepromin-H used throughout the study contained 175 million bacilli/ml. This is slightly stronger than "standard" lepromin (160 million bacilli/ml) recommended at the Eighth International Congress of Leprology (Rio de Janeiro, 1963). The initial 22 patients were tested with lepromin-A containing 220 million bacilli/ml; for the remaining 93 patients this suspension was adjusted to 175 million bacilli/ml. The human and armadillo preparations contained, respectively, 11% and 6% solid staining bacilli, which is assumed to be a measure of viable *M. leprae* in each original tissue. One-tenth ml of lepromin-H was injected intradermally on the flexor surface of the right forearm and an equal volume of lepromin-A was injected intradermally at the same sitting at the corresponding site of the left forearm. Since only rarely was more than one patient tested on the same day, the same syringe, filled to the same level, was used almost exclusively for each reagent. Lepromin ampoules were well shaken before filling the syringes and injections were made immediately. These precautions were observed to minimize dosage variations ([8]). The Fernandez reaction was read at 48 hours, and the Mitsuda reaction at 28 days, and recorded as the width of induration at the injected site measured at 90° to the long axis of the forearm. We obtained biopsy specimens (7 mm) of both the lepromin-H and lepromin-A reactions at 28 days from 42 patients and evaluated hematoxylin-eosin and Fite-Faraco stained sections. The test site in lepromatous patients, frequently inapparent at 28 days, was identified by the intradermal injection of minute amounts of sterile India ink one cm proximal to the point of lepromin injection. When normal armadillo tissue extract was employed, the injections were made in the same manner as those for the lepromins at a point about five cm proximal to the lepromin-A test site. In all cases where a patient was to receive a repeat injection with either lepromin-A or armadillo tissue extract, or any sequential combination of these reagents, a prior intradermal test injection of 0.1 ml of a 1/100 dilution of armadillo extract was administered and the patient observed for at least one hour.

RESULTS

In this series of leprosy patients representing the complete spectrum of established clinical forms of the disease, we noted the classic response to both lepromin-H and lepromin-A (Table 3), i.e., the intensity of the Mitsuda reactions progressed from a minimal response in LL patients to maximal levels in TT patients. In patients grouped according to class of leprosy, lepromin-A consistently provoked a more intense Mitsuda response than lepromin-H. The differences are significant for all categories of patients except the small group of BL pa-

TABLE 3. *Comparison of Mitsuda reactions to lepromin-H and lepromin-A.*

Class	Lepromin-H			Lepromin-A			Diff. of means[a]	Sig.[b]
	Patients	Mean[a]	S.D.	Patients	Mean[a]	S.D.		
All patients	112	5.8	4.89	111	8.2	6.12	2.4	<.005
LL	39	1.5	1.93	38	2.6	2.19	1.1	<.005
BL	7	4.1	1.24	7	4.7	2.18	0.6	NS
BB	20	5.3	2.45	20	7.6	3.40	2.3	<.025
BT	18	8.4	2.77	18	11.3	3.72	2.9	<.05
TT	28	11.0	4.87	28	15.1	5.10	4.1	<.005

[a] Millimeters of induration.
[b] t-test (two-tailed).

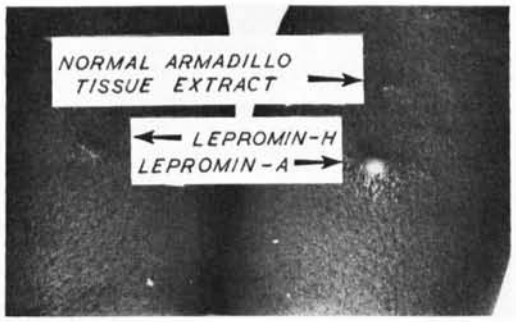


FIG. 1. Skin reactions, 28 days postintradermal injection to lepromin-H, lepromin-A and normal armadillo tissue extract in a tuberculoid leprosy patient, class TT. These reactions measured as follows: lepromin-H, 10 mm; lepromin-A, 17 mm; armadillo extract, 5 mm. AFIP 75-4760-4.

tients tested. Figure 1 demonstrates the Mitsuda reactions to both lepromin-H and lepromin-A and a 28-day reaction to armadillo tissue extract in a TT patient.

Although of doubtful practical usefulness, we have tabulated the results of the Fernandez reactions in Table 4. Reactions to lepromin-A again were consistently larger than those to the lepromin-H. In grouped patients these differences were significant in all but the BL and TT patients.

Reactions to the original lepromin-A (220 million bacilli/ml) and the diluted (175 million bacilli/ml) preparations are compared in Table 5. Lepromin-A produced stronger Fernandez and Mitsuda reactions than lep-

TABLE 4. *Comparison of Fernandez reactions to lepromin-H and lepromin-A.*

Class	Lepromin-H			Lepromin-A			Diff. of means[a]	Sig.[b]
	Patients	Mean[a]	S.D.	Patients	Mean[a]	S.D.		
All patients	112	4.7	2.94	109	6.6	2.78	1.9	<.001
LL	39	3.6	2.24	38	5.7	1.92	2.1	<.001
BL	7	5.4	2.38	6	6.7	1.60	1.3	NS
BB	20	4.6	2.25	20	6.2	2.22	1.6	<.05
BT	18	4.8	2.48	17	6.5	1.45	1.7	<.025
TT	28	6.0	3.63	28	7.1	2.91	1.1	NS

[a] Millimeters of induration.
[b] t-test (two-tailed).

TABLE 5. *Comparison of reactions to a standard lepromin-H (175×10^6 bacilli/ml) and two concentrations of lepromin-A.*

FERNANDEZ REACTION								
Lepromin-H				Lepromin-A				
Patients	Mean[a]	S.D.	Bacillary concentration lepromin-A	Patients	Mean[a]	S.D.	Diff. of means[a]	Sig.[b]
22	5.3	2.96	220×10^6/ml	22	8.0	2.95	2.7	<.01
90	4.5	2.85	175×10^6/ml	87	6.2	2.61	1.7	<.001
MITSUDA REACTION								
22	7.2	5.7	220×10^6/ml	22	11.0	6.36	3.8	NS
90	5.5	4.6	175×10^6/ml	89	7.5	5.83	2.0	<.025

[a] Millimeters of induration.
[b] t-test (two-tailed).

romin-H at both concentrations of lepromin-A; however, the differences are less at equal bacillary concentrations. This stresses the importance of a strict control of bacillary counts of each lepromin preparation employed.

Table 6 gives individual responses to normal armadillo extract compared with lepromin-A in 35 patients of the different classes of leprosy. The results of this series suggest that normal armadillo tissue components participate in the reaction to lepromin-A. This is particularly true in the BT and TT patients. There is no convincing evidence that previous experience with lepromin-A regularly sensitized leprosy patients to armadillo tissue. However, seven of the eight patients who had previous contact with lepromin-A had borderline or lepromatous leprosy and may have been less capable of

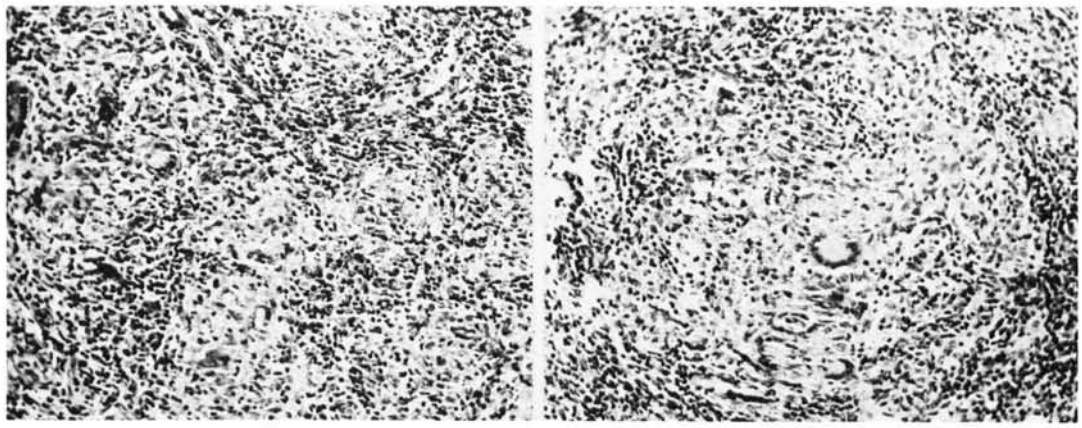
TABLE 6. *Comparison of reactions to lepromin-A and normal armadillo tissue extract in individual leprosy patients.*

Class	Lepromin-A[a]		Normal armadillo extract[a]	
	48 hours	28 days	48 hours	28 days
LL[c]	8	5	—	0
	7	2	10	0
	5	5	0	0
	7	6	3	3
	7	0	4	0
	5	0	4	0
	6	3	3	0
	5	0	0	0
	4[b]	5[b]	6	0
	7[b]	4[b]	3	0
BL	8	—	4	—
	4[b]	7[b]	7	0
	6[b]	5[b]	3	0
BB	8[b]	7[b]	4	1
	7	5	—	0
	4	6	7	3
	8	3	8	0
	4[b]	8[b]	4	3
	8[b]	7[b]	7	0
BT	6	20	5	14
	5	11	2	4
	8	—	5	—
	6	14	0	7
	4	8	2	5
	6	13	3	6
	6	11	2	6
	10	10	1	5
	9	15	—	8
TT	18	28	8	15
	0	8	—	10
	6	9	—	0
	8	17	—	5
	6	13	3	6
	4	19	0	13
	6[b]	9[b]	5	0

[a] All reactions in millimeters.

[b] Values from a previous lepromin-A test. All other patients were injected with lepromin-A and normal armadillo extract simultaneously.

[c] Of the ten LL patients, four show Mitsuda reactions of 5 or 6 mm. These reactions were 2 to 3 mm to lepromin-H and all were long-standing lepromatous patients who may be showing an upgrading of their immunologic status. Thus the initial diagnosis of LL leprosy in these patients does not conflict with these data.



A B

FIG. 2. Comparison of tissue reactions at 28 days to lepromin-H (A), and lepromin-A (B), in a tuberculoid leprosy patient, class TT. Reactions are similar and are characterized by foci of epithelioid cells with a few giant cells surrounded by lymphocytes. H & E stain, X165. AFIP (A) 74-6321 and (B) 74-6319.

developing a sensitivity than patients with higher resistant forms of leprosy. The one TT patient who had a repeat injection of armadillo tissue, did not react.

In another series of 36 patients of all classes of leprosy, we injected a second dose of both lepromin-H and lepromin-A six to eight weeks after the initial test with the same reagents. There were no significant differences in the early or late readings to lepromin-H or lepromin-A in the repeat test. This series provides additional evidence that the armadillo tissue component of lepromin-A probably has a low sensitizing potential when used in this way. One LL patient, however, was eliminated from the study because a wheal developed at the site of the injection of a 1/100 dilution of normal armadillo extract within 30 minutes. There was no systemic reaction in this patient.

Histologic evaluations of the biopsy specimens of Mitsuda reactions to lepromin-H and lepromin-A, over the range of the clinical forms of leprosy, revealed similar cellular responses in each instance. Figure 2 shows the similarity of the histopathologic features of reactions to lepromin-H and lepromin-A in a TT patient. One late reaction to armadillo tissue extract was biopsied. This specimen was from a TT patient and showed an intense giant cell, epithelioid cell and lymphocytic response, quite similar to those in Figure 2.

DISCUSSION

The clinical importance of the lepromin reaction must be emphasized. The worldwide distribution of standardized lepromin would permit clinicians everywhere to assess the status of CMI in their patients and plan a regimen of optimal therapy and management. The results of clinical drug trials and "immunization" attempts (e.g., BCG vaccination) in different geographic areas and different ethnic groups may be more meaningful with the added information obtained from reactions to a standardized lepromin. A single infected armadillo can probably supply enough *M. leprae* for 15 million doses of lepromin. Thus there is every expectation that a standardized lepromin-A can be supplied in amounts adequate for world need. There are, however, many factors which must be investigated before lepromin-A can be introduced for general use. In fact, lepromin-H has not yet been satisfactorily standardized, nor has the optimal dosage been determined (9).

The two most urgent needs for the development of a suitable lepromin-A are: 1) removal of as much soluble armadillo material and tissue debris as possible, and 2) to determine the most stable method of storage. Our studies suggest that armadillo tissue in crude Mitsuda-Hayashi-Wade lepromin-A may contribute to an enhancement of the lepromin reaction and hence diminish the specificity of the reagent. This is not unexpected since "positive" skin reactions to normal human tissue have been reported in leprosy patients. These reactions, studied by Kooij

and Gerritsen ([13]), were more frequent and more pronounced in tuberculoid leprosy patients, as were those we observed to normal armadillo tissue. Methods of storage of lepromin have been studied by Abe *et al* ([1]) who showed that lyophilized lepromin-H may be stable for at least five years. Studies of purified lyophilized lepromin-A are therefore urgently needed.

In addition to the clinical usefulness of lepromin-A, our findings help confirm that the acid-fast bacillus in the infected armadillo is *M. leprae*. No other known acid-fast bacillus gives the classic lepromin response pattern over the clinical spectrum of forms of leprosy. In a limited study, Convit and Pinardi ([3]) found that lepromin-A produced reactions only in tuberculoid leprosy patients. Thus the evidence to date that the acid-fast bacilli in the infected armadillos are *M. leprae* is as follows: 1) pattern of reaction to lepromin-A in leprosy patients, 2) pyridine extractability of acid-fastness ([18]) and the dopaoxidase activity ([16]) of bacilli recovered from infected armadillo tissue, and 3) selective invasion of nerves in the armadillo by the acid-fast bacilli ([11]).

SUMMARY

To assess the usefulness of *Mycobacterium leprae*-infected armadillo tissue as a substitute for human lepromas for the manufacture of lepromin, we compared skin reactions to preparations from these two sources in 115 leprosy patients. The patient sample represented all the primary clinical forms of leprosy. Lepromin derived from the armadillo (lepromin-A) provoked the same pattern of responses as human derived lepromin (lepromin-H), i.e., lepromatous patients gave the weakest reactions and tuberculoid patients the strongest reactions. Lepromin-A reactions were consistently more intense than those to lepromin-H. We conclude that lepromin-A is a promising alternative to lepromin-H and may make the worldwide distribution of a standardized skin testing reagent feasible.

RESUMEN

Para determinar la utilidad del tejido de armadillo infectado con *Mycobacterium leprae* como substituto de lepromas humanos para la preparación de lepromina, comparamos las reacciones de la piel a preparaciones de estos dos materiales en 115 enfermos con lepra. La muestra de pacientes comprendía todas las formas clínicas de lepra. La lepromina derivada de armadillo (lepromina-A) produjo el mismo patrón de respuestas que la lepromina derivada de humanos (lepromina-H), o sea, los pacientes lepromatosos dieron las reacciones más débiles y los pacientes tuberculoides las reacciones más fuertes. Las reacciones a la Lepromina-A fueron consistentemente más intensas que las a la Lepromina-H. Concluímos que la Lepromina-A es una alternativa promisoria para la Lepromina-H, que puede hacer posible una distribución mundial de un antígeno estandardizado para pruebas cutáneas.

RÉSUMÉ

Dans le but d'évaluer l'utilité des tissus d'armadillos infectés par *Mycobacterium leprae*, comme substitut de lépromes humains pour la fabrication de lépromine, on a comparé des réactions cutanées à des préparations obtenues à partir de ces deux sources. Cette étude a été menée chez 115 malades de la lèpre. L'échantillon de malades comprenait toutes les formes cliniques primaires de la lèpre. La lépromine dérivée de l'armadillo (lépromine A) a entraîné le même type de réponse que la lépromine d'origine humaine (lépromine H), c'est à dire que les malades lépromateux ont présenté des réactions les plus faibles et les malades tuberculoïdes les réactions les plus fortes. Les réactions à la lépromine A ont été régulièrement plus prononcées que celles à la lépromine H. On en conclut que la lépromine A constitue une alternative pleine de promesses pour la lépromine H, et pourrait rendre possible une distribution à l'échelle mondiale d'un réactif cutané standardisé pour les épreuves lépromíniques.

Acknowledgments. We wish to thank Mr. T. K. M. Nkelele and Miss Edna M. Staple, S.R.N., of Kivuvu, who helped perform the skin tests and provided administrative assistance, and Mr. Mbongi Kiletu Mandiangu, General Director, Institut Médical Evangélique, Kimpese, for his support. We are especially grateful to all the leprosy patients who so willingly cooperated after being informed of the details of the study.

REFERENCES

1. Abe, M., Yoshino, Y., Kobayashi, S. and Hokibara, H. Studies on the preparation, standardization and preservation of lepromin. VII. Changes in the bacterial count and the potency of lepromin during preservation. Lepro **39** (1970) 1-6.
2. Bullock, W. E. Studies of immune mechanisms in leprosy. I. Depression of delayed allergic response to skin test antigens. N. Engl. J. Med. **278** (1968) 298-304.

3. CONVIT, J. AND PINARDI, M. E. Leprosy: confirmation in the armadillo. Science **184** (1974) 1191-1192.
4. DHARMENDRA. The immunological skin tests in leprosy: the isolation of a protein antigen of *Mycobacterium leprae*. Indian J. Med. Res. **30** (1942) 1-7.
5. DRAPER, R., REES, R. J. W. AND WATERS, M. F. R. Comparison in man of lepromins prepared from leprosy infections in man and mice. Clin. Exp. Immunol. **3** (1968) 809-816.
6. FERNANDEZ, J. M. M. The early reaction induced by lepromin. Int. J. Lepr. **8** (1940) 1-14.
7. GOIHMAN-YAHR, M., FERRARESI, R. W. AND RAFFEL, S. Passive transfer of hypersensitivity to lepromin. Proc. Soc. Exp. Biol. Med. **130** (1969) 390-393.
8. HANKS, J. H. Precautions for injecting uniform doses of lepromin. Int. J. Lepr. **36** (1968) 64-65.
9. HANKS, J. H., ABE, M., NAKAYAMA, T., TUMA, M., BECHELLI, L. M. AND MARTINEZ-DOMINGUEZ, V. Studies towards the standardization of lepromin. Progress and prospects. Bull. WHO **42** (1970) 703-709.
10. HAYASHI, Y. On a pure culture of leprosy bacilli and skin reactions by means of the pure culture suspension. Saikin-gaku Zasshi (J. Bacteriol.) **272** (1918) 51-53 (in Japanese). English translation in Int. J. Lepr. **21** (1953) 370-372.
11. KIRCHHEIMER, W. F. AND STORRS, E. E. Attempts to establish the armadillo (*Dasypus novemcinctus* Linn.) as a model for the study of leprosy. I. Report of lepromatoid leprosy in an experimentally infected armadillo. Int. J. Lepr. **39** (1971) 693-702.
12. KIRCHHEIMER, W. F., STORRS, E. E. AND BINFORD, C. H. Attempts to establish the armadillo (*Dasypus novemcinctus* Linn.) as a model for the study of leprosy. II. Histopathologic and bacteriologic postmortem findings in lepromatoid leprosy in the armadillo. Int. J. Lepr. **40** (1972) 229-242.
13. KOOIJ, R. AND GERRITSEN, T. Positive "lepromin" reactions with suspension of normal tissue particles. Int. J. Lepr. **24** (1956) 171-181.
14. LEW, J., YANG, Y. T. AND PYUN, W. S. Experimental infection of the Korean chipmunk (*Tamias sibiricus asiaticus*, Gmelin) with *M. leprae*. Int. J. Lepr. **42** (1974) 193-202.
15. MITSUDA, K. On the value of a skin reaction to a suspension of leprous nodules. Jap. J. Dermatol. Urol. **19** (1919) 697-708. English translation in Int. J. Lepr. **21** (1953) 347-358.
16. PRABHAKARAN, K. A rapid identification test of *Mycobacterium leprae*. Int. J. Lepr. **41** (1973) 121.
17. RIDLEY, D. S. AND JOPLING, W. H. Classification of leprosy according to immunity. A five-group system. Int. J. Lepr. **34** (1966) 255-273.
18. WADE, H. W. Preparation of the Mitsuda antigen. First report, Expert Committee on Leprosy. WHO Tech. Rep. Ser., 1953, No. 71 [method reprinted in Int. J. Lepr. **21** (1953) 535].
19. WHO Memoranda. Immunologic problems in leprosy research: 1, 2. Bull. WHO **48** (1973) 345-354, 483-494.