# THE LEPROMIN REACTION IN NORMAL DOGS; PRELIMINARY REPORT

F. 2, 2496

# By H. W. WADE, M.D. From the Leonard Wood Memorial Laboratory Culion, Philippines

Study of the nature of the lepromin reaction has heretofore been confined entirely to human subjects, despite the obvious limitations of experimentation upon them, and the equally obvious fact that the phenomenon presents features which still call for active investigation. Such studies, if there is to be a full understanding of the phenomenon, are not to be limited solely to effects induced by lepromin itself, but must include those of analogous antigens made from other acid-fast microorganisms. It need only be recalled that Hayashi (8) reported positive reactions with suspensions of nonpathogens from cultures (M. *phlei* and others), and that Muir (15) and Kawamura (10) investigated the reactions to an antigen made from the leproma of rat leprosy. Others have investigated such reactions since then.

Because this phenomenon is a general one, and since it is different from any of the ordinary skin reactions, there is need of a general name for it. To call it the "lepromin-type" reaction, in analogy with the term "tuberculin-type reaction" for those of that class, would perhaps at times be objectionable because of prejudice against anything that denotes leprosy. It would seem permissible, and appropriate, to call it the "Mitsuda-type reaction," or the "Mitsuda phenomenon."

One of the most important questions awaiting further elucidation is that of the nature of the reaction itself. It is usually if not universally regarded as one of allergy, yet curiously little emphasis has been laid on the conspicuous peculiarities of the concentrated and particulate nature of the antigen and the marked lag in the development of the positive reaction lesion, which ordinarily does not begin to appear until a week or more after the injection. There is here a striking and undoubtedly significant contrast to all of the familiar skin tests designed to determine allergic sensitization, in which are used dilute, soluble

antigens and which are read in a matter of hours or, in the case of the "delayed" type represented by the tuberculin reaction, after two days at longest. It is true that Mitsuda (14), Hayashi (8), Rodriguez (17), and, more recently, Fernández (7), have reported that relatively prompt and brief erythematous reactions occur after injection of the antigen or a filtrate of it, in cases that are later to show a strong Mitsuda-type reaction, but the latter is of a very different character. It is also true that Villela (20) and Rabello et al. (16) have reported positive results with a protein fraction of lepromin, but their work has not been published in detail, or, apparently, followed up even by them.

In line with this question is the generally accepted fact that a large proportion of normal persons who have had no contact with leprosy react positively to lepromin, at least beyond a certain early age. That has repeatedly been demonstrated among peoples in leprous regions. On the other hand there has been comparatively little study of the matter in regions where the disease is not endemic (Wales, 4 Belgium, 5 Italy 2); public prejudice being as it is, it would obviously be most difficult in any such region to investigate this question adequately with lepromin itself. Rotberg (18) has questioned the correctness of the reported positive findings where leprosy is absent, asserting that this reaction in apparently normal persons must result from the effects of generalization of the infection in the populace.

Another widely-accepted belief is that the reaction denotes resistance to the infection. This idea derives in part at least from the fact that, dealing with the disease itself, it is the neuraltype case, relatively resistant to its progress, in which positive reactions are to be expected. It perhaps finds support in repeated observations, by Mitsuda (14), Bargehr (1), Stein and Steperin (19) and others that persons much exposed to leprosy are apt to react more strongly than noncontacts, a fact which is generally accepted as evidence of its allergic nature but is difficult to explain precisely in the present state of knowledge of the phenomenon. Also in support of this idea, Chiyuto (3) and Manalang (13) have pointed to the fact that very young children-regarded by most workers, though not by all, as especially susceptible to infection-usually react negatively, the frequency of positive findings increasing during the second and third years; and they go so far as to insist that infection takes place only during that early period.

.On the other hand Lara (12) has found that, in Culion-born children, the reaction is no index of resistance to the development of the disease. An entirely different line of inquiry is suggested by his further findings in children, by those of Ignacio (9) with actual cases, and by those of Lagrosa (11) with "negatives." In all of them they find that, on repeated testing at intervals of several months, originally negative reactors tend to become positive and those originally giving weakly positive results tend to react more strongly. Yet the children in whom the reactivity is thus modified are no less liable than others to develop manifestations of the disease, the actual patients whose reactivity is so modified show no evidence that that condition is beneficial with respect to progress of the disease, and the negatives may undergo relapse without the reaction becoming negative. These findings are of interest in connection with those to be reviewed in a later article, of sensitization induced by lepromin.

This question of the significance of the reaction in connection with resistance to infection led Rodriguez (17) to test experimentally several kinds of animals, some of them of ordinary laboratory species and others less usual. The findings, which he presented briefly in an article dealing with an extensive study of the reaction in patients, are summarized in Table 1. In discussing these findings he pointed out that the species of animals

#### TABLE 1.-Reactions of animals to lepromin (Rodriguez).

Kind	Age	Number	Results
Dogs	Adult	6	1 + to 3 +
Puppies	Week to month	3	— or +
Rabbits	Adult	2	1+ and 2+
Goat	Adult	1	3+
Monkeys	Adult	3	Negative
Cats	Adult	2	Negative
Kittens	Week	2	Negative
Rats, house	Adult	4	Negative
Hogs	Adult	2	Negative
Pigs	Weeks (3?)	2	Negative
Chickens	Adult	3	Negative
Turtle	(?)	1	Negative

that proved to be nonresponsive are as lacking in susceptibility to experimental infection with leprosy as those that reacted positively. The work with the latter kind was not extended to an investigation of the nature of the reaction itself, though it was recognized that its mechanism is imperfectly understood; "for

this reason," he added, "its true significance can not be fully evaluated."

In view of the questions awaiting investigation on a more intensive basis than would be generally practicable or possible with human subjects alone, it might have been thought that the possibilities thus indicated of experimental study of this reaction in laboratory animals would have been taken up promptly. So far as is evident from the literature, however, the matter has been ignored.

#### PRESENT WORK

This work was entered into more or less fortuitously. The dog being the most available animal among those found by Rodriguez to react positively, a number of them were given multiple lepromin injections and biopsied serially for the purpose of studying the histology of the reaction lesions. Several days later, still with the same end in view, they were given further injections. The immediate reactions to these injections differed markedly from those to the original ones, indicating that sensitization had occurred. A considerable series of experiments was thereupon made in connection with that feature.

Much of this work will be reported elsewhere, but it is desired to bring the main findings especially to the attention of leprosy workers, and to discuss certain of its features in relation to the lepromin reaction as seen in patients. The present article is a preliminary and summary report on the reactions in new, untreated adult dogs, from which a "normal average curve" of the course of the reaction is derived; notes on certain factors that might be expected to influence the reaction are included. Experiments referring to the influence on the reaction of the skin site tested and of dilution of the antigen, and one concerning the reactions in families (mothers and puppies), are to be reported in more detail shortly. The findings regarding sensitization, of more interest in relation to general immunology than to leprosy work, will be summarized briefly later. The matter of technique is gone into somewhat fully here.

#### TECHNIQUE

Animals.—The animals, the common mongrel dogs that infest the streets of this colony, were used consecutively as brought in, without selection as to size, sex, color or any other feature. Because it had been shown (17) that individuals differ considerably in reactivity, they were invariably used in groups, usually of three. Chance proved favorable in this matter, as a rule.

42

Antigens.—A single lot of lepromin, made by the technique of Hayashi, <sup>1</sup> was used throughout, eliminating any possible variation on that account. Other antigens, from a rat leproma, nonleprous tissues, and certain acid-fast cultures, were also used in testing sensitized animals.

Injections.—The injections were made superficially, after clipping and shaving, to produce discrete flat, pale wheals. Their locations were marked immediately by spots tattooed with India ink,<sup>2</sup> their patterns differing to identify different kinds of injections given in the same region. The standard amount (0.1 cc.) was used for each injection, but the total dosages given varied widely. For certain purposes three doses were used, seldom fewer, to permit cancelling out variations of individual lesions by averaging. In other experiments from 15 to 20 were given simultaneously.

Observations.—Except in the first experiment, the lesions were observed from the beginning, usually two or three times on the day of injection. The first observation was a measurement of the injection wheal (properly made within five minutes or less), ordinarily found to be between 9 and 10 mm. The others were made after one and four hours, to determine the immediate tissue reaction; an attempted 3-hour compromise proved unsatisfactory. After the first two or three days the examinations were made at increasing intervals of days, for at least five or six weeks. More frequent records would have determined more precisely the times when changes in the course of the reactions took place, and rounded out curves now too angular, but those that were made suffice to establish the general course of events.

Measurement.—The most important record is the measurement, done with calipers, to within 0.5 mm. when possible. The lesions being usually round or broad ovoid, only single measurements were made as a rule; it was not practicable either to make two measurements in all instances or to determine the actual areas. When, however, exuberant reaction lesions became notably elongate or asymmetrical, two measurements were made and averaged in analysis. Often the measurements recorded were qualified by " $\pm$ " because of diffusion of outline in the active phase, or loss of definition in the last stage. The personal equation also must have introduced some variation, though probably seldom more than 1 mm. Variations due to those factors tend usually to cancel out in averaging, but temporary, minor fluctuations of 1 mm. or less in the curves, particularly when few lesions are involved, are often of doubtful significance.

Other records.—Records were also made of the degree of elevation; the presence, degree and extent (central or general) of erythema; the presence of notable scaling, or necrosis, or erosion or ulceration, the last being measured; and other features such as frank edema, diffusion of outline, peculiarities of shape, secondary extension, abscess formation, recession, etc. Such features are important mainly for following the course of events at the time.

Analysis.—The course of the reactions can be demonstrated systematically, by curves, only on the basis of the measurements, though that leaves

<sup>1</sup>This material was kindly supplied by Dr. J. O. Nolasco, head of the pathological section of this colony, to whom thanks are due for this courtesy.

<sup>2</sup> An efficient implement for tattooing is made from five ordinary small sewing needles, bound firmly together with fine copper wire to within about 0.5 cm. of the points, enough wire being used to provide a convenient hold.

much to be desired. Lesions of the same diameters vary markedly in volume (elevation and depth), and in character (early active as against late recessive ones); and average figures do not suggest the often wide variations among multiple lesions in a given animal. The important feature of ulceration, practically universal in strong reactions (a large lesion may actually appear as a narrow-rimmed crater), cannot very well be indicated in a graph. While in the earlier, progressive stages size is fairly closely correlated with activity, once the peak has passed the lesion recedes in size only to a certain point. The area of disturbance seen after the lesion has become flat and residual may actually measure larger than that of elevation during the late period of recession, so that some curves have a misleading upward trend at the end. There is not much point, therefore, in making systematic measurements after recession has progressed to a certain stage.

#### THE REACTION IN NORMAL DOGS

As a base line for the evaluation of things seen under special circumstances, it is necessary to establish a norm for the course of the reaction in normal animals. These must not have been subjected to any previous manipulation, must be representative of the range of reactivity that may be met with, and must be sufficient in number so that, in averaging, the major effects of irregularities in individuals and extremes in the group are cancelled out. For this purpose can be used the data, entirely valid for the first three weeks or more, of a total of ten animals, tested in three consecutive experiments. The findings, as stated, are considered here only in summary.

One group of five dogs (Nos. 6-10) were given three injections each, with this end expressly in view. This dosage was decided upon because, on the one hand, it was desired to avoid any effect on the course of the reaction that might possibly result from more massive dosage, while on the other hand it was considered undesirable to depend upon one injection per dog in so limited a number because of variations among different lesions in the same animal that had been seen earlier. It was hoped that this relatively large group would be satisfactorily representative of the normal range of reactivity, but because three reacted slowly, one of them (Dog 8) being an exceptionally poor reactor, the average curve of the lot is on the low side. Another group of three that is also used (Nos. 11-13) had two full doses each (and, in addition, two doses of each of two dilutions); its average curve is on the high side, mainly because two of the animals were unusually prompt and rapid reactors. Finally, there is available a group of two animals which received massive dosage at the outset (Nos. 4-5, 18 doses each), and which proved to be very alike and of a medium degree of reactivity.

In total, these ten animals serve the purpose reasonably well. The unusually reactive group serves to compensate for the low first one but not, it is believed, to an excessive degree. It is not to be expected that the results would be duplicated precisely by any other similar lot of animals, even from the same

source, but the differences would probably be only quantitative and of moderate degree; the essential characteristics would be similar. Whether or not dogs of other kinds, or from other sources, would give like results it would be interesting to know.

The tabulated data of these three groups are not given here, but their averages are shown in a graph (Text-fig. 1), together with a "general average" one derived from the lot. To illustrate the range of reactivity found among these animals there are also included the essential parts of the curves of the most prompt and rapid reactor and the most slow and poor oneactually the extremes so far encountered. The average curve is begun at the 24-hour point, but the curve of the Nos. 6-10 group in the first day is entirely characteristic of what is usually found during that period in normal dogs.

In constructing the general average curve account was taken of the number of dogs in each group. Only at two points (1st and 14th days) did the observation schedules of all three coincide, to give a direct figure. At several other points observations of one or another pair of groups coincided, it being necessary to calculate the data of the third one from its curve. At three points, all after the third week, that had to be done for two groups; the result seems quite valid, but in any case that part of the curve is of least interest, because of the natural variability of developments in that period if not also because all of these animals received reinjections then, as indicated in the graph.

Characteristics of the reaction.-These curves reveal certain general characteristics of the course of the reaction. These features represent different stages of the process, three of the period before onset of the reaction, three of the positive reaction itself. Variations that are cancelled out in averaging, or any clinical peculiarities that may have been seen, do not affect these features.

(1) Enlargement of the injection wheals in the first hour. This initial reaction, which varies somewhat according to the region injected, is typical and practically always evident in averages such as these. It is due to nonerythematous edema, usually symmetrical, evidently nonspecific. It is of brief duration, ordinarily lessening markedly within a very few hours.

(2) Reduction of size of the injection lesion. This reduction, or resorption, is a general feature which occurs whether or not there was increase during the first hour or so. It almost always continues until at least the second day after injection, and sometimes longer.

(3) Quiescent or latent period. In this period, which lasts

45

for several days, ending with the onset of reaction, individual variations may become conspicuous. None of the average curves in this graph go below the 5 mm. level, and only one approaches it closely. In individual animals some or all lesions have gone lower, and it has been necessary to read a few as quite negative for a time. The length of this period is decidedly variable. In the curve of the Nos. 11-13 group the onset of the reaction is evident as early as the fourth day, though it could not have been identified as such at that time because so small a change in the curve might have been due to fortuitous variations in measurement. In neither of the other groups was onset evident in the average figures until the 10th day, and in Dog 8 not until the 17th day. The rise seen in the general curve on the 7th day is valid for a general average, but it can be said that in most instances onset is not evident until a little later.

(4) Primary development of the definitive reaction (first phase of the positive reaction). Once begun, in normal animals such as these the reaction progresses, with rather wide variations as regards rapidity and the point reached, to a high level generally reached between the 14th and 21st days-occasionally earlier and sometimes later, but on the average at about the end of the third week. Dog 13, at its height on the 11th day, is almost as unusual as Dog 8, the extremely slow and low course of which is largely responsible for the more gradual progression after the 14th day of the curve of the group to which it belonged. In the curves as constructed it would seem as if the definitive progression was at first slower than later, but that appearance may be fallacious; because of the intervals of observation, it can seldom be said on exactly what day the increase really began. As will be seen, the point reached at the end of this primary phase need not be the final maximum.

(6) Height of the definitive reaction (second phase of the positive reaction). This phase, in general, extends from about the 15th to the 30th days, or, otherwise stated, between the ends of the second and fourth weeks. The fact that a higher maximum may be reached in this phase than at the end of that of primary development is only suggested in the general average curve, though it is conspicuous in the Nos. 11-13 curve, and more so in those of several individual dogs. This late, second-ary increase may be lacking; recession may start sooner or later after the primary phase ends (usually soonest when the early progression was most vigorous and rapid) and continue without

interruption. When late increase does occur, it may be seen after either a period of slow progression (as in the Nos. 6-10 curve after the 17th day), or of relative stability (curve flattened, much as in that of Nos. 4-5) or of recession (the Nos. 11-13 curve after the third week). This late development is in general due to reactivation or exacerbation, often with extension, of usually a small proportion of the lesions present in an animal; it may occur either spontaneously, without evident explanation, or after reinjections such as these animals were given. This phase of the reaction is, as a rule, one of obvious instability, in contrast with the first and last phases, and it is the one in which essential ulceration usually becomes general.

(7) Phase of recession (third phase of the positive reaction). This refers to the final or terminal recession, after the unstable phase of the height of the reaction is past. On the whole, it cannot be expected to begin before the end of the fourth week. During recession the lesions undergo considerable changes in appearance and ulcers become smaller and heal, though often that does not occur until very late. The areas of elevation lessen in size, though not proportionately to the degree of actual resolution; and when that is finished the resulting level disturbed area, more or less cicatricial, may, as has been said, be larger than that of elevation in its late stage.

#### FACTORS POSSIBLY INFLUENCING REACTION

Certain factors which might be thought possibly to influence the course of the reaction, or which have been found to do so, will be dealt with summarily here.

Influence of dosage.—As has been seen, Dogs 6-13 (two experiments) each received three doses or slightly less, while Nos. 4-5 had 18 doses each; the first dogs used (Nos. 1-3) also had amounts on that order, at least before the effective dosage was reduced by biopsies of the injection sites. The average curves of the two small-dose lots differ quite markedly, but that can only be ascribed to the factor of individual reactivity. The curve of the smaller of the large-dose groups was of intermediate character; and, though the other three dogs of that category differ rather widely among themselves, their average curve is essentially similar. The conclusion seems clear that the initial dosage has no influence on the definitive reaction, either with respect to the time of its onset or its rate of progression.

As for what occurs afterward, the situation may be different. It happens that the curves of the large-dose animals during the second period (height of reaction) are relatively smooth, at least before the later reinjections were made, and the changes after that are not marked. In both of the small-dose groups, on the other hand, the curves of some of the animals show more or less wide fluctuations, both before and after reinjection. Though the numbers of animals involved are too small to permit drawing a definite conclusion, it would seem that the situation is decidedly less stable after small than after large initial dosage.

Influence of reinjections.—Sooner or later, in all of the experiments, further injections of the same or other antigens were given, usually in considerable amounts. The analysis of the data pertains to the three phases of the positive reaction.

(1) With respect to the period of development, the experience with Dogs 1 to 3 is pertinent. One lot of reinjections, six doses per dog, was made about ten days after the original ones (actually nine to eleven days, since the three had been started on successive days). Because of the peculiarities of the reactions that followed, a test for sensitization (three doses per dog) was made two days later, and that was repeated after another three days. There is no evidence, in the curves or other data, that this intervention had any effect whatever on the original reaction lesions.

(2) At the other end of the sequence, the third phase of the reaction, injections were given several of these animals after recession had begun or was about to begin. This interference was also without actual reactivating effect, so far as can be seen in the individual curves or the detailed records. The only thing to be seen is that in two or three instances there was an apparent interruption of recession, indicated by levelling off of the curves for a time.

(3) The situation is otherwise with respect to the unstable second phase. An interesting observation was made in Dogs 4-5. Reinjected on the 21st day and measured a day later (not done in any other experiment), the curves of both showed a rather small but apparently significant average decrease, without any evident clinical peculiarity. That drop, however, was only temporary; it occurred in the period represented by the broken part of their curve in Text-fig. 1.

In other instances the effect, if any, was stimulative rather

48

than depressive. Here again there is a difference between the dogs given few injections and those given many. In Dogs 1-3, after reinjections made on about the 27th day (26th to 28th), the curves of all three rose moderately to a late maximum. Whether that change was induced or spontaneous it is impossible to say; the antigens used then were heterologous ones, and the same ones when given Dogs 4-5 were without any such effect. Of the three-dose animals, the reinjections given the Nos. 6-10 group had no obvious effect in any individual. On the other hand the marked late increase in the curve of the Nos. 11-13 group, due to changes in two of them, followed the reinjections given them. The apparent connection cannot be ignored, even though the lesions of those animals had run particularly erratic courses earlier; the reactivation may have been spontaneous, but the particularly unstable condition that apparently existed in these animals may have been disturbed by the interference.

More direct evidence of a stimulative or reactive effect of such reinjections given in this unstable phase of the reaction is to be found in the clinical notes. Repeatedly there was apparent flare-up in individual lesions, expressed mainly in increased elevation and erythema. Since size was not necessarily increased to a material degree, and especially since when there were many lesions only a minority showed any evident change, the condition is not always evident even in the curves of the individuals, much less in the average ones of the groups.

Influence of surgical intervention.—Little need be said on this point. In the first three dogs from 7 to 13 injection sites and early reaction lesions had been removed by biopsy before the 14th day. None of them showed any indication that this interference affected the course of events, despite the fact that usually the wounds were torn open and became superficially infected. The only interesting feature observed was that the skin of the most promptly and rapidly reactive dog of the group (No. 3) seemed also most sensitive to this secondary infection, a superficial dermatitis developing around several of the wounds.

Influence of location.—This is a matter that has been found to be of some importance, and it will be dealt with in detail in a later article. Suffice it to say here that there are material differences in the reactions in regions of the body in which the skin differs notably in texture. In the relatively firm, thick skin of the chest there is typically somewhat more recession of

the injection lesion in the stage of resorption and quiescence, and a considerably greater development of the definitive reaction, than in the thin, soft, elastic skin of the abdomen.

## SUMMARY AND DISCUSSION

The findings here considered in preliminary summary afford ample confirmation of the observation of Rodriguez that dogs react positively to lepromin. Other animals found positive by him have not been tested. The fact that a convenient laboratory animal is reactive to this antigen makes feasible experimentation with it and related ones—in the whole field of what may be called the Mitsuda-type reaction, or Mitsuda phenomenon more extensive and intensive than would be possible with the human subject.

Both for consideration of the nature of the reaction itself and for the evaluation of findings under special conditions, it is necessary to establish a base-line or norm for the reaction in the animals used. This has been derived from findings in a total of ten animals, used in three experiments, which include individuals of an extreme range of reactivity. Though the findings are wholly valid for only the first three weeks or so, because all of the animals were given further injections after that time, even the later part of the curve that has been constructed is believed to be essentially characteristic of what would have been found in any case.

Aside from the immediate temporary, nonspecific reaction that is typically seen in average data for the first few hours, followed by recession due to resorption—usually to the end of the second day if not longer—the first feature of interest is a period of quiescence before the definitive, positive development begins. That period varies widely, and onset may be seen though it may not be identifiable as such immediately—as early as the 4th or not until after the 14th day. In the average curve it is definite on the 7th day, but in a majority of individuals it occurs a little later. In this period, then, there is a clear parallel in these animals with what is usually seen in human beings.

It is evident from the further findings, though so far as I am aware it has not been discussed in connection with those in the human being, that in the aggregate the definitive, positive reaction is divisible into three phases, those of (a) primary progression, (b) height of the reaction, and (c) final recession and resolution. Especially noteworthy is the marked variability 9, 1

of the time required to reach the maximal point, which may be early, at the end of the primary phase of development, or later, in the second phase. In general it occurs during the third week, and typically (average) at the end of it, but it may be sooner—occasionally even before the end of the second week—or considerably later, at the end of the fourth week or even after that. In this, again, there is a clear parallel with what ordinarily occurs in man. The period of resolution, on the whole, shows less marked variability. It cannot be expected to begin before the end of the fourth week, though it sometimes does so sooner; it has seldom been delayed very much longer in these experiments, and the condition has usually been residual in from six to eight weeks.

Interesting features of these three phases have been brought out by an analysis of the effects of certain factors that might be thought would influence the reaction, with reference particularly to the time of onset and the course of its primary progression. With regard to the influence of original dosage, it seems clear that there is no difference ascribable to that factor between the groups of animals given few injections and those with more massive dosage. It would appear that a certain amount of time is required for the production of the immunological factor upon which the reaction obviously depends, that time being wholly dependent upon the inherent characteristics of the individual animal. If there are any immunological differences referable to dosage during this phase, they are not expressed in the rate of the changes that occur within the individual reaction lesions. Later, in the phase of height of reaction, the situation is probably different, though the numbers of animals involved in the observations are too small to permit any conclusion to be drawn. From the relative frequency of spontaneous fluctuations seen in the lesions in the small-dosage groups, it would seem as if the immunological set-up were less stable in them than in those given massive dosages.

Similar indications are seen in the experience with reinjections. Those made repeatedly in Dogs 1-3 during the developmental phase of the original reaction lesions were without any perceptible effect on them, though there was prompt and vigorous response to the new injections themselves. In the second phase, on the other hand, after the reaction is well established, it appears that reinjections may affect its course. A single observation indicates that there may be a temporary lessening of activity after 24 hours. More interesting is the evidence of occasional reactivation or flare-up of lesions. That evidence is found more in the detailed observations of changes in occasional individual lesions than in the curves, but changes in some of them are not to be ignored, especially among the small-dose animals—which again appear to be in a particularly unstable condition. The situation seems to have become stabilized by the time final recession has begun, at or after the end of the fourth week, for reinjections given then have in no instance shown a notable reactivating effect, if any at all.

Another feature to be mentioned in this connection is an apparent inherent difference in different individuals with regard to spontaneous variations, dependent upon the degree of reactivity. The data have not been presented, and the observations are too few to permit a definite statement, but it is a fact that the curves of the slower reactors are, in general, relatively smooth and free from fluctuations, while those of the more prompt and rapid reactors are sometimes quite irregular, indicating relative instability. Whether anything of the sort has been observed in man is not known.

A question that arises is whether or not the animals used in this work are in any way peculiar. Rodriguez recorded two of his six adult dogs as 1+, two as 2+, and only 2 as  $3+.^3$  Without record of his actual measurements, it is uncertain whether or not the animals used in the present experiments actually reacted more strongly than did his, but that would seem to have been the case. In both instances the animals were from leprous communities. It cannot be said whether or not dogs from elsewhere would have reacted differently, but it is difficult to see how these animals could be affected in this respect by contact with lepers. Notoriously resistant even to experimental tuberculosis, they are certainly not affected by leprosy. If there were anything peculiar about the dogs used, it would be in the ultimate degree of reactivity rather than in an acceleration of reaction. It would be interesting to know how dogs in other regions. and-since evidence of an hereditary influence in this matter has been seen-also those of other kinds, compare with these in their rate and degree of reaction to a similar antigen.

<sup>3</sup> It has been learned (personal communication) that the criteria which he used in that connection were: 1+, definitely raised area, with inducation and erythema, to 5 mm. diameter; 2+, similar, with inducation up to 10 mm. diameter; 3+, the same with central pustulation. Observations were terminated on the 28th day.

With respect to the relation of these observations and the lepromin reaction in man, discussion will be limited to the question of the essential nature of that reaction-or, more generally, the Mitsuda phenomenon. The relatively early (tuberculin-like?) erythematous response that has been observed repeatedly in patients may be ignored, for it is fundamentally different and not seen in normal dogs. It is obvious, in the first place, that reactions of this type are not dependent upon previous allergic hypersensitization of the individual; otherwise, for one thing, there would not be the long period of quiescence preceding the onset of the definitive reaction. That period, which for reasons not apparent is highly variable in different individuals, lasts in general for a week or longer-the time usually required for allergic sensitization to occur in a normal animal capable of responding in that way to such a foreign element. The reaction lesion itself, when it does develop, may be regarded as a probably complicated manifestation of allergy induced by an antigen that, because of its physical character, is removed with more or less difficulty and that therefore—as serial examinations by bacteriological smears shows to be the case-remains in situ for a considerable period of time. Therefore, while the actual reaction itself may be correctly spoken of as one of allergic nature, it seems beyond question that the test is not one of the existence of allergic sensitization, but rather one of capability of reacting in that way. The peculiar feature in leprosy is the specific loss of that capability in lepromatous cases.

Thus nothing strange is seen in the fact that normal persons, never in contact with leprosy, as well as some animals, may give positive reactions. There is no need to assume that general dissemination of leprosy in a community is necessary to explain the occurrence of such reactions, or reason to deny the validity of those that have been reported from nonleprous regions. Nor need the theory of cross-sensitization be invoked to explain the fact that similar reactions can be produced by other antigens of similar general nature in all kinds of individuals, including those with the lepromatous type of leprosy who are ("specifically") nonreactive to human lepromin. The fact that reactions to that antigen are not known to be affected by the existence of active tuberculosis is pertinent.

That there are peculiarities in the picture is beyond question. Examples are seen in the fact that apparently normal persons long in contact with lepers may react more strongly than others

(though if more rapidly, it has not been shown); that in actual cases of leprosy which react positively there is seen the same lag, or period of quiescence, as in nonlepers, and that the principal difference between different classes of lepromin-positive cases (simple neural and tuberculoid) is the degree of ultimate reaction; that, as Fernández has reported (6) the administration of BCG to a nonreactive child results in its becoming a strong reactor; and the observations of Lara and his colleagues, cited earlier, that positivity induced by repeated testing does not interfere with the ordinary course of events in children of lepers, positive cases or negatives. These peculiarities simply indicate the lack of a complete understanding of this reaction.

# SUMMARY AND CONCLUSIONS

1. In this preliminary communication it is shown that the finding of Rodriguez that the dog reacts positively to intracutaneous injection to lepromin has been confirmed and extended. This animal, therefore, offers a convenient medium for the study of this and other reactions of the general Mitsuda type.

2. From a summarization of the data of ten previously unmolested animals, comprising individuals of an extreme range of reactivity, a "normal average" curve of the course of the reaction has been constructed.

3. The principal features of this curve are: an immediate nonspecific edematous reaction, followed by recession by resorption, usually for two days; a period of latency, of highly variable length but usually lasting about seven days, during which a banal foreign-body lesion may be present and which is ended by onset of the definitive reaction; and, finally, three phases of the latter: primary development, height of activity and recession.

4. It is noted, without the supporting data, that the factors of original dosage (number of injections), early reinjections (of the same or other antigens), and surgical intervention (multiple biopsies) with secondary inflammation, have no apparent influence on the time of onset or on the primary phase of development of the positive reaction. Reinjections may have a disturbing effect in the relatively unstable second period, but not materially in the recessive stage. The fact that the site of injection influences to some extent the degree of reaction is also noted.

5. Indications have been seen that the condition which ensues in animals given few injections is less stable as regards fluctuations in the course of the reaction than in those given massive total dosages, and also that it may be naturally less stable in prompt, vigorous reactors than in slow ones. The question of whether or not dogs from a highly leprous community may react more vigorously than others cannot be answered.

6. It is concluded that this reaction, while undoubtedly one of allergic nature, is not a test of the existence of allergic hypersensitiveness, but rather one of capability of developing an allergic state after the introduction of the antigen. The specific feature in leprosy is the loss of that capability in lepromatous cases. Mention is made of certain peculiarities of it, as seen in its practical application, that require elucidation.

#### REFERENCES

- BARGEHR, P. Künstliche lepraespezifische Allergie und aktive Immunisierung gegen Lepra. Ztschr. f. Immun. u. Exper. Therap. 49 (1926) 346-353.
- (2) BONCINELLI, U. Ricerche ed osservazioni sulla reattività cutanea dei lebbrosi alle cosidette "lepromine." Giorn. italiana Dermat. e Sifil.
   78 (1937) 629-651; abstract in Urol. & Cutan. Rev. 41 (1937) 825.
- (3) CHIYUTO, S. Leprolin test. Month. Bull. Philippine Health Serv. 12 (1932) 300-307.
- (4) CUMMINS, S. L. AND WILLIAMS, E. M. Cutaneous sensitivity to acidfast lepra bacilli in suspension. British Med. Jour. 1 (1934) 702-703.
- (5) DUBOIS, A. La réaction de Mitsuda (note complémentaire). Bull. Soc. Path. exot. 29 (1936) 649-651.
- (6) FERNÁNDEZ, J. M. M. Estudio comparativo de la reacción de Mitsuda con las reacciones tuberculínicas. Rev. Argentina Dermatosif. 23 (1939) 425-453.
- (7) FERNÁNDEZ, J. M. M. The early reaction induced by lepromin. Internat. Jour. Lep. 8 (1940) 1-14.
- (8) HAYASHI, F. Mitsuda's skin reaction in leprosy. Internat. Jour. Lep. I (1933) 31-38.
- (9) IGNACIO, J. L. Observations on the leprolin test in clinically active, bacteriologically positive lepers. Month. Bull., Bur. Health (Manila) 19 (1939) 95-105.
- (10) KAWAMURA. Cited by Kitano and Inoué, Internat. Jour. Lep. 9 (1941) 29-38; see Ohtawara, T. and Kawamura, M. Studien der Lepra. II. Mitteilung. Wie reagirt der Leprakranke auf die intrakutane Injektion von Rattenlepra bazillen? Zentralbl. f. Bakt. I. Orig. 134 (1935) 312.
- (11) LAGROSA, M. The leprolin (Mitsuda) reaction in "negative" lepers.
   I. Observations over a period of one year. Month. Bull., Bur. Health (Manila) 19 (1939) 83-93.

- (12) LARA, C. B. Mitsuda's skin reaction (leprolin test) in young children of leprous parents. I. Observations on children from one to five years old. Month. Bull., Bur. Health (Manila) 19 (1939) 15-47.
- (13) MANALANG, C. Significance of leprolin reaction in the natural and experimental transmission of leprosy. Month. Bull, Philippine Health Serv. 12 (1932) 308-310.
- (14) MITSUDA, K. Les lépreux maculo-nerveux, d'une part, les tubéreux, d'autre part, se comportent différement a la suite d'une inoculation d'émulsion de tubercle lépreux. III<sup>e</sup> Conf. Internat. de la Lèpre, Strasbourg, 1923. Paris, 1924, Bailliere et Fils, pp. 219-220.
- (15) MUIR, E. The leprolin test. Lep. in India 5 (1933) 204-218.
- (16) RABELLO, JR., VILLELA, G. G. AND TOSTES, J. Recherches sur la fraction antigénique specifique de l'antigène lépromateux de Mitsuda. Bull. Soc. française Dermat. Syphilig. 46 (1939) 1386-1387.
- (17) RODRIGUEZ, J. N. Observations on the leprolin (Mitsuda) reaction. Internat. Jour. Lep. 6 (1938) 11-32.
- (18) ROTBERG, A. Some aspects of immunity in leprosy and their importance in epidemiology, pathogenesis and classification of forms of the disease; based on 1529 lepromin tested cases. Rev. Brasileira Leprol. 5 (1937) Spec. No., pp. 45-97.
- (19) STEIN, A. A. AND STEPERIN, M. I. Die spezifische allergie bei leprösen. Nederlandsch Tijdschr. Hyg. Microbiol. Serol. I (1934) 209-218; also (in English) Urol. & Cutan. Rev. 38 (1934) 860-863.
- (20) VILLELA, G. G. Sur la fraction active de l'antigène de Mitsuda. Bull. Soc. française Dermat. et Syph. 46 (1939) 1387-1388; also (in Portuguese) Acta Méd. (Rio de Janeiro) 4 (1939) 272.



