

# OBSERVATIONS ON THE LEPROLIN (MITSUDA) REACTION

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## INTRODUCTION

Interest in the leprolin or Mitsuda reaction has become world-wide, particularly since the extensive tour made in 1932 by F. Hayashi, a pupil of Mitsuda. The material for this simple test can be prepared even in distant stations without elaborate laboratory equipment, and its use will probably become even more widespread in the future. Its principal interest at the present time lies in its possible usefulness in the study of so-called "tuberculoid" leprosy.

It is very probable that extracts of boiled lepromatous tissue had been employed by others before Mitsuda used it (12). There can be no doubt, however, that he worked with this material ("leprolin") more persistently and more thoroughly than any other investigator, and as early as 1917 he had already tested with it 403 lepers in the Zensei Hospital, near Tokyo (13). He was also the first to establish the one finding in connection with this test which has been confirmed by all successive workers, namely, that it is negative in the large majority of cases of the cutaneous type of leprosy and positive in most of those belonging to the neural type.

Using a fresh emulsion of lepromata, Mariani in 1924 (11), reported that the reaction in eight cases of the nodular form of the disease was slight and passing, while in two patients with the neural type it was more intense though late. Bargehr, who at first used fresh and finally boiled leproma extracts, published his work in several articles the earliest of which appeared in 1926 (1). He used a skin reaction applied in the same manner as the von Pirquet test. All subsequent workers, including the present writer, who have used that method have found it unreliable; and Bargehr's findings, especially as regards the differences in the reactivity among contacts and noncontacts, have not been generally confirmed. More recently excellent articles on the leprolin test have been published by Souza-Araujo (14), Chiyuto (4), F. Hayashi (8), Muir (13), Fernandez (6) and K. R. Chatterji (3).

The most significant finding with regard to this test in recent years was made by Chiyuto (4) and Manalang (10). They found it negative in normal infants, variable in children between one and three years of age, and always positive in adults. These findings were interpreted as indicating susceptibility to leprosy in infants and most young children, with absolute immunity among adults.

From the work of all the above-mentioned investigators, the following points appear to be more or less definitely established:

1. The reaction is negative in the majority of cutaneous-type cases (65 to 92 percent) and positive in the overwhelming majority of lepers belonging to the neural type (66 to 97 percent).

2. The reaction is negative in infants under 1 year of age, variable in older children, and positive among adults. According to Chiyuto 100 percent of 10 adults reacted positively, though Fernandez found only 77.24 percent positive among contacts (22 cases) and 75.75 percent among noncontacts (33 cases) of adult age.

3. The results of the test in contacts and noncontacts in endemic countries is variable. When the data of Fernandez cited above are tested statistically, his results are found to be the same in both groups. Chatterji found the percentage of positive reactors to be highest among neighbor contacts, lowest among family contacts, with noncontacts in between; he presents interesting differences in the age curves of the three groups.

4. Among older children of lepers (intimate contacts) a positive reaction is the rule (Muir 63 percent, Chiyuto 84 percent).

5. When leprolin is passed through a bacterial filter the filtrate does not produce a reaction (Hayashi). If, instead of a lep-

roma rich in bacilli, lepromatous tissue poor in bacilli such as a lymph node with few bacilli (Hayashi), tuberculoid macule (Fernandez), or skin from the site of a former infiltration in a negative case (Rodriguez), is used as the test antigen the resulting reaction is the same but the response is definitely feebler. The reaction, then, would seem to depend partly on the bacilli themselves, and partly on some product of interaction between the bacilli and the body cells.

6. When leprolin from rat leprosy (Muir's "Stephansky leprolin") is employed among *nonlepers*, the results are similar to those obtained with the ordinary leprolin. That is, infants and very young children are negative or weakly positive, while adults show positive reactions of variable intensity.

7. When the Stephansky leprolin, or preparations of cultures of saprophytic acid-fast organisms such as timothy hay, smegma or acid-fasts derived from milk, water and feces, as well as preparations of cultures of supposed leprosy bacilli obtained by the methods of Clegg, Needham, McCoy, Duval, or Kedrowsky, are used as test antigens they give as a rule *positive* reactions in cutaneous leprosy, in contrast with the negative reaction to leprolin.

Thus the leprolin test has been found to give fairly consistent results along certain lines. However, in using the test it must be remembered that, because of the very nature of the test material itself, there are certain objections to it. In the first place, unlike tuberculin, which consists of an extract from a pure culture of the tubercle bacillus, leprolin consists of a mixture of presumably dead leprosy bacilli and human tissue from the host. The admixture of proteins, lipins, and other material from the tissues may influence considerably the resulting reaction, at least in individuals sensitive to such substances. Also, in spite of precautions to standardize the material by roughly counting the number of bacteria per field as suggested by Muir, it cannot be guaranteed that every batch of leprolin will give exactly the same results as all the others. We have observed differences even in samples made from nodules and leprotic infiltrations obtained from different parts of the body of the same patient. Perhaps this is due to the fact that, on account of differences in thickness and texture of the skin, as well as in the amount of fibrosis present depending on the duration of the lesion, some nodules are easier to triturate and grind than others. The amount and fineness of the suspended macerated tissue has to be judged merely by sight.

Because of the factors stated, re-tests performed with different batches of leprolin from different patients or from different parts of the body of the same patient may not give exactly comparable results. The differences become important when dealing with cases that give weakly positive and doubtful reactions; among those giving markedly positive reactions such minor differences are of no consequence.

#### TECHNIQUE

The method of preparing the vaccine described by Hayashi (8) has been employed in the work here reported, with one modification suggested by Mr. Epifanio Mabalay, laboratory technician of the Cebu Skin Dispensary. He found that a fresh nodule boiled for 30 to 60 minutes in physiologic solution was slippery and difficult to grind in a mortar without using sterile sand. After trying various methods he discovered that the nodule became much easier to grind if it was first heated in an autoclave for one hour under five pounds pressure. The prepared test material was kept in the ice box. It was not used if more than three months old.

In reading the test the method of evaluating the degrees of reaction adopted by Muir (13) was followed. Readings were made on the seventh, fourteenth, and twenty-first days, and usually the reaction was again examined after one and two months from the date of the last reading.

Though the first reading is not made for a week, it must be noted that in most positive reactions there is an earlier, initial redness and edema, though usually it is not marked and may easily be missed unless specially looked for. Starting a few hours after the injection, this passing reaction is usually at its maximum intensity after 24 hours; thereafter it subsides rapidly and is usually gone by the fifth day. In some cases, however, this early reaction merges with the typical nodule formation, which becomes noticeable at about the end of the first week. The initial redness corresponds to the period of intensive edema, capillary hemorrhage, and microscopic abscess formation shown by biopsy during the first few days after injection.

#### RESULTS OF THE LEPROLIN TEST IN HOSPITAL PATIENTS

##### I. THE TEST IN NEWLY ADMITTED PATIENTS

A large majority of all patients admitted to the Eversley Childs Treatment Station are—necessarily, under the regulations in force in the Philippines—of the cutaneous type. Sometimes, however, tuberculoid cases, especially those in the "reaction" state (which incidentally is infrequent in Cebu) have been found to show bacilli in one or more of many smears taken and have been segregated. In exceptional instances bacteriologically negative neural cases with macular lesions have been admitted for study and confirmation of the diagnosis.

For the purposes of this study the recently admitted patients

were classified into two main groups: *Group 1.* Cutaneous type cases showing either no sensory disturbances at all or only anesthesia, and without noticeable trophic involvement such as atrophies, contractures or trophic ulcers, and without markedly thickened nerves (C and CN1). *Group 2.* "Mixed" cases showing noticeable neural involvement in addition to their cutaneous lesions (CN2 and CN3). The results of the leprolin test in these cases are shown in Table 1.

TABLE 1.—Results of the leprolin test in newly admitted positive patients.

Degree of reaction	Group 1, C or CN1		Group 2, CN2 or CN3	
	Number	Percent	Number	Percent
Negative (—) .....	10	71.4	13	68.4
Doubtful (±) .....	0	0.0	1	5.3
Positive, + .....	4	28.6	3	15.7
Positive, ++ .....	0	0.0	1	5.3
Positive, +++ .....	0	0.0	1	5.3
TOTALS.....	14	100.0	19	100.0

Among the newly-admitted cases with unmixed or practically unmixed cutaneous-type leprosy (Group 1, C and CN1), positive reactions were infrequent and the few that occurred were weak. None of the negative reactions was found to have become positive when the test sites were re-examined two months after the third reading.

On the other hand, among the mixed cases (Group 2, CN2 and CN3), there were several that reacted positively. Furthermore, when the injected sites were re-examined two months afterward three of those that previously were negative were found to have become 2-plus and one was considered 1-plus; another became ±, the rest continuing to be negative. Delayed reactions were more frequent among those showing noticeable neural symptoms.

The leprolin test was also performed on many other new patients, but the results have not been included in this report because the patients were not consecutively admitted and there may have been unconscious selection of them.

## 2. THE TEST IN LONG-SEGREGATED POSITIVE LEPERS

Desiring to compare the results of the test obtained in newly admitted patients with those in cases that had been in segregation for two years or more and that had received active antileprosy treatment during that time, two other groups were tested. These

cases were classified as were the previous ones, the type and advancement given being those recorded at the time of admission. The results are shown in Table 2.

TABLE 2.—Results of the leprolin test in bacteriologically positive patients under segregation and treatment for two years or more.

Degree of reaction	Group 1, C or CN1		Group 2, CN2 or CN3	
	Number	Percent	Number	Percent
Negative (—) .....	19	95.0	11	36.7
Doubtful (±) .....	0	0.0	6	20.0
Positive, + .....	1	5.0	3	10.0
Positive, ++ .....	0	0.0	6	20.0
Positive, +++ .....	0	0.0	4	13.3
TOTALS.....	20	100.0	30	100.0

Comparing the results in the first, or unmixed, group of old cases with those given by the similar group of newly admitted ones (Table 1), it is seen that no significant difference in reactivity to leprolin was evident, in spite of the fact that on the whole the old patients were somewhat more advanced as regards leprosy than the new ones, and, as said, they had been under treatment for two years or more. However, unlike the new group, three of the old cases that were negative after three weeks had become ± after two months.

Of the mixed group of old cases, on the other hand, no less than thirteen gave positive reactions of some degree, and six others were ±; only eleven (37 percent) were entirely negative. When re-examined after two months the reaction nodules in the four cases with 3-plus reactions remained unchanged. Among the six cases with 2-plus reactions three remained unchanged, one had become converted to a healed ulcer, one was considered to have increased to 3-plus, and the remaining one had become reduced to 1-plus. Of those having 1-plus reactions, one had died, another had become 2-plus, and the third one negative. Among the six with a ± result, three were unchanged, one had become 3-plus, and the two remaining ones were negative. Of the eleven negative cases, seven continued to be negative; one had become 1-plus; and three became 2-plus.

It therefore seems that some of the cases with marked neural manifestations tend to become positive after prolonged hospitalization and treatment, and delayed reactions are also more frequent among them.

## 3. THE TEST IN PATIENTS ON THE NEGATIVE LIST

A third class of patients available for testing was those who, once bacteriologically positive and clinically active, had become clinically inactive and bacteriologically negative and were therefore on the "negative list." This group was also subdivided as were those previously discussed, the classification again being based on the type and advancement of the disease at the time of admission. The average duration of the segregation period at the time of testing was almost exactly 2.5 years. The results are given in Table 3.

TABLE 3.—Results of the leprolin test in patients that had become bacteriologically negative.

Degree of reaction	Group 1, C or CN1		Group 2, CN2 or CN3	
	Number	Percent	Number	Percent
Negative (—) .....	48	42.1	23	33.8
Doubtful (±) .....	6	5.3	1	1.5
Positive, +.....	36	31.6	27	39.7
Positive, ++.....	8	7.0	7	10.3
Positive, +++.....	16	14.0	10	14.7
TOTALS.....	114	100.0	68	100.0

It is apparent that there is a marked difference in the results of the Mitsuda test among "negatives" from those obtained in bacteriologically positive cases. Many more cases reacted positively, and the results in the two subgroups did not vary significantly—that is, the proportion of positive reactors is about the same among those who had no obvious neural changes on admission as among those who showed such changes. It is evident that cutaneous cases which show marked improvement under treatment and hospital care tend to become leprolin positive.

## 4. THE TEST IN "LEPRA REACTION"

Green (11), working at Kuala Lumpur, reported that in patients in "lepra reaction" the intradermal injection of fresh, unboiled lep-rotic tissue was followed by a reaction at the site of injection. With that finding in mind a considerable group of lepers, 72 in all, who were in varying grades of reaction ranging from a few acute papules to a highly febrile generalized condition, were tested.

It was found that the proportion of positive results among them was about the same as in bacteriologically positive nonreacting cases, and that there was no relation between the result of the test and the severity of the lepra reaction. The impression derived from these tests was that cases that are negative to leprolin continue,

as a rule, to be negative during the reaction phase. It is regretted that they had not been tested before the onset of the reaction. An observation of interest was that in two cases of recurrent lepra reaction the sites of the injection, which previously was only slightly positive, became reactivated during a subsequent attack.

Almost exactly two years after the first test was made (that is, the one made during lepra reaction), as many as possible of the 72 cases were retested. The results indicated that some few cutaneous cases, previously negative or weakly positive, become positive to leprolin during lepra reaction; but as a general rule the result of the test remains unchanged during an attack of lepra reaction of the "cutaneous type."

#### 5. PROGNOSTIC VALUE OF THE TEST

The usefulness of the leprolin test with regard to prognosis has already been emphasized by Muir (13). In the present study its prognostic value was determined in a group of 179 cases which were on the "negative list" during the period from October 26, 1933 to March 26, 1934. Exactly one year after the date of the last tests (that is, on March 26, 1935), the patients were re-examined to determine whether or not they had relapsed. The relation between reactivity to leprolin and relapses among these cases is shown in Table 4. In interpreting the data shown it is important to bear in mind that all of the cases had become negative before they were tested, and that the observation period after the time of the test was only one year; relapses occurring after that year are not included.

TABLE 4.—Relation between reactivity to leprolin and relapses among "negative" patients.

Degree of reaction	Number tested	Number relapsing	Percent <sup>a</sup> relapsing
Negative (—) .....	69	30	43.5
Doubtful (±).....	7	3	43.0
Positive, +.....	62	8	12.9
Positive, ++.....	15	1	6.7
Positive, +++.....	26	0	0.0
TOTAL CASES.....	179	42	23.5

<sup>a</sup> Percentages of the individual groups concerned.

Nearly one-half of the cases that did not react at all, or gave only doubtful reactions, had relapsed within one year. On the other hand only 13 percent of those reacting weakly (+) relapsed, and



but one out of the fifteen 2-plus cases; in none of the 26 strongly positive ones did that occur. It may therefore be stated, as a general rule, that a strongly positive reaction indicates a good prognosis. A weakly positive reaction is also a fairly good indication, though some relapses may be expected among such cases.

#### EXPERIMENTAL INVESTIGATIONS OF THE REACTION

##### 1. REACTIONS IN LOCALLY-INJECTED LESIONS

In view of the finding that the test tended to become positive among cases that had become bacteriologically and clinically "negative" after prolonged treatment, the question arose whether or not that change in reactivity might be due to the drug treatment itself. Obviously, it is impossible to eliminate such factors as the natural course of the disease, diet, improved hygiene, etc. It seemed possible, however, that if the effect were due to injections there might be discovered a difference in the reactivity of patches of skin which had been repeatedly infiltrated with the drug, as compared with comparable areas which had not been treated locally. To investigate this possibility tests were performed in 15 cases whose lesions had been injected intradermally with chaulmoogra ethyl esters (*H. wightiana*) for periods ranging from one month to two years, applying it in every instance to a treated lesion and for control to the opposite side of the body. The tested lesions had not been injected for periods varying from one month to one year.

In no instance was it possible to detect any difference in the reactivity of the treated patch as compared with the control area.

##### 2. NATURE AND SITE OF THE REACTION

It is generally held today that bacterial allergy is the result of a cellular reaction between a whole or partial antigen and a specific sessile antibody or reagin which has been developed as a result of previous contact or sensitization (16). It may be supposed that sensitivity to leprolin also is the result of such a reaction.

(a) *Result of the Prausnitz-Küstner test.*—This test was performed as follows: A small quantity (0.1 cc.) of the blood serum of a patient who had given a strong reaction to leprolin was injected intradermally into each of 6 patients with cutaneous leprosy who had reacted negatively. After 24 hours, 0.1 cc. of leprolin was injected into the same sites as the serum had been given. At the same time 0.1 cc. of leprolin and 0.1 cc. of the serum were injected separately into the opposite arm as controls.

In four out of the six cases the Prausnitz-Küstner test as well

as the two controls (leprolin and serum) were negative. In two remaining cases the test injection as well as the leprolin were both  $\pm$ , while the serum injection remained negative. The results are not very clear cut, but since the Prausnitz-Küstner test was not definitely positive in any instance there was no demonstration of passive transfer of reactivity to leprolin. Other authors report that there is no passive transfer of reactivity to tuberculin. This result may also be taken to indicate that the antibodies responsible for the reaction to leprolin are not circulating freely in the blood serum.

(b) "*Patch test*" with leprolin.—After a preliminary washing of the skin site with soap and water, small pieces of gauze about 1.5 cm. square and two layers thick were soaked with leprolin and placed on the skin in the interscapular area, covered with oil paper of convenient size and attached firmly to the skin with adhesive plaster. The gauze was removed after 24 hours and the site was observed for three weeks.

This test was performed on four patients who had been strongly positive to injections of leprolin. In not a single instance was there any reaction to the patch test. The result indicates that the epidermis is not the site of the reaction to leprolin.

(c) *Subcutaneous injection of leprolin*.—After 0.1 cc. of leprolin had been drawn up into a tuberculin syringe, the needle was carefully wiped with sterile gauze and the injection was made rapidly through the dermis into the subcutaneous tissue. This test was performed on eight patients who previously had been found to react strongly to leprolin.

The results were as follows: Negative, two cases;  $\pm$ , two cases; 1-plus, three cases; 2-plus, one case; no case was 3-plus. Although care was taken to prevent leakage of the leprolin into the dermis during the puncture, it is possible that this occurred in some of the cases and may have been responsible for some of the positive reactions.

As a result of the experiments described in this section, it is believed that the sessile antibodies or reagins responsible for the leprolin reaction have been proved to exist mostly in the dermis, probably anchored to the cells of this layer of the skin.

### 3. EFFECT OF MULTIPLE DOSES

A few experiments were made for the purpose of determining whether or not multiple or repeated injections of leprolin might have a massive or cumulative effect on the reaction in bacteriologically positive patients positive to leprolin. The idea was to stimulate and

then to exhaust the "antibodies," if any, that are responsible for the leprolin reaction. It is regretted that there were very few strongly reacting cases available for this test; most of them were newly admitted cases with positive reactions. Sets of three patients were used, one patient receiving five injections simultaneously, another five injections at intervals of an hour, and the third five injections given daily. The readings were of the reactions to these injections, observed after one, two and three weeks. The protocol of one set of patients is given in Table 5. The dosage used in that experiment was 0.1 cc. In two others the doses were 0.15 and 0.2 cc., respectively. The results were about the same.

TABLE 5.—Results of multiple and rapidly repeated leprolin injections in a set of three positively-reacting, bacteriologically positive patients.

Method of injection	Site	Results		
		First week	Second week	Third week
CASE J. A. Five injections, 0.1 cc. each, given simultaneously	1	—	±	±
	2	—	—	+
	3	—	—	+
	4	—	—	±
	5	—	±	+
CASE E. E. Five injections, 0.1 cc. each, given at intervals of one hour	1	—	±	+
	2	—	—	+
	3	—	±	±
	4	±	±	±
	5	±	±	+
CASE N. B. Five injections, 0.1 cc. each, given at intervals of one day	1	+	+	++
	2	±	±	+++
	3	±	±	+++
	4	±	±	+++
	5	±	±	+++

As has been stated, there were available very few patients strongly positive to leprolin who were not on the negative list; hence the results of this experiment were inconclusive. Such an experiment could better be undertaken in an asylum where there are many neural or predominatingly neural "mixed" cases, many of which probably would be strongly positive to the test. It might be advisable also to try larger doses of leprolin than those here employed.

A considerable number of relapses occurred shortly after the patients on the negative list were tested in the ordinary way, and the patients believed that the injections produced the relapses. It seems possible that the tests may have precipitated the relapses in some cases, and although no data can be presented to support this

belief we refrained from using patients in the negative list for testing the effect of multiple doses of leprolin.

#### 4. EFFORTS TO DEMONSTRATE "ANTICUTINES"

Jadassohn and his pupils, after many years of experiments with the quantitative tuberculin reaction, came to the conclusion that the so-called tuberculides can be subdivided into three groups according to their reactivity to tuberculin:

(a) Lichen scrofulosum and rosacea-like tuberculides, which seem to be strongly hypersensitive to tuberculin.

(b) The papulo-necrotic tuberculides, erythema induratum, and lupus miliaris faciei, which are slightly less sensitive to tuberculin than normal, and much less sensitive than true skin tuberculides.

(c) The so-called sarcoid group, which is very decidedly less sensitive to tuberculin than normal individuals.

In explanation of the above conditions, Sulzberger and Wise (15) may be quoted:

The lessened reaction to tuberculin such as is found in sarcoids is called "anergy", in contradistinction to heightened reaction which is called "hyperergy." The particular type of anergy presented by tuberculous sarcoids is called specific anergy (J. Jadassohn, cited by Martenstein) or positive anergy (Hayek, cited by Martenstein), in contrast to a type of (negative) anergy which we will mention below.

In these diseases with positive specific anergy, substances have been found in the blood serum, in the lesions themselves, and elsewhere, which when mixed with tuberculin in vitro counteract the action of the tuberculin. These have been called *anticutines* by Pickert and Loewenstein who first described them. In other words, patients with positive anergy have elaborated substances which combat the harmful action of tuberculin. It is tempting to assume that it is through these substances that the tuberculin injected into their skins in testing is rendered less harmful. Thus these persons can stand higher concentrations of tuberculin than the norm. . . .

In addition to the positive anergy which is observed in the sarcoids ("positive anergy" because it is due to the presence of bodies counteracting the tuberculin poison), there is a group of cases which do not react because no immunological bodies of any kind are formed, either because none have been necessary, i. e., no infection has taken place, or because the power to form them has been lost (marasmus, cachexia, measles, acute miliary tuberculosis, patients dying of pulmonary phthisis, etc.).

These cases also show no reaction to high concentrations of tuberculin, and are called "negative anergies," in contradistinction to the "positive anergy" described above. The search for pro- and anti-cutines in these conditions has never been carried out on a large group as far as we know. If the theory is to be "held upright," it should naturally be impossible to demonstrate these substances here.

In fairness to Sulzberger and Wise it must be stated that the above quotation merely states in schematic outline the very complex problem of the tuberculin reaction among the tuberculides; it is understood that many inevitable biological variations occur. However, it is the most satisfactory statement of this difficult subject that the writer has come across.

If the above conceptions are correct and if comparison with leprosy is permissible, it may be assumed that positive specific anergy exists in the earlier stages of the cutaneous type of the disease. After this stage there are two possible developments, depending on the outcome of the infection. If the infection is overcome as evidenced by the recession of the cutaneous lesions (coincident in many cases with appearance of neural involvement), the state of anergy changes to hyperergy. If on the other hand the infection becomes overwhelming, a condition of negative anergy of the exhaustion type may result.

On the supposition, then, that a condition of positive anergy due to the presence of anticutines exists in the early cutaneous type, an attempt was made to demonstrate the presence of such anticutines by means of the experiments described below. To each of four neural lepers previously proved to be positive to leprolin, the following materials were injected:

PATIENT A.—(1) A mixture of 0.1 cc. of blood serum from an early cutaneous case shown to be negative to leprolin, plus 0.1 leprolin, the mixture having been thoroughly shaken in the syringe for two minutes. (2) (Control) 0.1 cc. of above serum. (3) (Control) 0.1 cc. of leprolin.

PATIENT B.—Same as in Patient A, except that the mixture of serum and leprolin was allowed to stand at room temperature for 10 minutes.

PATIENT C.—Same as in Patient A, but the mixture was placed in the ice-box for 24 hours.

PATIENT D.—Instead of serum, 0.1 cc. of an aqueous suspension of a fresh (i.e., unboiled) piece of normal-looking skin from a leprolin-negative early cutaneous case was thoroughly mixed with 0.1 cc. leprolin and injected. Control injections of 0.1 cc. of the tissue suspension and the leprolin were made.

The result in each case was negative; that is, the reaction resulting from the mixture of leprolin and serum or suspension was the same as that produced by the leprolin alone (control). There appeared, therefore, to be no substance in the serum or the tissue of a leprolin-negative early cutaneous case which neutralized the effect of leprolin in a patient who was sensitive to it. The controls with the serum and the tissue suspension were also negative.

In other words, the attempt to demonstrate the presence of anticutines in early cutaneous leprosy was unsuccessful, and there-

fore the possibility that the anergy to leprolin in that form of the disease is of the positive type is unproved. It is possible, however, that with more patients and more refined technique these hypothetical substances may yet be shown to exist in leprosy.

##### 5. REACTIONS IN LABORATORY ANIMALS

The importance of performing the leprolin test on laboratory animals lies in the possibility of correlating the resultant reactions with the results of direct inoculation of the tested animals with unmodified leprous material, a crucial experiment which is of course impossible to make in children. It has long since been thoroughly demonstrated that many species of laboratory animals are immune to human leprosy, though in some instances lesions from which acid-fast organisms could be recovered for a considerable period of time have been produced at the sites of inoculation. It may be assumed that if the leprolin reaction is to be taken as a true index of immunity to the infection, all such noninfectible animals should react positively to the test. It therefore seemed desirable to investigate the matter.

The test was performed in exactly the same manner and with the same dose as in humans. To secure uniformity of results the same site of inoculation (the hairless undersurface of both hind legs) was used in all the experiments. The data on the animals used and the results of the tests are given in Table 6.

Though too few animals were used to permit drawing very definite conclusions, the results obtained do not support the assumption that immunity to infection is necessarily accompanied by the capability of reacting positively to leprolin.

It is interesting to note that the animals that were tested may be roughly divided into two groups: (a) those which react negatively (cats, monkeys, chicken, pigs, rats, and turtles), and (b) those reacting positively (dogs, goats and rabbits). If we were to accept the interpretation of some workers regarding the relation of reactivity to leprolin and susceptibility to leprosy, the first group should be susceptible and the second group resistant to infection. Judging from the result of countless inoculations done by previous workers, however, it is certain that that is not the case, inasmuch as animals in the first group have been demonstrated to be as resistant as those in the second. However, it is to be admitted freely that the reactivity of laboratory animals to leprolin may be different from that of humans.

It will be noted also that in dogs the reaction was negative

among the young and positive among adults, as is the case in human beings. It was not possible to test young goats and rabbits, the only other animals found to react positively.

TABLE 6.—Results of the leprolin test in laboratory animals.

Kind of animal	Age	Sex	Weight (kilograms)	Results
Cat.....	1 week	M	0.500	—
Cat.....	1 week	M	0.600	—
Cat, brown spots.....	(?)	F	1.400	—
Cat, white.....	3 years	M	1.450	—
Monkey.....	(?)	F	3.000	—
Monkey.....	(?)	F	3.600	—
Monkey.....	(?)	M	5.200	—
Fowl, rooster.....	(?)	M	1.700	—
Fowl, hen.....	(?)	F	1.100	—
Fowl, hen.....	(?)	F	1.200	—
Pig, black.....	3 weeks?	M	1.500	—
Pig, black.....	3 weeks?	F	1.600	—
Pig, black.....	1 year	F	75.000	—
Pig, white spots.....	1½ year?	M	87.600	—
Rat ( <i>R. norvegicus</i> ).....	(?)	M	(?)	—
Rat.....	(?)	F	(?)	—
Rat.....	(?)	F	(?)	—
Rat.....	(?)	F	(?)	—
Turtle.....	(?)	M	(?)	—
Rabbit, white.....	1 year	M	1.500	+
Rabbit, white, brown spots...	1 year	F	1.360	++
Goat, brown.....	young adult	F	(?)	+++
Dog, black.....	1 week	M	0.800	—
Dog, brown.....	1 month	M	1.800	±
Dog, brown.....	1 month	F	1.500	—
Dog, brown.....	(adult)	F	11.000	+
Dog, brown.....	(adult)	M	17.500	++
Dog, black.....	(adult)	F	9.500	++
Dog, black.....	(adult)	M	16.500	+++
Dog, white.....	(adult)	F	10.500	+
Dog, white.....	(adult)	M	10.000	+++

At one time we thought that there was a marked difference in reactivity between the four dark colored (black and brown) dogs on one hand and the two white ones on the other. The papules produced by the first inoculation in the latter were much smaller than those observed in the former. Since in general the major and minor tuberculoid lesions of leprosy seem to be most frequent among Negroes and other dark peoples and least common among the Whites, with the yellow and brown races coming in between, the difference in reactivity noted in the two groups of dogs seemed particularly in-

teresting. On repeating the injections on the same dogs, however, no significant differences in the size of the papules were observed, and in histological sections the cellular structure of the reaction nodules was apparently identical. However, this unique feature may be investigated further with profit.

With regard to the animal tests in general, it may be hoped that other workers in a better position to undertake such experiments will extend them. It would be interesting to know if the response to the leprolin test is in any way related to the distribution of the Forssman antigen among the different animals.

#### HISTOLOGY OF THE REACTION

The histopathology of the leprolin reaction has been dealt with in several important articles, but the successive tissue changes accompanying the development of the reaction papule have not received adequate attention. So far as the writer is aware Manalang, in 1932, was the first to study these changes. Examples of the conditions found in reaction lesions studied by me are shown in Plate 4.

A specimen removed *forty-eight hours* after injection gives the picture of an acute inflammatory reaction involving both the epidermis and the dermis. The cells of the cuboidal layer stain irregularly and there is marked intercellular edema. In some of the sections the horny layer is lifted up in many places by hemorrhages and serum which separate it from the stratum granulosum (Fig. 1). Collections of polymorphonuclear leucocytes are also sometimes present in the upper layers of the epidermis and the papillary layer is filled with serum and fibrin. The papillary and subpapillary vessels are congested and engorged with blood. In the corium there is moderate to marked focal infiltration with polymorphonuclears mixed with a few lymphocytes. These are collected chiefly around the capillaries and small blood vessels. The glandular structures are not distinct and are almost obliterated by infiltrations of polymorphonuclear leucocytes. This histological picture is essentially similar to that of a slight to moderate tuberculin reaction.

By the *fourth day* the hemorrhages and edema in the epidermis are not as a rule markedly diminished, but there is less cloudy swelling of the cells in the corium. Most of the polymorphonuclear leucocytes have usually become fragmented, and many of the previous collections of these cells in the cutis have disappeared. Their places are now occupied chiefly by lymphocytes.



At the end of the *first week* the edema in the epithelial layer may still persist, but usually it is slight. The edema in the corium has also greatly subsided, and the sites of the previous collections of polymorphonuclears are now occupied by large and small lymphocytes, a few fibroblasts, and some endothelial or epithelioid cells. There may be some remaining polymorphonuclears undergoing destruction or dissolution. Occasional giant cells may be observed.

By the *tenth day* desquamation of the horny layer of the epidermis is usually seen. The corium shows wide streaks of pale-staining tissue, probably the remnants of the degenerated structures observed in the earlier sections. In certain places there is distinct evidence of newly formed fibrous tissue presenting a linear or lamelated arrangement. By this time the predominating cells in the cutis are the epithelioid or endothelial cells, although there may be no distinct tubercle formation. Foreign body giant cells are also common (Fig. 2).

Toward the end of the *second week* collections of epithelioid cells and lymphocytes, together with a variable number of giant cells, are observed, so that a picture similar to that of tuberculoid leprosy results (Fig. 3). In some of the sections, however, this picture is lacking or is not distinct even by the end of the third week. In these sections the sites of the original collections of polynuclears are occupied rarely by newly-formed capillaries infiltrated with some epithelioid cells and lymphocytes. There is usually marked fibrous regeneration in such sections.

In dogs the character of the cellular elements found in the exudate after the first week is quite different. The most numerous cells consist of plasma-like cells, mixed with some endothelial cells and occasional eosinophiles (Fig. 4).

#### DISCUSSION

The total lack of response to leprolin among infants, followed by increasing reactivity with increase of age, recalls the phenomenon of "serologic ripening" presumed to occur in growing children. The investigations of Burky (2) and of Kobak and Pilot (12) are of interest in this connection. Burky found that young rabbits tolerated more than eight times the usual adult minimum lethal dose of staphylococcus filtrates, and that the results of intracutaneous tests with the same filtrates were invariably negative in rabbits less than four months old and always positive in adult rabbits. Kobak and Pilot reported that, while about 95 percent of all human

mothers are skin-reactive to *Staphylococcus aureus* vaccines, less than 2 percent of their children under two months of age are reactive.

On the basis of the above theory, the lack of reactivity among infants and the gradually developing response among growing children, not only to leprolin but also suspensions of other acid-fast bacteria, may be interpreted as a manifestation of this normal maturation cycle. The only exception, in so far as the acid-fast are concerned, seems to be the tubercle bacillus, since it is the consensus of opinion that the tuberculin test is always negative, even in adults, among those who have not been in contact with the disease; this occurs for instance among races who have not been exposed to tuberculosis. This exception may be one more indication that the leprosy bacillus is more closely related to the saprophytic acid-fast bacilli than to the highly pathogenic tubercle bacillus.

On the other hand the lack of response to leprolin among lepers with the cutaneous type of the disease seems to be different from the absence of reactivity among infants, inasmuch as these cases only fail to react to the ordinary leprolin while they usually react actively to preparations of saprophytic acid-fast bacilli. In other words, if these theories are correct we have in the cutaneous type of leprosy a condition of particular or *specific anergy*, in contrast with the negative reaction in infants which is nonspecific.

This brings us to the often repeated statement that the result of the leprolin reaction indicates adult immunity and infantile susceptibility to leprosy. Although there is much evidence that in many endemic countries infants are much more susceptible to infection than adults, it does not necessarily follow that the result of the leprolin test is a further proof of this, any more than similar results with *S. aureus* vaccine in infants and adults would support such a belief.

Perhaps confusion will be lessened if it is borne in mind that the leprolin test is probably an indication of cutaneous allergy and not necessarily of immunity. Although allergy and immunity usually go together, since both are the result of the same infectious process, it has been shown that it is possible for one to exist without the other. We seem to have fallen into the same error that occurred in the past among students of tuberculosis in confusing allergy with immunity, and vice versa. For this reason the caution given in a recent editorial (5) with regard to the interpretation of the tuberculin test in tuberculosis would appear timely. This editorial says, in part:

In the past it was generally assumed that a positive Mantoux test signified immunity (as well as allergy) to the tubercle bacillus and contrariwise that a negative test meant lack of infection, lack of allergy and absence of immunity. Experimental evidence now shows convincingly that this visible reaction is not a necessary concomitant of immunity and that the latter can function fully and effectively in the complete absence of evidence of this hypersensitivity. Further, it has even been shown that the allergic necrotizing-inflammatory response, far from being helpful or protective, may even be injurious. The so-called anergic, or nonreactive, phase has long been known—a negative response in the presence of highly active or overwhelming infection. These investigations [Rich et als., cited] and others tend to refute the frequently heard contention that a negative tuberculin test means absence of immunity. It is not improbable that an early infection even without the almost inevitable subclinical reinfections may leave an immunity lasting long after the positive skin test has completely disappeared or at least defies detection.

The leprolin reaction, then, is not necessarily a test of immunity but probably of allergy. The only way to determine whether a certain animal is susceptible or not to a certain infectious disease is to expose the animal to the causal agent of the disease under experimental conditions. It is apparent that this rigid test can never be applied in human infants with regard to leprosy.

It should not be inferred from the above discussion that the tuberculin and the leprolin reactions are necessarily similar or closely related. Except for their common allergic basis they are certainly different, since the tuberculin reaction reaches its maximum intensity and therefore the result is read at the 48th hour, whereas the leprolin test is read only after the end of the first week and preferably at the end of the second week.

Histologically the tuberculin reaction is characterized by severe edema and capillary hemorrhages, with marked polynucleosis, while the typical leprolin-positive nodule shows tubercle-like changes, with epithelioid cells and giant-cell formation. However, during the first few days the tuberculin and leprolin reactions are practically identical histologically, except perhaps in degree. Since the former is a protein reaction, being elicited by purified tuberculo-protein, it is suggested that the preliminary reddening in the leprolin test may also be due to a protein factor, but that the true leprolin reaction is produced by some other substance or substances, possibly partly derived from the waxy coating of the organism.

#### SUMMARY

The leprolin (Mitsuda) test in the hands of leprosy workers in different parts of the world has given fairly consistent results

along certain lines. However, the mechanism of the reaction is still very imperfectly understood, and for this reason its true significance can not be fully evaluated. The present report covers certain work done in an effort to obtain a further understanding of its nature.

The results of this study suggest that a positive reaction depends on the presence of sessile antibodies attached to the cells in the dermis. Efforts either to stimulate or to exhaust these hypothetical antibodies by repeated injections of the test material at varying intervals were unsuccessful, but the experiments in this respect were not entirely satisfactory because of insufficiency of material.

A positive leprolin reactive in a case of the cutaneous type of the disease may be considered a favorable prognostic sign, but it is not necessarily a guarantee that the case will improve to the bacillus-free stage within a reasonable time. Among bacteriologically negative candidates for parole, strongly positive reactors are seldom found to relapse.

The usual negative result of the leprolin test among cutaneous-type lepers seems to be based on positive or specific energy, though we have failed to demonstrate the presence of hypothetical substances analogous to the anticutines shown by Picker and Loewenstein to exist in tuberculosis. On the other hand the negative result in infants and young children seems probably to be nonspecific.

The leprolin and the tuberculin reactions have some points in common, but they are so dissimilar in certain important respects that in all probability they are fundamentally different. Neither can be transmitted by passive transfer and both are more delayed than the usual cutaneous tests. However, one of their important differences lies in the fact that the leprolin reaction has been found to be positive in most normal adults, whether they have been in contact with the leprosy or not, whereas the tuberculin reaction is positive only in those who have acquired an infection. Furthermore, the tuberculin reaction is usually at its maximum and is read at the end of 48 hours; the leprolin reaction is much more delayed and is ordinarily read at the end of the second week. The reaction to tuberculin is to a protein; that to leprolin very probably is not.

Experiments here reported show that some laboratory animals that are resistant to infection by human leprosy fail to react to leprolin.

Although leprolin is an interesting substance with which to

study the different phases of leprosy, it is well to bear in mind, in interpreting the results obtained with it, that it is composed of a combination of many substances derived from the tissues of the lep-rotic nodule as well as from the leprosy bacillus.

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## DESCRIPTION OF PLATE

## PLATE 4

Fig. 1. Section showing reaction at the end of 48 hours. Severe papillary edema and focal infiltration with polymorphonuclear leucocytes mixed with a few lymphocytes.

Fig. 2. On the tenth day after injection. Cellular infiltration consist of lymphocytes, epithelioid cells, and young fibroblasts. Several giant cells present.

Fig. 3. At the end of the second week. A typical small tubercle with numerous giant cells.

Fig. 4. High power magnification of a section through a nodule at the site of leprolin injection by the end of the first week in a dog. Notice the numerous plasma-like cells.

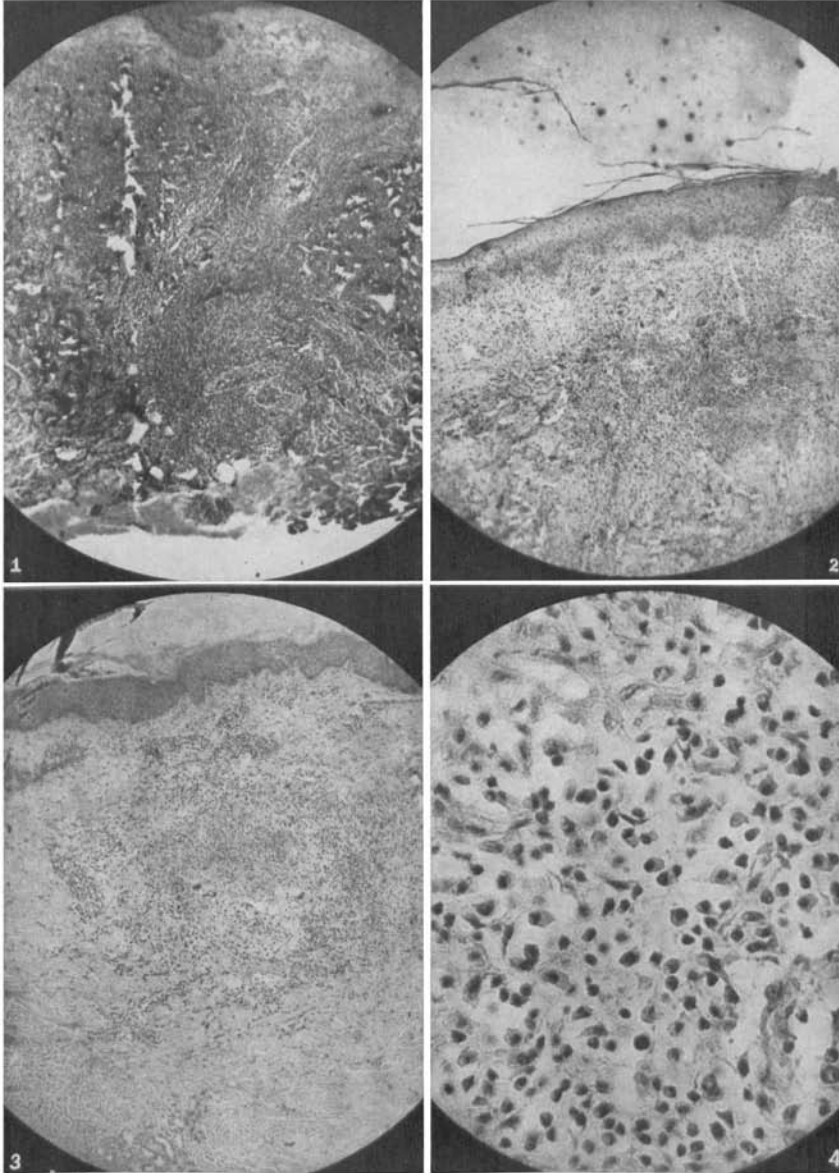


PLATE 4