

The Leprolin Test.

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INTRODUCTORY.

IT is sought in this paper to review briefly the literature on the subject, to describe the technique, and to describe certain experiments which the writer has carried out by means of the test. The main object of the paper is, however, to suggest lines along which the leprolin test may be used with a view to elucidating certain problems connected with the resistance to leprosy, the method of transmission, and the factors determining the type of leprosy. A list of these suggestions will be found at the end of the paper. There are many leprosy institutions and clinics in India which furnish excellent clinical material for investigation along the lines we have indicated. It is hoped that this paper will be of use to the doctors in charge of these institutions who are interested in these problems.

WORK DONE BY OTHERS.

The leprolin test has been used by Mitsuda in Japan for many years. Hayashi (1933) has recently developed the use of this test. An article by him was abstracted in the July, 1933, number of this journal, so we have not detailed his methods further here.

Mariani (1924) made a suspension from a leprous nodule and inoculated it intradermally in 10 cases of leprosy, 8 of which were of the nodular and 2 of the nerve type. In the former there were no signs except a little erythema and infiltration which disappeared by the 8—12th day. In the nerve cases after the 10th day there was infiltration and swelling locally, followed by the formation of a central vesicle. On the 20th it reached the size of an orange pip and was surrounded by a red dense border. It cleared up with some loss of tissue by the 60th day. There was no fever or constitutional disturbance.

Bargehr (1926) originally recommended using a suspension similar to Mitsuda's; but instead of injecting he applied it by sacrificing the skin and rubbing in the suspension. In our own experience and that of others, this method of carrying out the test is not as satisfactory as that of intradermal injection. Bargehr tested 162 patients with the following results: Of 82 showing bacilli, all gave a negative test; of 80 showing no bacilli (a large number of whom showed definite signs of leprosy), 45 gave negative results, and 35

gave positive results; the 35 giving positive reactions either showed no signs of active leprosy (though they had been in contact with lepers) or were lepers with mutilated fingers and toes apparently recovered from the disease. Bargehr does not mention the type of case (common in India, Japan, and other countries) with red, swollen, annular lesions, which invariably gives a positive reaction. This may be due to absence of this type of case from the leprosy institution in which he worked.

de Langen (1929) prepared a dried powder prepared from nodules and stored in vacuum tubes. From this he prepared his leprolin. He concluded that the reaction is specific and that it may be of use in the diagnosis of early cases. He used it for distinguishing between active and inactive cases, as the former gave a negative and the latter a positive result. He considered that a positive reaction in a person who had frequently been in contact with lepers justifies the opinion that he has acquired resistance against the infection. He also suggested that the test may be of use in taking prophylactic measures.

Chiyuto tried out the leprolin test in three groups: (1) 169 children of leprous parents, their ages varying from under 1 to over 16 years. There were signs suggestive of leprosy in 168 of these, but bacteriological examinations were negative. Under 2 years all were negative; over 16 only 5.5% were negative. Between 2 and 16 results varied. (2) 97 healthy children. Those under 1 year all were negative. Those from 3 to 14 years were all positive, as compared with only 85.5% positive in the children of lepers of the same age. (3) 10 healthy adults without history of leprosy. Of these all were positive, though one was only slightly positive.

A positive reaction is indicated by an inflamed, button-like induration at the point of leprolin inoculation, which does not appear when the result is negative. This is explained below in detail.

It must be clearly understood that the specific nature of the test is shown not by the positive results, but by the negative results obtained in leprous cases of the cutaneous type. Suspensions of other acid-fast bacilli, such as Koch's bacillus, Stefansky's bacillus (*M. Lep. mur.*), etc., give positive results in non-lepers and also in leprous patients both of the neural and cutaneous type. Hansen's leprolin gives positive results, varying in degree, in many non-lepers, more strongly positive results in neural cases of leprosy, and negative results in skin cases. The leprolin

test is therefore not entirely dependent on an allergic condition as is the tuberculin test in tuberculosis, though in cases of leprosy the reaction may possibly be increased or diminished by the action of specific immunity or of allergy. The objection may be raised that positive results in non-lepers are due to their having unknowingly been subjected to small infections, which have produced some specific immunity. To prove or disprove the validity of this objection, we are arranging to have the test tried out in a country where leprosy is non-endemic.

With non-leprosy subjects, negative results are more common in children, especially very young children, than in adults.

The strongest reactions with the leprolin test are in patients with indurated, raised, erythematous lesions of the nerve type, *i.e.*, the tuberculoid lesion of Klingmüller (1900, 1930) and others, which is very common in Japan and N. India, but less familiar in some other endemic countries. Clinically the indurated nodule produced by the intradermal injection of Hansen's bacillus suspension resembles a small lesion of this type (see Figs. 1 to 6). Histologically there is a difference. In the former, if we examine it a few days after inoculation, we find an extensive hyaline area at the centre, which may or may not become necrotic, and round this there is a dense cellular infiltration, in which it is difficult or impossible to find bacilli. In the natural lesion the granuloma is arranged in a cord-like form round the vessels and nerves, and instead of the large hyaline area there are giant cells, though occasionally small hyaline areas are found. This difference is accounted for by the trauma caused by the needle and the massive number of bacilli suddenly introduced by inoculation.

TECHNIQUE.

Preparation of Leprolin.—We have adopted the following technique in preparing leproma suspension for the leprolin test. Many cases of the C₃ type have thickened pendulous ear lobules which have a very high content of lepra bacilli. These patients are generally eager to have their ears trimmed. This is done as follows: After sterilising the skin of the ear lobe with tinct. iodi, a special curved clamp is applied, leaving exposed on its conclave margin the superfluous tissue. This tissue is removed with a sharp knife and placed in a Petri dish. Pure carbolic is applied to the cut surface and the clamp removed. Several pieces of tissue are removed in a similar manner from other

patients. These are boiled in water for 45 minutes and then cut up into small pieces, which are dried for a few hours under a fan and thereafter in a vacuum dessicator over pure sulphuric acid. The dried material is ground up to a fine powder in a glass mortar and stored in a dessicator.

In preparing the suspension, 0.4 gramme of the dry powder is ground up with about 10 c.c. of saline ; the fluid suspension is pipetted off ; the solid residue in the mortar is again ground up with saline, and the fluid suspension pipetted off and added to the rest of the suspension, this process being repeated three or four times. The whole suspension is then shaken up in a large test tube and allowed to sediment for 10 minutes, after which the fluid is again pipetted off, the sediment being discarded. Saline is added to make 100 c.c. along with 0.5% carbolic. The suspension is then made up in 1 c.c. ampoules which are sealed and heated at 120 deg. C. for half-an-hour. This forms Hansen's (H) leprolin.

The control leprolin is made by the same method, the spleen and liver of a rat which had been inoculated intra-abdominally with rat leprosy five or six months previously, being used in place of the lepromatous skin. This forms the Stefansky (S) leprolin.

It is difficult to understand leprolin accurately, as in neither of the organisms used is an *in vitro* culture yet available. For practical purposes, however, the above technique is sufficiently accurate. This is shown by making a series of intradermal tests with full strength standard leprolin and dilutions 1 in 2, 1 in 4, 1 in 8. While the stronger suspensions give a somewhat stronger reaction, the difference in the reaction is not in proportion to the difference in dilution, 1 in 8 giving + where the full strength gives ++. For the sake of uniformity, however, we keep a standard smear of each suspension, which has been prepared by spreading out a standard loopful over a given area of microscopic slide. When fresh leprolin is prepared, a similar loopful of the suspension is spread over an equal area of slide and the concentration of bacilli compared. No attempt at counting the bacilli is made, but it is possible to tell whether the numbers are approximately equal. By making several such examinations before diluting the suspension, it is possible to adjust the strength by adding more or less saline.

It is important in preparing the powder for leprolin to make a large amount at one time, so as to ensure, as far as possible, uniformity of results.

The usual dose used for inoculation is 0.2 c.c. Care should be taken that it is injected into and not under the skin.

Reading the Results.—In doing this it must be remembered that even in cutaneous cases there is slight serious effusion due to local irritation, which lasts a few days. The actual results should be read off at weekly intervals for six weeks after inoculation. Four criteria may be used : (a) The size of the red flush round the point of inoculation ; (b) the feeling of a raised area on passing the finger over the infiltrated spot ; (c) the button-like feeling of induration on picking up the affected skin between the finger and thumb ; (d) desquamation, vesication, and pustulation in the more severe reactions.

The degree of reaction is indicated as follows :—

- (negative) with no reaction showing.
- + (doubtful) slightly raised skin felt on passing the finger over the area, slight erythema noticeable in light coloured skins.
- + (one plus) definitely raised skin, erythema and induration to the extent of 5 mm. diameter.
- ++ (two plus) the same as the last with induration up to 10 mm. diameter.
- +++ (three plus) as the last with pustulation at the centre.

Those who carry out the test in all types of cases will soon learn how to divide their results into the five groups. Some idea can also be gained from the photographs, Figs. 1 to 6, which have been enlarged to life size. The reaction frequently is definite by the 7th day, though it may be delayed till the 21st day. Thereafter it takes 1 or 2 weeks to reach its maximum.

The reactions to Hansen's and to Stefansky's leprolin are similar in type, though they may vary in degree.

EXPERIMENTS.

Six experiments with the leprolin test are recorded in this paper. The first of these was carried out with the single leprolin test, using H leprolin alone. In experiments 2 to 4, the double leprolin test was used with both H and S leprolin. Unfortunately, the former of these had a much lower bacillary content than the latter, and thus in cases in which the disturbing specific factors connected with leprous infection were absent (*i.e.*, in non-lepers), it tended to give a weaker reaction.

The sixth experiment shows the effect of injections of H. leprolin filtrates and of a leprolin prepared from Kedrowsky's bacillus.

Experiment (1).—The leprolin test was carried out in 84 children of leprous parents in the Purulia Leper Home. These are divided into 5 series : (a) Inmates of a home for the

healthy girls of lepers. The girls themselves did not show any signs of leprosy, though some of them had in the past shown such signs. (b) Infants in a nursery connected with the last-mentioned home. These were free from signs of leprosy. (c) Inmates of a boys' home similar to that of the girls. (d) Girls under observation, *i.e.*, showing slight signs of leprosy, though negative to bacteriological examination. (e) Boys under observation, similar to the last.

This experiment was carried out with the single leprolin test, only H leprolin being used.

The results are shown in Table I.

TABLE I.

	+++	++	+	±	—	Total	Average age in years
(a) Healthy girls	15 ; 42.8%	1 ; 2.8%	12 ; 34.2%	5 ; 14.2%	2 ; 5.6%	35	13.4
(b) Children in nursery	0 ; 0%	1 ; 12.5%	0 ; 0%	1 ; 12.5%	6 ; 75%	8	3.5
(c) Healthy boys	3 ; 15%	3 ; 15%	5 ; 25%	7 ; 35%	2 ; 10%	20	11.2
(d) Observation girls	0 ; 0%	2 ; 13.3%	5 ; 33.3%	1 ; 6.6%	7 ; 46.6%	15	12.2
(e) Observation boys	1 ; 66.6%	1 ; 16.6%	2 ; 33.3%	0 ; 0%	2 ; 33.3%	6	11.1
TOTAL	19	8	24	14	19	84	

In series (a), the average age of which was 13.4 years, there were only two negatives, while 42.8 per cent. gave very strong positives. These reactions are seen to be stronger than are generally found in cases which have not been in contact with leprosy (compare Tables II and III), and may probably be taken as an indication of increased resistance to leprosy.

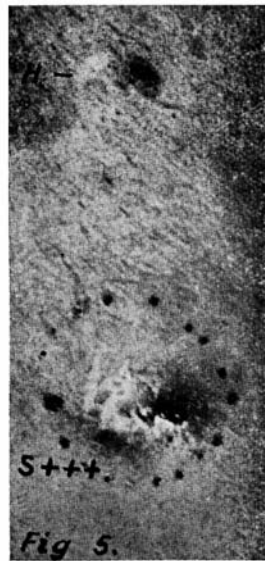
In series (b), *i.e.*, the nursery children, 6 out of 8 were negative. The other two were a child of three years of age with ++, and a child of six years, with +.

Series (c) shows a slightly higher number of negatives, but this may be accounted for by the lower average age.

The last two series (d) and (e) show a very different condition, having respectively 46.6 and 33.3 per cent. of negatives. Apparently they contain the less resistant children.

This experiment seems to indicate that the leprolin test may be of value in making a prognosis in the case of leprous contacts, whether they show slight signs of leprosy or not. These results are similar to those in Chiyuto's first group.

If the negative leprolin test in infants indicates low resistance to leprosy, it helps to explain the generally



FIGS. 1-5. H=test made with Hansen's leprolin, S=test made with Stefansky's leprolin. The dotted lines indicate the limit of induration as felt by picking up the skin between finger and thumb.

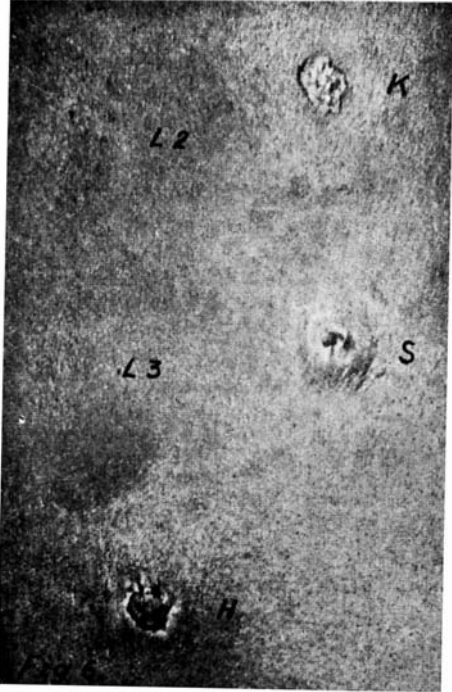


FIG. 6. Shows the leprolin reaction.

- K = test with leprolin prepared with Kedrowsky's culture.
 S = " " " " Stefansky's "
 H = " " " " Hansen's "
 L₂ = " " filtrate from suspension of Hansen's bacillus
 filtered through an L₂ Chamberland candle.
 L₁ = " " filtrate from suspension of Hansen's bacillus
 filtered through an L₃ candle.

acknowledged greater frequency of infection in children than adults. We have discussed below the possible connection between this and the types of disease.

Experiment (2).—Twenty-nine children, average age 3.4 years, at a children's clinic in Calcutta were inoculated, the double test with H and S leprolins being used. The results were as follow :—

Result	H leprolin	S leprolin
—	15	5
+	7	10
+	5	7
++	2	4
+++	0	3

Among the 21 children of three years old and under there were 15 negative to H leprolin, *i.e.*, 76.2 per cent. Of the 8 who were over three years none were negative to H leprolin. Of the 5 giving negative results to S leprolin 4 were under one year and 1 was 18 months of age. It was noticed, however, that 3 children gave unusually high results, viz :—

No. 9,	age 3,	H leprolin	+	S leprolin	+++;
" 18,	" 3,	" "	++,	" "	+++;
" 19,	" 1,	" "	++,	" "	+++.

On enquiry it was found that numbers 9 and 19 were sisters, and on examining their relations, a single anæsthetic macule, diagnostic of leprosy, was found on the body of their father. Careful enquiry, however, failed to reveal any other sign of leprosy among relations or other contacts. It should be mentioned that those attending this children's clinic are drawn from the homes of coolies working in a large industrial concern. A careful survey of the labour staff of this factory two years previously showed 0.75 per cent. of leprosy.

It was noticed that almost without exception the result of H leprolin bore a definite proportion to that of S leprolin, the former being one point lower than the latter except when both were negative. This is accounted for by the fact that the former contained fewer bacilli than the latter. Thus the two children who gave ++ with H leprolin gave +++ with S leprolin, the other child giving +++ with S leprolin (the sister of one of the latter) was an exception, and gave only + with H leprolin.

This experiment suggests that reaction to Hansen's bacilli, when it occurs in non-lepers, is generally not due to previous contact with human leprosy, any more than a positive reaction to Stefansky's bacillus is due to previous contact with rat leprosy, but is of the nature of a non-specific reaction to acid-fast bacilli. Conversely, the

lowered or absent reaction to Hansen's bacillus in very young children is of the same nature as the lowered or absent reaction to rat leprosy organisms in such children.

Experiment (3).—We found the same relationship between H leprolin and S leprolin in six older patients who were sent to our leprosy clinic with a mistaken diagnosis of leprosy. The following table gives the particulars:—

TABLE III

	Name	Age	Disease	H leprolin	S leprolin
1.	Surju	45	nil	+	++
2.	C.N.	11	Lichen	—	+
3.	A.M.	30	„	++	+++
4.	A.D.	35	Syphilis	+	++
5.	Akhay	32	„	+	++
6.	Bishnu	28	„	+	++

From this, as well as from the previous experiment, it is seen that the degree of reaction to both H and S leprolin varies in strength in non-leprous individuals.

As is mentioned above, the S leprolin had a higher bacillary content than the H leprolin, and therefore gave a stronger reaction; but there is a definite relation between the reactions to the two types of bacilli, a stronger or weaker reaction with the one being linked respectively with a stronger or weaker reaction with the other.

Experiment (4).—In this experiment the double leprolin test was carried out in three cases of neural and two cases of cutaneous leprosy. The readings recorded week by week are given in Table IV.

TABLE IV

No.	Name	Age	Type	H Leprolin Reading in days after injection				S Leprolin Reading in days after				
				7	14	21	28	7	14	21	28	
1	S.R.	36	N ₁	—	+	+	++	+	+	+	+	(See Fig. 1)
2	R.M.	32	N ₁	+	+	+	++	+	+	+	++	(See Fig. 2)
3	D.S.	21	N ₁	+	+	++	++	+	++	++	++	(See Fig. 3)
4	M.K.	32	C ₁	+	+	+	+	+	++	++	++	(See Fig. 4)
5	K.U.	34	C ₂	+	—	—	—	+	++	++	+++	(See Fig. 5)

We see here that in neural and cutaneous leprosy the relative degree of reaction given with H and S leprolin is different from the relative degree of reaction given in non-lepers, as shown in Tables II and III.

In case 1 (see Fig. 1) the H reaction is stronger than the S, though the former took a longer time than the latter to develop its full strength. In cases 2 and 3 (see Figs. 2 and 3)

they are equal. In case 4 (see Fig. 4), one of the slight cutaneous leprosy, S is stronger than H, as it is in non-lepers. In case 5, a C₂ case, in spite of a +++ reaction with S leprolin, the H leprolin gave a negative result.

Thus, by carrying out a double test with H and S suspensions, we have :—

- (a) in neural cases a relatively strong H leprolin reaction,
- (b) in cutaneous cases a relatively weak or negative H leprolin reaction,
- (c) in non-lepers an intermediate place, the two reactions being approximately equal, if the bacillary content of the suspensions is the same.

The weekly readings show that the H leprolin reaction came on more slowly than the S leprolin reaction. Thus the former was at first weaker than the latter, while in the fourth reading it had become stronger. This is found in most neural cases of leprosy, but not in non-lepers.

This would seem to show that, while in non-lepers the positive results with both suspensions indicate a non-specific reaction due to natural immunity, in neural cases there is in addition to this an element of acquired immunity or allergy which supplements the natural reaction to H leprolin and makes it stronger. This factor, however, takes longer to act than the non-specific reaction. Hence the delay of increased reaction with H leprolin in these cases. From this we may argue that there is in cutaneous cases an analogous force acting in the opposite direction, which removes or cancels out the natural reacting power.

Experiment (5).—In this experiment the double leprolin test was carried out in 120 cases of all types of leprosy at the Gobra Hospital and the Calcutta School of Tropical Medicine leprosy clinic. The results with H leprolin, classified under the subtype of case and strength of reaction, are shown in Table V.

TABLE V

Subtype	Maximum reading of test					Total
	+++	++	+	+	—	
N ₁ ...	11	14	7	3	1	36
N ₂ ...	8	11	12	0	0	31
N ₃ ...	1	0	0	1	0	2
C ₁ ...	0	0	4	4	8	16
C ₂ ...	0	0	2	1	23	26
C ₃ ...	0	1	0	0	8	9
TOTAL ...	20	26	25	9	40	120

There are 86.2 per cent. of negatives and doubtfuls in the cutaneous cases, but only 7.2 per cent. in the neural cases. There is only one completely negative among the neural cases, and this case when re-inoculated with a slightly stronger leprolin gave a $\bar{+}$ result. Notice that in this case the reaction to leprolin was only $+$.

While advanced cutaneous cases almost always give a negative result with H leprolin, it will be noticed that one C₃ case gave a positive. This nodular case had been given a prolonged course of intravenous injections of 1 per cent. mercurochrome. This, after the first few injections, improved the patient's condition, but later it caused necrosis, liquefaction, and bursting of cutaneous and subcutaneous nodules producing lesions similar to those found in very strong reactions to leprolin. In spite of this the general condition of the patient remained good. It was then found that H leprolin produced a $++$ reaction. Unfortunately this test was not carried out before the course of mercurochrome. The above would seem to indicate that this line of treatment had increased the patient's resistance to the disease.

The other cutaneous cases giving positive H leprolin tests were chiefly patients in excellent health making satisfactory progress towards recovery.

In some other cases, which were at first supposed to be of the neural type and in which the routine bacteriological examination had been negative, the H leprolin test was negative; but on repeated, very careful bacteriological examinations, lepra bacilli were found in the skin and nasal mucosa, showing them to be actually cutaneous cases. This shows the usefulness of the test in classifying cases.

Experiment (6).—A quantity of H leprolin was divided into three portions, *a*, *b* and *c*; *a* was filtered through a Seitz filter, *b* through a Chamberland L₂ filter, and *c* through an L₃ filter. Eight patients suffering from different types of leprosy were each inoculated with 0.2 c.c. of each of these filtrates, and also at the same time with H leprolin, S leprolin, and a leprolin prepared from Kedrowsky's so-called culture of Hansen's bacillus. None of the cases reacted to any of the filtrates; all of them, both neural and cutaneous types, reacted strongly to both S leprolin and Kedrowsky's suspension; the neural cases reacted to H leprolin, but the cutaneous cases did not (see Fig. 6).

This experiment confirms Hayashi's findings, and shows that the leprolin is not caused by any filtrable element in

the suspension. It also seems to prove that Kedrowsky's so-called culture is not actually a culture of *M. lepræ*, and that a culture of non-pathogenic acid-fast bacillus (which we believe this culture to be) gives a positive leprolin reaction similar to that produced by Stefansky's bacillus.

DISCUSSION AND CONCLUSIONS.

We have tabulated and explained six series of experiments with the leprolin test and tried to indicate the nature of the test. We also suggest certain lines along which it may be usefully employed. These may be summarised as follows :—

1. Acid-fast bacilli, when injected in suspension intradermally in healthy human subjects, tend to produce at the site of injection a reaction indicated by a raised, red, indurated swelling, from 5—20 mm. in diameter, sometimes followed by vesication, pustulation, and loss of tissue, but without noticeable general constitutional disturbance. This is generally true of both pathogenic and non-pathogenic organisms. It applies also to suspensions of leproma taken from lepers, and to suspensions of tissue of rats suffering from rat leprosy, if these contain large numbers of acid-fast bacilli.

2. Experiments 2 and 3 seem to indicate that in those who have not been infected with leprosy, the degree of reaction to Hansen's and to Stefansky's bacilli is approximately the same, the one or the other being greater according to the number of bacilli inoculated. The degree of reaction is not, however, in exact proportion to the number of bacilli, a dilution of 1 in 8 giving a reaction of about half the strength given by an undiluted suspension.

3. In very young children the reactions both with Hansen's and Stefansky's leprolins are as a rule either negative or very much less than those observed in adults. If this indicates weak resistance to leprosy, it will help to explain the frequency of leprous infections in early childhood compared with those in adult life.

4. In cases of nerve leprosy with few bacilli, especially in those of the macular type, the reaction to Hansen's leprolin is increased, but that to Stefansky's leprolin remains the same. The increased reacting power is, however, delayed in showing itself.

5. In cases of cutaneous leprosy with numerous bacilli, the reaction to Hansen's leprolin is diminished or absent, but that to Stefansky's leprolin is apparently not diminished.

6. This relative increase and diminution of reaction to Hansen's leprolin seems to indicate the presence of a specific factor connected with immunity or allergy.

It is generally acknowledged that neural leprosy, especially of the macular type, is associated with a higher degree of resistance to the disease than cutaneous leprosy, the lesion of the former showing few or no bacilli and those of the latter many. For this reason the strong leprolin reaction in neural leprosy may be taken to indicate comparatively strong resistance to the disease.

7. We cannot agree with those who consider that a positive test, carried out with H leprolin alone, is, in the case of non-lepers, an indication of a previous infection with leprosy. Apparently in non-contacts the reaction may be present or absent, and, when present, may vary in strength to a considerable degree. Using the double leprolin test, however, with two leprolins of approximately equal bacillary content, a reaction with H leprolin stronger than that with S leprolin justifies a suspicion of immunization through previous infection with leprosy in the case of non-lepers.

8. A strong positive leprolin reaction may be taken as an indication for a favourable prognosis, as it indicates resistance to the infecting organism. This would seem to indicate that any drug or line of treatment which changes a negative reaction with H leprolin into a positive, or strengthens the degree of reaction, may be looked upon as of therapeutic value in leprosy.

9. Experiment 6 suggests that the leprolin test may be used to show whether or not a culture obtained from leprotic tissue is a true culture of *M. lepræ*.

SUGGESTIONS FOR FURTHER INVESTIGATIONS.

1. Improvement in preparation of leprolin. It may be argued from the statements made in paragraphs 4 and 7 of the *Discussion* that a dilution of H leprolin, too weak to give a reaction in a non-contact, should react positively in a case of neural (N_1 or N_2) leprosy, the signs beginning to appear later than with a stronger suspension. S leprolin of equal bacillary content would, however, fail to give a reaction. Such a modification of the leprolin test would require delicate uniform standardisation. If recent claims to have cultured *M. lepræ* are confirmed, it may be possible to obtain more uniformly standardised leprolin prepared from such a culture.

2. Acid-fast bacilli, supposed to be *M. lepræ*, have been found by various workers in newly-born infants. It is generally agreed that children separated from their leprosy mothers at birth and kept free from leprosy infection thereafter, seldom, if ever, develop leprosy. If young infants have low resistance to leprosy, as is indicated by experiments 1 and 2, why does the pre-natal infection not develop into active leprosy in children who have been protected from post-natal infection? Is it possible that immediately after birth there is, as in certain other diseases, a short period of high resistance, followed by a longer period of low resistance? This is a matter for careful investigation.

3. Experiments 2 and 3 seem to show that the degree of reaction to Hansen's bacilli, and therefore presumably resistance to leprosy, varies in non-contacts. What is the reason for this? Is this reacting power hereditary; is it connected with families, tribes or races; is there a sex variance; is it raised or lowered by the general health of the patient; is it changed by disease, diet or habits?

4. Are those who have naturally an absent or weak leprolin reaction before contact with leprosy more liable to develop leprosy after infection than those who give a strong leprolin reaction previous to infection?

5. Our experiments seem to show that in non-contacts equal quantities of Hansen's and Stefansky's bacilli produce approximately equal reactions. We have shown that in cases of neural leprosy there is an increased reaction with H leprolin. Our experiments show that a stronger reaction to Hansen's bacilli than to an equal number of Stefansky's bacilli is characteristic of neural leprosy and also probably of increased resistance to leprosy. Are we justified in assuming, however, that those non-contacts who give a strong reaction to S leprolin (and also therefore to H leprolin) are more resistant to human leprosy than those who give a weak reaction or none at all? Also, conversely, are we justified in assuming (as we have been inclined to do in this paper) that very young children with weak or negative reactions to both forms of leprolin have a weak resistance to leprosy?

6. If a weak or negative leprolin test in early childhood indicates weak resistance to leprosy, then infants in contact with infectious cases are likely to become highly infected before they reach the more resistant condition found in maturer age. A high degree of infection also causes a weak leprolin test, which, we assume, indicates low resistance to

leprosy. There is thus primarily low resistance due to the age factor, and secondarily low resistance maintained by the heavy infection which has taken place during infancy. We should therefore expect such cases to develop the more severe cutaneous form of leprosy. If our hypotheses are true we may take it that high infection in infancy and early childhood leads to the cutaneous type of leprosy with numerous bacilli; whereas later infection, taking place after greater natural resistance has developed, is more likely to produce the less severe neural type. This is a matter waiting for confirmation or disproof. We would suggest careful enquiry as to the likely age of infection in every case, and comparison of this with the type of disease.

7. Attention is drawn to the interesting case (mentioned under experiment 5) of C_3 leprosy with a +++ leprolin test after treatment with mercurochrome, and to the remarks in paragraph 8 of the *Discussion*. This suggests a wide avenue for investigation.

8. In this paper we have associated a negative leprolin test, as found in C_2 and C_3 cases of leprosy, with low resistance to leprosy, because in these cases there is comparatively little reaction to *M. lepræ*—whether it be the bacilli which are found in large numbers in such cases, or whether it be the dead bacilli of the leprolin.

How is it then that sometimes with or without special treatment, such cases become bacteriologically negative, though it is only after the disappearance of the bacilli, or when they have become very much reduced in number, that the leprolin test begins to become positive? What then is the relationship of the factor which causes the elimination of Hansen's bacilli to the factor which causes the reaction to H leprolin?

9. This suggests another question: Is there any connection between the condition commonly known as "lepra reaction" and the factor which causes a positive leprolin test? Can it be that, while in C_3 cases this factor is ordinarily absent, under certain circumstances it becomes temporarily present, and that "lepra reaction" is analogous to the positive leprolin test, the bacilli present in the tissues acting in a manner similar to those inoculated in the leprolin test?

If this is so, the debility produced by "lepra reaction" may be responsible for destroying the above-mentioned factor, resulting in the usual spontaneous subsidence of "lepra reaction." This hypothesis is put forward for

investigation as a possible explanation of this mysterious phenomenon for which no satisfactory explanation has yet been offered.

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