

## THE EFFECT OF TREATING LEPROMIN WITH LEPROMATOUS SERUM

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In an interesting experiment, Ridley<sup>(1)</sup> found that serum from two patients suffering from lepromatous leprosy, when added to lepromin, tended to inhibit the normal response to the lepromin test in two individuals suffering from tuberculoid leprosy.

If found to apply generally, this observation would have implications which might considerably modify accepted ideas regarding the immunology of leprosy, and Ridley suggested the desirability of confirming and extending his experiment. This paper reports the findings of one such experiment in Nigeria.

### LEPROMIN

The lepromin was part of a batch of purified lepromin prepared by a modification of Dharmendra's method,<sup>(2)</sup> and in this case actually prepared under the personal supervision of Dr. Lowe.\* It was in use for routine purposes before, during and after the experiment to be described, and was of proved activity. All the lepromin used in the experiment came from one single batch.

### LEPROMATOUS SERUM

Samples of serum from 12 cases of typical lepromatous leprosy were used. It was thought desirable to specify the reaction of the individuals concerned to tuberculin as well as to lepromin, and patients were selected as follows:

Cases 1-3. Patients lepromin-negative and tuberculin-negative, who had had considerable sulphone treatment.

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\* (It should be stated that this lepromin was prepared by the method (short treatment with chloroform) designed to give a lepromin with a good late (Mitsuda) reaction, rather than a good early (Fernandez) reaction.

Ridley's work referred particularly to the early reaction.—J. Lowe.)

Cases 4-6. Patients lepromin-negative and tuberculin-negative, who were in the early stages of sulphone treatment.

Cases 7-9. Patients lepromin-negative but tuberculin-positive.

Cases 10-12. Patients formerly lepromin-negative and tuberculin-negative, but who had shown conversion to positive after B.C.G. inoculation.

#### RECIPIENTS

Each of these 12 samples of serum, when added to lepromin, was injected into 10 individuals whose reaction to lepromin was known. In practice it was found convenient to test two samples simultaneously in the same patient. Sixty recipients were thus sufficient for the whole series of tests. All were leprosy patients, of whom 55 were of tuberculoid type (24 Major, 31 Minor), 3 were indeterminate, 1 was borderline and 1 was lepromatous in type.

#### CONTROL TESTS

Three control tests were considered to be the minimum acceptable. They were as follows:

1. Lepromin diluted with normal saline in place of serum.
2. Lepromin diluted with serum from a healthy individual who was lepromin negative.
3. Lepromin diluted with serum from a healthy individual who was lepromin positive.

These three control tests were made in all 60 recipients, using the same batch of each for the entire series.

#### METHOD

Whether the lepromin used in the experiment was being diluted with saline, control serum, or the lepromatous sera being tested, the same technique was followed, the required volume of lepromin being diluted with an equal volume of diluent.

In its subsequent preparation, Ridley's method was followed closely. The samples of diluted lepromin were incubated simultaneously for  $1\frac{1}{2}$  hours at 37 C., kept overnight in a refrigerator, and used the following day.

All injections were administered on the same occasion, 0.1 c.c. being injected intradermally on the inner side of the upper arm. Each of the 60 recipients received 5 injections, suitably spaced, as follows, from above downwards.

1. Control: Lepromin plus saline.
2. Lepromin plus a test serum.
3. Lepromin plus another test serum.
4. Control: Lepromin plus lepromin negative serum of a healthy person.
5. Control: Lepromin plus lepromin positive serum of a healthy person.

Arranged in this way, comparison between the test sera and controls presented no difficulty.

The early lepromin reaction was read after 24 and 48 hours.

The late reaction was recorded after 7, 14, 21 and 28 days.

To aid objectivity in assessing the degree of response, a minimum of three qualified observers was used on each occasion, the recorded result being their agreed judgment.

In each recipient, the response to the lepromin-saline mixture indicated the normal reaction of the individual at that time to lepromin in a dosage half that usually employed. This response was the standard with which the other four responses had to be compared.

The results may be summarised as follows:

[In the original manuscript the results are given in detailed tables covering four pages. These are here omitted.—Editor.]

## RESPONSE TO LEPROMIN TEST

	<i>Early Reaction</i>			<i>Late Reaction</i>		
	Enhanced	Normal	Diminished	Enhanced	Normal	Diminished
1st control (lepromin-saline) (60 persons)	—	60	—	—	60	—
1st lepromatous serum (lepromin and tuberculin negative; much treatmt.) (10 persons)	5	5	nil	nil	10	nil
2nd lepromatous serum (lepromin and tuberculin negative; much treatmt.) (10 persons)	4	6	nil	nil	10	nil
3rd lepromatous serum (lepromin and tuberculin negative; much treatmt.) (10 persons)	3	4	3	1	9	nil
4th lepromatous serum (lepromin and tuberculin negative; little treatmt.) (10 persons)	2	6	2	1	9	nil
5th lepromatous serum (lepromin and tuberculin negative; little treatmt.) (10 persons)	3	6	1	1	9	nil
6th lepromatous serum (lepromin and tuberculin negative; little treatmt.) (10 persons)	3	5	2	1	9	nil
7th lepromatous serum (lepromin negative and tuberculin positive) (10 persons) ...	3	7	nil	nil	9	1
8th lepromatous serum (lepromin negative and tuberculin positive) (10 persons) ...	4	5	1	nil	9	1
9th lepromatous serum (lepromin negative and tuberculin positive) (10 persons) ...	1	8	1	2	8	nil
10th lepromatous serum (lepromin positive and tuberculin positive) (10 persons) ...	7	3	nil	nil	10	nil
11th lepromatous serum (lepromin positive and tuberculin positive) (10 persons) ...	5	4	1	nil	9	1
12th lepromatous serum (lepromin positive and tuberculin positive) (10 persons) ...	3	7	nil	nil	10	nil
2nd control (lepromin neg. normal serum) (60 persons) ...	25	33	2	7	51	2
3rd control (lepromin pos. normal serum) (60 persons) ...	25	32	3	15	45	nil

## COMMENTS

The results of both early and late lepromin reactions follow the same general pattern, though there are differences in detail. The early reaction is not easy to read in dark-skinned individuals, and in Nigeria the late reaction has proved to be the more reliable.

In this experiment the late reaction yielded results which are quite unequivocal, and can be stated as follows:

1. Out of 120 lepromin tests in which the antigen used was lepromin diluted with an equal volume of lepromatous serum, 111 showed a normal reaction and 9 showed minor degrees of deviation. Out of 120 controls undertaken simultaneously in the same patients, and in which lepromatous serum was replaced by normal serum, 96 showed a normal reaction and 24 showed minor degrees of deviation.

2. Of the 9 abnormal reactions in the test series, 6 showed enhancement of the normal reaction, and 3 showed diminution. Of the 24 abnormal reactions in the control group, 22 showed enhancement and 2 showed diminution.

3. The 9 deviations in the test series were not exhibited by a single lepromatous serum, or by a group of sera, but were distributed through 8 of the 12 sera tested. Duration of treatment, reaction to tuberculin, and even reaction to lepromin itself on the part of the donors thus did not appear to influence the effect of their serum on the lepromin test.

4. With a diminution in response appearing in only 3 out of 120 tests with lepromatous sera, as against two in the same number of control tests, it cannot be considered that any of the 12 lepromatous sera tested had an inactivating action on lepromin.

Similar observations can be made in respect of the early lepromin reaction. Although here deviations were more numerous, they were always of minor degree.

1. Out of 120 test reactions, 66 were normal, 43 showed enhancement and 11 showed diminution. Out of 120 control reactions, 65 were normal, 50 showed enhancement and 5 showed diminution. Thus 54 deviations in the test series compared with 55 in the control.

2. Deviations among the test series were distributed throughout the 12 test groups with no concentration in respect of any single serum or group or sera.

3. No relationship could be detected in the test series between deviations in the early reaction and deviations in the late reaction. No case either of enhancement or diminution in the early reaction reappeared as such in the late reaction.

4. These observations strongly suggest that deviations were non-specific in character.

5. The relatively large number of enhanced early reactions both in the test and control groups is noticeable. It is not surprising. The addition of serum to lepromin inevitably introduces a factor capable of stimulating a non-specific early response.

6. One curious finding was that 6 of the 25 lepromin-positive serum controls exhibiting an enhanced early reaction also exhibited an enhanced late reaction. It is probably without significance.

#### CONCLUSION

This experiment yielded no evidence of a specific inactivating action on lepromin on the part of 12 samples of serum taken from 12 cases of lepromatous leprosy.

#### SUMMARY

In order to test the suggestion that the serum of patients suffering from lepromatous leprosy may inactivate lepromin, samples of serum from 12 such patients were added to lepromin, and each tested in 10 individuals. The 12 donors were selected in relation to duration of treatment, reaction to tuberculin, and reaction to lepromin itself. Controls tested in each recipient consisted of lepromin diluted in the same proportion with normal saline, with serum from a lepromin-negative healthy individual, and with serum from a lepromin-positive healthy individual.

Both early and late lepromin reactions yielded similar findings, but as usual in dark skinned individuals, the late reaction gave a clearer picture. Using the reaction to the lepromin-saline mixture as a basis of comparison, out of 120 tests with lepromatous serum, 111 conformed to the normal, and of the 9 deviating results, all of which were small in degree, only 3 exhibited diminution in response. Of 120 control tests with normal serum, 96 gave normal results, 22 showed enhancement and 2 diminution.

All the evidence suggests that deviations were non-specific in character. It cannot be considered that any of the 12 lepromatous sera tested had an inactivating action on lepromin. The duration of treatment, the reaction to tuberculin, and the reaction to lepromin on the part of the donors had no detectable influence on this finding.

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