IMMUNOLOGY OF LEPROY

Antigens derived from Mycobacterium lepra in oily suspension* by

DRS. J. M. M. FERNANDEZ, Chief of the Women’s Section,
Ex-Professor of Clinical Dermatology of the
Medical Faculty of Rosario

and

RODOLFO MERCAY, Chief of Clinic of the Service**

The investigations which form the basis of the present paper were undertaken to determine whether the use of an oily base would modify the antigenic activity of Mycobacterium lepra in the human organism. In other words, we have tried to establish whether lepromin in oil behaves in a different manner from the usual lepromin which has a water base.

We were led to undertake the present study by the surprising results obtained by Saenz and Canetti (1) — although in a different field—who inoculated rabbits intratesticularly with killed Koch bacilli suspended in liquid vaseline. The authors proved that the use of this vehicle increases the toxicity of Koch’s bacillus to the extreme of producing lesions at a distance — in the lungs — of extraordinary gravity. Inspired by these results, we outlined a plan of work limited to the field of immunology covering two series of investigations:

(a) In experimental animals (guinea pigs) with oily suspensions of the bacillus of Koch.

(b) In patients with leprosy with oily suspensions of the bacillus of Hansen.

As regards the first series, the results will be reported later; the results of the second are described in the present paper.

MATERIAL OF STUDY AND TECHNIQUE EMPLOYED

The subjects of our investigations were patients suffering from leprosy of the lepromatous and neural (tuberculoid) types as well as a control group consisting of persons supposed to be free from this disease.

The antigens under study and control substances were injected intradermally in doses of from 0.1 cc. to 0.2 cc.

Readings of the reactions were made after forty-eight hours and at the third week. In some cases, readings were also made after eight hours, twenty-four hours, and in the sixth week.

*Translated from the Spanish by Dr. J. N. Rodriguez.

**From the Leprosy Service of the “Professor Enrique P. Fidanza” Hospital, Carrasco, Rosario, Argentina. Chiefs: Prof. Salomon Schajman, Prof. J. M. M. Fernandez.
With respect to the evaluation (or reading) of the results, the usual criteria were accepted, all early reactions which within forty-eight hours showed an erythematous infiltrated halo with a diameter of not less than 10 mm. being considered as positive. Also considered positive were all late reactions producing at the third week a papule, nodule, or plaque of a diameter of 5 mm. or greater.

Adopting this procedure we studied the cutaneous reactivity of the following antigens, making comparison in each case with the integral, bacillary, or purified lepromin:

1. Suspension of heat-killed Hansen bacilli in liquid vaseline.
2. Suspension of the same killed bacilli in olive oil.
3. Suspension of the same in benzylic esters of chaulmoogra oil (neochaulmestrol).

For control, we used:
2. Sterilized liquid vaseline.
3. Sterilized olive oil.
4. Sterilized benzylic esters of chaulmoogra oil.
5. Integral and bacillary lepromin.

RESULTS

I. Suspension in liquid vaseline of heat-killed Hansen bacilli (Lepromin oleosa, L.a.v.)

1. Preparation of antigen.

To obtain the amount of pure bacilli necessary to prepare the suspension in liquid vaseline, the method of Fernandez-Olmos (2) or that of Dharmendra (3) may be used. In the present study we followed the second method which is as follows:

(a) Secure nodules rich in bacilli and boil in water for thirty minutes. Grind in a mortar in chloroform; remove the chloroform with a pipette, and place in a ("probeta") test tube. Add more chloroform to the residue, grind and pipette off. Repeat the operation until bacilli are no longer found in the residue. Separate the chloroform pipetted off and discard the residue.

Smears from the chloroform suspension should show bacilli only and no tissue detritus.

The chloroform is evaporated in a water-bath, leaving behind a residue consisting of lipoids and bacilli. Suspend this residue in ether and centrifuge at the rate of 3,000 revolutions per minute. The lipoids are dissolved in the supernatant fluid while the bacilli settle at the bottom; pipette off the ethereal
extract. To remove the lipoids completely, the bacillary residue is again suspended in ether and centrifuged. The bacillary sediment is separated and dried in an oven. The deposit thus obtained may be compressed into a dry block consisting of pure bacilli.

(b) The material consisting of pure and desiccated bacilli thus obtained is placed in a mortar and ground to a fine powder. To this powder is added liquid vaseline in small amounts and with continuous grinding. Thus a homogeneous and stable suspension is obtained. The product is placed in ampules and autoclaved for one-half hour at 120° C.

The concentration of the suspension we employed in these experiments was 20 mgm. of powdered bacilli in 100 cc. of liquid vaseline, or 1 in 5,000.

2. Biological study

Intradermal injection of 0.1 cc. of this antigen into patients with leprosy of the tuberculoid and lepromatous types and in individuals apparently free from leprosy produced the following results:

(a) Cases of tuberculoid leprosy

In twenty-four hours: Frank positive reaction consisting of an erythematous plaque of a diameter practically almost always larger than 20 mm., very much infiltrated and with diffuse borders. At times this plaque presented a vesicle at its central portion which was surrounded by a wide erythematous zone.

In forty-eight hours: The process became intensified with increase of infiltration and a central necrotic zone was noted in some cases (Figure 1).

At the third week: A deeply infiltrated plaque was observed of about the same size as that seen after forty-eight hours, frequently with central ulceration. Striking features of the late reaction were the hardness and depth of the infiltration (Figure 3).

In all cases studied, the reactions provoked by this oily antigen, both early and late were more intense than those following the usual integral and bacillary antigens in aqueous suspension.

Control intradermal injections with pure liquid vaseline produced the following results:

In twenty-four hours and forty-eight hours in the major-
ity of cases the reaction was negative. Exceptionally, in a few patients there was a discrete early reaction consisting of a pink papule. At the end of the first week, on the other hand a reaction appeared consisting of a raised nodule or plaque much infiltrated and rose-colored. In the third week, there was a nodule or plaque red in color, well localized, hard in consistency and frankly raised above the surrounding skin. This lesion persisted unchanged for many months presenting the characteristics of a small tumor.

(b) Cases of lepromatous leprosy

In a group of lepromatous cases, the intradermal injection of the L.a.v. antigen in clinically uninvolved skin gave the following results:

In twenty-four hours: An erythematosus nodule or plaque was observed, infiltrated, with a diameter of over 10 mm. and with fairly well defined edges. In all lepromatous cases studied so far, the early reaction provoked by this antigen has been approximately one-half as intense as in the tuberculoid cases.

In forty-eight hours: The erythematosus nodule or plaque persisted and at times was intensified (Figure 8).

In the third week: There persisted at the site of the intradermal injection a non-inflammatory nodule or plaque, well localized and rose-colored. Ulceration was not observed in a single case, although the nodule itself persisted for several months.

(c) Individuals considered non-leprous.

In a group of more than 50 adults, considered non-leprous, an intradermal injection of this antigen provoked the following results:

In twenty-four hours: In all cases without exception, there was a frankly positive reaction consisting of a nodule or erythematosus plaque, infiltrated, with a diameter always greater than 10 mm. and at times surrounded by a large erythematous halo.

In forty-eight hours: The positive reaction was increased and in some cases small vesicles or areas of necrosis appeared at the central portion.

In the third week: Markedly infiltrated nodules or plaques were seen, at times accompanied by ulceration at the center. Several months after the injection, a plaque
or nodule, well localized, elevated, non-inflammatory, and rose-colored remained.

Control intradermal tests with pure liquid vaseline, in lepromatous patients as well as in non-leprous persons, produced the same results as in the tuberculoid cases, that is, there occurred a discrete or else absent early reaction followed by the formation of a nodule or plaque, of a tumor-like appearance, rose-colored, with well-delimited borders. Appearing after the first week, it persisted practically indefinitely.

Summarizing this first series of experiments, we have shown that the intradermal injection of a suspension of killed Hansen bacilli in liquid vaseline produces:

(a) In cases of tuberculoid leprosy, a frank early and late reaction, ulcerated at times, always more intense than that produced by the ordinary integral or bacillary lepromin in aqueous suspension.

(b) In cases of the lepromatous type, there is an early inflammatory reaction, less intense than in the tuberculoid cases, but invariably present, and a late reaction of a non-inflammatory character which persists indefinitely, without ulceration.

(c) In persons considered to be free from leprosy, a frank early reaction is obtained, less intense than in tuberculoid cases but on the other hand more intense than that in the lepromatous type, followed by a persistent late reaction which at times leads to ulceration.

Control intradermal injections with pure liquid vaseline do not produce any appreciable early reaction in any cases. On the other hand, a late non-inflammatory nodule of a tumor-like aspect, which persists indefinitely without undergoing ulceration, is produced.

II. Suspension of heat-killed Hansen bacilli in olive oil (Lepromin oleosa, L.a.o.)

1. Production of antigen

To obtain the antigen, the same procedures as were employed in the preceding experiments were followed, the only change being the substitution of olive oil as a vehicle for liquid vaseline.

This antigen, therefore, consists of a suspension in olive oil of Hansen bacilli killed by heat, obtained by extraction with chloroform, and reduced to a fine powder. The concentration in the same, being 1:5,000.
2. Biological study

The former procedure was followed injecting intradermally from 0.1 cc. to 0.2 cc. of the antigen in each of a group of tuberculoid and lepromatous patients, and in individuals supposed to be free from leprosy. Control injections of pure sterilized olive oil and of ordinary integral and bacillary lepromin were used also.

The following results were obtained:

(a) Cases of tuberculoid leprosy

In twenty-four hours: A frankly positive reaction occurred with the same characteristics as those produced by the leprosy antigen in liquid vaseline, although of somewhat less intensity.

In forty-eight hours: More marked reactions were observed; in some cases there was central vesiculation (Figure 1).

In the third week: Marked nodules or plaques were the rule, ulcerated at times, similar to the late reaction provoked by the antigen in liquid vaseline and always of greater intensity, particularly as regards infiltration, than the reactions produced by the ordinary lepromin in aqueous suspension.

After a few weeks: the nodule or plaque persisted but was reduced in size, without signs of inflammation, and showed a marked tendency to be absorbed.

(b) Cases of lepromatous leprosy

In twenty-four hours: Similar to the experience with the antigen in liquid vaseline, there was an evident early inflammatory reaction consisting of a nodule or plaque which was frankly erythematous and almost always surrounded by a reddish halo.

In forty-eight hours: The reaction was less intense.

In the third week: The inflammatory reaction subsided and only a non-inflammatory nodule or plaque remained, of a diminished size and with a tendency to complete absorption.

(c) Individuals considered non-leprous

In twenty-four hours: A frankly positive reaction was the rule consisting of an inflammatory nodule or plaque, surrounded by an erythematous halo and at times exhibiting central vesiculation.

In forty-eight hours: The area of reaction was increased,
both as regards erythema and infiltration. In some instances, there was a zone of central necrosis.

In the third week: Infiltrated nodules or plaques of a reddish-violet color, sometimes ulcerated at the center, were the rule.

Control intradermal injections with pure sterilized olive oil produced in all cases of leprosy an early positive reaction followed in the third week by discrete semi-inflammatory induration and a definite tendency towards absorption. Among the individuals considered to be free from leprosy, the intradermal injection of olive oil produced in some cases a positive early reaction which was not observed in others.

Summarizing this second series of experiments, we can say that the intradermal injection of a suspension in olive oil of heat-killed ground Hansen bacilli produced in all the patients studied an early reaction very similar in its characteristics to that produced by the bacillary suspension in liquid vaseline. The late nodule, however, is less persistent and after a period of time, is reabsorbed, without leaving any trace in those suffering from the lepromatous type and leaving behind scar tissue in patients with tuberculoid leprosy and in individuals considered to be free from leprosy. The scar formation is especially conspicuous in those in whom the reaction was frankly positive in its early stages (that is, in whom there has been a strong early reaction).

Control injections with pure olive oil almost always gave early positive reactions, especially in patients with leprosy, followed by a late induration which was afterwards absorbed.

III. Suspension of heat-killed Hansen bacillus in the benzylic esters of chaulmoogra oil (neo-chaulmestrol) (L.a.ch.)

1. Production of the antigen

The same method of preparation of the antigen was followed as that utilized in Experiments I and II, with the difference that the ethyl esters of chaulmoogra oil (neo-chaulmestrol "Bayer") were substituted for liquid vaseline or olive oil. This suspension, however, was higher than the preceding ones, being of the order of 1:2500.
2. Biological study

(a) Cases of tuberculoid leprosy

In twenty-four hours: There was an intense early reaction, consisting of an infiltrated nodule or erythematous plaque, surrounded by a halo of intense inflammation, in some cases reaching a diameter of 50 mm.

In forty-eight hours: The reaction persisted with the same intensity, frequently accompanied by vesiculation and central necrosis.

In the third week: A nodule of variable size was the rule, often ulcerated.

(b) Cases of lepromatous leprosy

In twenty-four hours: A frankly positive reaction occurred consisting of a nodule or plaque surrounded by an intense erythematous halo from 20-30 mm. in diameter.

In forty-eight hours: The reaction persisted with undiminished intensity, frequently with vesiculation and central necrosis.

In the third week: A nodule smaller in size than in tuberculoid cases was observed.

Control intradermal injections with pure neochaulmos-terol in the majority of cases did not produce any reaction in twenty-four or forty-eight hours and only in a few patients was there observed a discrete inflammatory reaction much less evident than that produced by the antigen under study.

Intradermal tests with aqueous bacillary and integral lepromins were invariably negative (both in the early and late reaction) in lepromatous cases and positive in tuberculoid, but the intensity of the reaction in the latter with the usual lepromin is always less in tuberculoid cases than that observed in the reaction following the use of the antigens under study.

As a summary of this series of experiments, we can say that the intradermal injection of a suspension of heat-killed Hansen bacilli in benzylic esters of chaulmoogra provoked in all patients studied a frankly positive early reaction, a result not observed with control intradermal injections with pure esters nor with aqueous integral and bacillary antigens.
IV. Suspension of heat-killed Eberth bacilli in oil or vaseline (E.v.)

1. Preparation of antigen

A 24-hour culture of Eberth bacilli on agar is used. Wash off culture with physiologic salt solution, heat washings to 60°, centrifuge, wash and dry. Grind in a mortar to a fine powder and mix with liquid vaseline. The strength of the oily suspension was 0.5:1000.

For control a suspension of powdered Eberth bacilli prepared in the same way in normal salt solution was used.

2. Biological study:

Tests were conducted in the same manner as in the preceding experiments on patients of the lepromatous and tuberculoid types and on individuals considered to be non-leprous.

(a) Cases of tuberculoid leprosy

In twenty-four hours: A frankly positive reaction followed the injection, consisting of a nodule or plaque, pinkish, surrounded by an ample erythematous halo.

In forty-eight hours: The reaction persisted with the same intensity.

In the third week: The reaction was limited to a non-inflammatory papule, pinkish, markedly infiltrated, which persisted without change for months.

(b) Cases of lepromatous leprosy

In twenty-four hours: Positive reaction consisting of an infiltrated nodule or plaque, surrounded by an intense inflammatory halo.

In forty-eight hours: The previous reaction persisted but with diminished intensity.

In the third week: Simple non-inflammatory nodule, pinkish, much infiltrated.

(c) Individuals considered non-leprous.

In twenty-four hours: In all cases, a positive reaction was observed consisting of an infiltrated nodule or plaque, erythematous, and at times surrounded by a pinkish halo.

In forty-eight hours: The reaction persisted, sometimes increased and in other cases decreased in intensity.

In the third week: A non-inflammatory nodule or plaque remained, much infiltrated.

Control injections of saline and aqueous suspensions of Eberth bacilli provoked a few weak reactions in twenty-four and forty-eight hours; no evidence of reaction was present in the third week.
Judging from the results of this series of experiments we can say that a suspension of Eberth bacilli in liquid vaseline provokes in twenty-four and forty-eight hours, in all cases, whether or not the individual has leprosy, an early fairly marked reaction, of less intensity than that produced by Hansen bacilli in the same vehicle. On the contrary, saline and aqueous suspensions of Eberth bacilli only exceptionally produced an early positive reaction.

V. Complementary investigations

A. Time required for absorption of a papule produced by intradermal injection of normal salt solution, liquid vaseline, and olive oil in patients with leprosy and in non-leprous individuals.

1. In two groups of patients comprising 6 lepromatous and 6 of the tuberculoid type, all adult females of twenty to forty years of age, we performed the McClure and Aldrich test (called by Labbe and Vialle a "cutaneous test for hydrophylia"), using the technic of Urquijo (4). This test is performed by injecting 0.2 cc. of physiological salt solution and determining the time required for the complete resorption of the papule thus formed. The following were the results obtained:

<table>
<thead>
<tr>
<th>Observation</th>
<th>Absorption time: physiological salt solution</th>
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<tbody>
<tr>
<td>Lepromatous cases</td>
<td></td>
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<tr>
<td>Tuberculoid cases (Nt.)</td>
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<tr>
<td>Time in minutes</td>
<td>Time in minutes</td>
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<tr>
<td>1</td>
<td>47</td>
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<td>2</td>
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<td>53</td>
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<tr>
<td>6</td>
<td>54</td>
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<tr>
<td>Average of 6 cases</td>
<td>49.7</td>
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</table>

2. In both lepromatous and tuberculoid cases we produced simultaneously two papules using 0.2 cc. of liquid vaseline and olive oil. Both papules persisted long after the papules produced by the normal salt solution disappeared. In fact, in not one instance could the absorption of the papule produced by oil suspension be demonstrated since it always persisted until after the termination of the experiments.

3. In a group of non-leprous persons the preceding experiments were repeated. None of the papules produced by
the two oils was absorbed in two hours. In 6 individuals those produced by the normal salt solution were absorbed in 58, 57, 60, 66, 95 and 70 minutes respectively for an average absorption time of 71 minutes.

B. Experiments with concentrated bacillary lepromin, in oily suspension (olive oil) and in physiological salt solution.

Two lots of powdered pure Hansen bacilli obtained by extraction with chloroform according to the method of Dhar-mendra were prepared as follows:

(a) Suspension of the bacillary powder in olive oil at a concentration of 2:1000. This was sterilized in an autoclave at 120° C. for half an hour.

(b) Suspension of the bacillary powder in physiological salt solution at the same concentration of 2:1000 to which 0.5 per cent of phenol had been added. This was likewise sterilized in the autoclave at 120° C. for half an hour.

Experiment No. 1. Intradermal injections of two papules with 0.1 cc. of bacillary lepromin suspended in saline provoked in 4 tuberculoid cases an intense local reaction in twenty-four hours at the site of the injection. This reaction consisted of an erythematous area over 20 mm. in diameter with a focal reactivation of the pre-existing cicatrix from the previous lepromin reaction. Old tuberculoid lesions in regression were also reactivated as evidenced by a perifocal erythematous and infiltrated halo, and in the majority of the cases a general reaction manifested by a feeling of fatigue, or fever, and a sensation of heat over the reactivated lesion was produced.

Experiment No. 2. Intradermal injection of two papules with 0.1 cc. of bacillary lepromin in oily suspension in 4 other tuberculoid cases provoked in twenty-four hours only an intense local reaction consisting of a markedly infiltrated, erythematous plaque measuring 20 x 20 mm. There was no focal reactivation of pre-existing lesions nor of scars of previous lepromin tests even when located very close to the site of injection of the oily suspension. Nor was any general reaction observed such as that mentioned above.

Experiment No. 3. To confirm the findings reported under Experiments 1 and 2 "Cross tests" were performed as follows:

(a) In patient Ana B., a tuberculoid case, who in Experiment 1 had a triple reaction (local, focal, and general) follow-
ing injections of two papules with bacillary lepromin in physiological salt solution, we injected two papules with bacillary lepromin suspended in oil. In twenty-four hours, there was only an infiltrated erythematous plaque at the site of the injection measuring 20 x 20 mm. There was no focal nor general reaction.

(b) In patient Elena P., a tuberculoid case, who in Experiment 2 showed a local reaction (an infiltrated erythematous plaque measuring more than 20 x 20 mm.) following intradermal injection of bacillary lepromin in oily suspension, we injected two intradermal papules with bacillary lepromin in normal salt solution. In twenty-four hours, there was an intense local reaction (an infiltrated erythematous plaque more than 20 x 20 mm. in diameter) and a focal reactivation at a distance in cicatrices of previous lepromin reactions. There were, however, no subjective manifestations of a general reaction.

The logical deduction from these experiments is that aqueous lepromin injected intradermally diffuses and acts at a distance while an oily lepromin does not.

VI. Anatomopathological studies.

Histopathological studies of the reactions provoked by antigens in oily suspension may be summarized as follows:

In forty-eight hours: In tuberculoid cases, the reactions produced with oily lepromin (vaseline, olive, chaulmoogra) are characterized by a violent acute inflammatory process, intense capillary vasodilatation, swelling of the endothelium, edema of the papillary layer of the dermis, enormous collection of polymorphonuclear cells and numerous eosinophiles with which is associated mechanical traumatic splitting by the oil of the collagenous tissue fibers.

In lepromatous cases, the inflammatory process is much less intense. In twenty-one days: In tuberculoid cases, two well defined processes developed:

(a) A typical tuberculoid-granuloma consisting of large accumulations of epitheloid cells surrounded by a lymphocytic layer and showing at their central portions giant cells of the Langhans type.

(b) A granuloma of the foreign-body-type-vaselinoma—with wide cavities in which the mineral oil has been encysted and the cellular infiltrate is constituted by giant cells of the foreign-body type (Figure 11).
In lepromatous cases, only the typical histology of a vaselinoma (Figure 13) is observed which demonstrates that the later nodule which is produced in these cases by the oily lepromin has no significance as an expression of resistance.

**SENSITIZING EFFECT OF OILY LEPROMIN IN LEPROMATOUS CASES**

In several patients with the lepromatous type (Lc) on whom we had performed intradermal injections of suspension in liquid vaseline, we found positive reactions to the standard lepromin after a period of three to five months. All of these showed a doubtful or negative Fernandez (forty-eight hours) reaction and frankly positive Mitsuda (twenty-one days) reaction. The latter consisted of a persistent violet-colored nodule of the size of a corn kernel following the previous injection of the antigen (Figures 9 and 10).

We proved also that this surprising change in the allergic state is only transitory since on repeating the tests over a period of several months, the intradermal injection of bacillary lepromin gave negative results.

That a lepromatous patient can be, apparently, sensitized to lepromin is a most interesting observation. We wish however to reserve further discussion until we have accumulated more data regarding it.

**DISCUSSION**

The results of our experiments show that *M. leprae* in oily suspension provoked early reactions (twenty-four to forty-eight hours) in all cases studied, even in lepromatous cases which regularly give negative results to ordinary lepromin.

In tuberculoid cases, however, positive reactions are of much greater intensity than those produced by the "standard" antigen in aqueous suspension.

The constancy of positive reactions in forty-eight hours, even when a suspension of Eberth's bacillus is used, suggests that these early reactions provoked by oily lepromin are not specific. Moreover the results of control tests show that these reactions are not attributable to a simple irritating effect of the oils used in vehicles, because the intense reactions in tuberculoid cases permit us to state that the reactogenic activity of *M. leprae* is increased when suspended in an oily vehicle.

For the interpretation of these findings, in our judgment, there are two hypotheses which may be considered:

a. That the oily vehicle acts by disassociating and thereby liberating all or most of the nonspecific antigenic frac-
tions which are present in M. leprae, while the aqueous vehicle acts in the same manner but only with regard to the protein fraction which is responsible for the specific action.

b. That the oily vehicle acts mechanically due to its lower diffusibility, thereby maintaining contact between the bacillus and the dermis at the site of injection for a longer time. For this reason, a more intense local reaction is provoked.

Both hypotheses may be defended by arguments of weight but we are inclined to the second, without however entirely disregarding the possibility that the two factors may act simultaneously.

Our preference for the second hypothesis is based upon our complementary investigations. These have shown that while a concentrated antigen in aqueous suspension provokes local, focal, and general reactions in patients with tuberculoid leprosy, the same antigen suspended in oil produces in these patients only an intense local reaction, which supports the hypothesis that the oily vehicle acts by delaying diffusion of the antigen.

SUMMARY

Suspensions of Hansen bacilli in mineral oil, olive oil, and ethyl esters of chaulmoogra oil, killed by heating, were injected intradermally in concentrations of 1:2500 for the chaulmoogra suspensions, of 1:5000 for the suspension in other oils, to establish their antigenic activity. A suspension of Eberth bacilli in oil was used as control.

The three types of lepromin thus prepared provoked intense local reaction in twenty-four to forty-eight hours in all cases of leprosy, even in the lepromatous form. Later the oil provoked the formation of a tumor, to which was added in persons allergic to lepromin the specific nodular reaction which M. leprae provokes. The suspension of Eberth bacilli also provoked an early positive reaction, but this was less marked than the lepromin reaction. Controls injected with the three oil vehicles alone showed that these substances have little or no irritating effect. Some patients with lepromatous leprosy gave a positive reaction to the ordinary lepromin a few months after injection with lepromin suspended in oil.

It is concluded that the early reactions to oily lepromin are not specific. Evidently the antigenic properties of M. leprae are increased in an oily medium. This is attributable to the greater and more prolonged contact of the bacilli with the dermis, when
oil preparations are injected because these do not diffuse as readily as aqueous suspensions.

RESUME

Utilizando suspensiones de bacilos de Hansen muertos por el calor en aceites de vaselina y oliva y en ésteres etílicos del aceite de chaulmoogra a concentraciones de 1:5000 para los primeros y 1:2500 para el derivado chaulmoogrico, los A.A. estudian su actividad antígenica, mediante intradermo-inyecciones de 0.10 de cc., en enfermos de lepra e individuos sanos. Emplean además, comparativamente una suspensión oleosa de bacilos de Eberth.

Comprueban que estas tres variedades de lepromina oleosa provocaron en todos los casos (aun en los lepromatosos) intensas reacciones locales entre las 24 y 48 horas; tardiamente observan la formación de un vaselinoma al que se agrega la reacción especifica nodular que provoca el M.L. en los casos alérgicos a la lepromina. La suspensión de bacilos tíficos también provoca reacciones pruebas positivas aunque menos intensas. En cambio las reacciones de control efectuadas con los tres vehículos oleosos demuestra que su acción irritante es discreta o nula. Algunos casos lepromatosos evidencian al cabo de unos meses, reacciones positivas a la lepromina corriente.

De acuerdo a los resultados de esta investigación los A.A. consideran que estas reacciones provocadas por la lepromina oleosa carecen de especificidad, pero admiten que la actividad reactogénea del M.L. se acrecienta cuando actúa en un vehículo oleoso. Atribuyen este fenómeno a la lenta difusibilidad del aceite que permite un mayor y más prolongado contacto "in situ" del bacilo con la dermis.

REFERENCES

1. SAENZ, A. and CANETTI, G. El problema de la neumonia oleosa en el adulto y en el niño.—La Semana Médica. 46 (1941) 8.
3. DHARMENDRA. Studies of the lepromin test. The active principle of lepromin is a protein antigen of the bacillus. Leprosy in India. 13 (1941) 89.
ILLUSTRATIONS

Figure 1. L.a.v., L.b., and L.a.o. reactions in forty-eight hours in a tuberculoid patient, provoked by lepromin in liquid vaseline, bacillary lepromin (ordinary) and lepromin in olive oil respectively, all in concentrations of 1:5000.

Figure 2. The same reactions as in figure 1 in a person considered to be non-leprous.

Figure 4 and 3. Reaction in forty-eight hours and twenty-one days respectively produced in a non-leprous person, by lepromin in liquid vaseline (L.a.v.), pure liquid vaseline (A.x.) and ordinary bacillary lepromin (L.b.). In this person whose allergy to M. leprae is explained by her tuberculosis of the skin, it is clearly demonstrated that the vaselinoma produced by liquid vaseline does not become apparent in forty-eight hours, but much later.

Figure 5. The same injections as in figure 7 in a tuberculoid patient. Here it is observed that the antigens in oily suspension (L.a.v. and L.a.o.) produce reactions which are stronger than those shown in Figure 7.

Figure 6. L.a.ch.; Ch, Li, and L.a.o. reactions in twenty-four hours in a lepromatous patient, produced by lepromin in esters of chaulmoogra, pure esters of chaulmoogra (control), integral lepromin, and lepromin in olive oil. As will be seen in this case, the only positive reactions correspond to oily suspension of M. leprae, while the common integral lepromin and the pure esters are completely negative.

Figure 7. L.a.v. reaction in forty-eight hours produced by lepromin in liquid vaseline in a lepromatous patient.

Figure 8. Vaselinoma of seventy days still in evolution provoked by intradermal injection of lepromin in a non-leprous person.

Figure 9 and 10. Positive Mitsuda (21 days) reactions produced by the "standard" bacillary lepromin in lepromatous (Lq) patients who had been previously injected intradermally with oily lepromin (L.a.v.).
Figure 11. Biopsy of a nodule twenty-one days after intradermal injection of lepromin in liquid vaseline, in a patient with tuberculoid leprosy. In this microphotograph, it is possible to see the double tubular reaction provoked by the antigen: in (a) a typical structure of a vaselinoma attributable to the oily vehicle, in which a central cavity is shown surrounded by an annular infiltration principally made up of giant cells of the foreign-body type; and in (b) a classical follicular tuberculoid structure, which reflects the specific reaction of an allergic individual (tuberculoid case) to the bacilli contained in the antigen.

Figure 12. A field of figure 11 under higher magnification which permits a more detailed study of the tuberculoid structure of the infiltration. At the center is a Langhans type of giant cell.

Figure 13. Biopsy of a nodule twenty-one days after intradermal injection of lepromin in liquid vaseline in a patient with lepromatous leprosy. In contrast with figures 11 and 12, only the structure of a vaselinoma with its characteristic cavities and collections of giant cells of foreign-body type are seen. Since the case is anergic to lepromin, being lepromatous, there is absence of tuberculoid reaction.