ON THE POLYSACCHARIDE OF *LEISHMANIA TROPICA* 1, 2

BY

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(Received for publication June 12th, 1940)

Whereas the antigenic composition of bacteria has been extensively studied, little experimental work has as yet been done on the antigenic composition of protozoa.

**Experimental**

*Extraction of the polysaccharide.* The Nahid strain of *Leishmania tropica* is grown on our leishmania medium (Senekji, 1939a) in 32-oz. medical flats for 5 days at 22° C. The colonies are emulsified in 10 cc of saline and smears are then made from all the bottles to check on the bacterial sterility. The growths in all the bottles are pooled together, and are centrifuged at 2,500 R. P. M. to collect the leptomonads.

The sediment is extracted with 4 volumes of pure acetone overnight at 37° C in the incubator. The acetone is decanted and the residue is dried at 37° C, reduced to a fine powder in a sterile mortar, and kept over calcium chloride in the dark.

One gram of the fine powder of leptomonads is extracted in 250 cc of N/4 trichloroacetic acid (Dennis and Senekji, 1939) at 37 degrees C in the incubator for 48 hours. The supernatant is a white and opalescent solution, which is separated from the residue by centrifuging. It is then neutralized with 20 per cent NaOH, and immediately a cotton wool precipitate is obtained which has a faint blue tinge. The flask is then returned to the icechest overnight, and the precipitate is collected by centrifuging. It is washed twice in saline, twice with absolute alcohol and finally with ether. Reduced to a fine powder after drying in the incubator at 37° C, it is weighed and stored over calcium chloride in the dark. The yield is 3 to 5 per cent of the powdered leptomonads. This is the S fraction.

All the polysaccharide was precipitated by the neutralization of the trichloroacetic-acid supernatant, because the further addition of one, two, three or four, volumes of 95 per cent alcohol did not produce any precipitate.

A second extraction of the trichloroacetic acid residue with 250 cc of N/4 trichloroacetic acid gave rise to no precipitate on neutralization with NaOH, indicating that all the polysaccharide was already extracted.

The trichloroacetic acid residue is washed in saline twice. The sediment is dissolved in 250 cc of N/1 NaOH which was previously heated to 55° C. The resulting solution, which is quite opalescent, is kept overnight in the incubator at 37° C. It is then filtered through several layers of gauze to remove the debris, neutralized with N/1 HCl and reprecipitated by crystals of trichloroacetic acid and returned to the icechest overnight. The sediment is obtained by centrifuging, washed once with saline, twice in absolute alcohol and once in ether, dried at 37° C, powdered and stored over calcium chloride in the dark. The yield is 5 to 7 per cent. This is the H fraction.

The chemical and physical properties of the fractions. The S fraction is a
whitish powder, insoluble in distilled water and saline. Five cc of 1:1,000 concentration is prepared in the following manner. Five milligrams are ground up in a drop of normal HCl, and are dissolved in an excess of normal HCl. It is carefully neutralized with normal NaOH. If an excess of NaOH is added, the polysaccharide is immediately precipitated. The volume is made up to 5 cc by the addition of distilled water. Phenol is added to give a final concentration of 0.5 per cent. The resulting solution is cloudy and colloidal, and cannot be filtered through Seitz or porcelain filters. Consequently the solution is prepared under strictly aseptic conditions.

The S fraction gives a strong naphthol Molisch's test. Biuret and Millon's tests are negative. The phenylhydrazine test is negative, and hydrolysis does not give rise to reducing sugars.

The H fraction is a pale white powder, insoluble in distilled water and saline. Five milligrams are dissolved in a small volume of normal NaOH, and then carefully neutralized with normal HCl, and the volume is made up to 5 cc. It gives a strong biuret and Millon's tests, but the naphthol Molisch's test is negative.

Preparation of the Leishmania vaccine for the skin tests. Five-day-old cultures are emulsified in saline, and the leptomonads are collected by centrifuging. They are then re-suspended in 0.3 per cent formalin-saline, and kept overnight at room temperature. The sediment is collected by centrifuging and is re-suspended in 0.5 per cent phenol saline. The suspension should be standardized to contain 40 million per cc (Senekji, 1939b). Bacterial sterility tests are performed and the material is bottled.

The allergic skin test. In performing the skin tests for the Oriental sore, the following antigens were used. 1:1,000 dilution of S fraction; 1:1,000 dilution of H fraction; killed and living leptomonads.

The skin test was performed by injecting intradermally 0.1 cc of the antigens. In a negative reaction, no change was observed. In a positive reaction, 15 to 30 minutes after the injection a red wheal appeared which reached its height after 24 hours, and faded away in 4 to 5 days. The intensity of the reaction was about the same in the case of the living and killed leptomonads. The S fraction gave less intense reaction than the H substance. In carrying out the routine allergic skin tests, we used the S fraction and the killed leptomonads. The positive reaction with the S fraction varied from 0.5 to 3 centimeters in diameter, and lasted about 3 days, while the positive reaction with the leptomonads varied from 2 to 8 centimeters in diameter, and lasted about 5 days. In some cases, especially when living leptomonads were used, there were slight constitutional symptoms as fever, headache and general weakness.

RESULTS

Rabbits. Two rabbits which were immunized by injecting living leptomonads (40,000,000 per dose) over a period of 1.5 months, and which received 10 doses, gave the following serological findings.

<table>
<thead>
<tr>
<th>Fraction</th>
<th>Leptomonads</th>
<th>Complement fixation</th>
<th>Agglutination</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>S</td>
<td>H</td>
<td>Killed</td>
</tr>
<tr>
<td>Rabbit I</td>
<td>+</td>
<td>++</td>
<td>++++</td>
</tr>
<tr>
<td>Rabbit II</td>
<td>+</td>
<td>+</td>
<td>++</td>
</tr>
</tbody>
</table>
POLYSACCHARIDE OF L. TROPICA

SKIN TESTS

<table>
<thead>
<tr>
<th></th>
<th>S fraction</th>
<th>Leptomonads</th>
<th>Positive smears (per cent)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>30 min.</td>
<td>24 hrs.</td>
<td>30 min.</td>
</tr>
<tr>
<td>115 patients</td>
<td>+</td>
<td>+</td>
<td>++</td>
</tr>
<tr>
<td>11 nonimmune controls</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>24 immune controls</td>
<td>- or ±</td>
<td>+</td>
<td>+ or ++</td>
</tr>
</tbody>
</table>

Men. 150 individuals were skin tested in this series. Eleven were nonimmune control children and adults who were foreigners and had never had Oriental sore. 24 were immune control adults who had had Oriental sore in their infancy and childhood, and had the scars of the previous boils. One hundred and fifteen patients were typical cases of clinical Oriental sore.

The ages of the patients were as follows:

- 2 patients were 0-6 months old.
- 29 patients were 7-12 months old.
- 59 patients were 1-5 years old.
- 14 patients were 6-12 years old.
- 11 patients were 13 years and above.

The average number of boils per patient was 3.495, but the incidence in this series was as follows:

- 32 patients had 1 boil.
- 30 patients had 2 boils.
- 15 patients had 3 boils.
- 12 patients had 4 boils.
- 5 patients had 5 boils.
- 7 patients had 6 boils.
- 5 patients had 7 boils.
- 2 patients had 8 boils.
- 1 patient had 9 boils.
- 6 patients had 10 to 23 boils.

The shortest duration of the Oriental sore prior to the time the patient was skin tested was 20 days, and the longest was 2 years. The average duration of the boils of the 115 patients was 6.38 months prior to the date of skin test.

Thirty-three (28.7 per cent) out of 115 clinical cases of Oriental sore showed *Leishmania tropica* in smears made from the lesions. We tried to make smears from all the boils, and the number of examinations, at different intervals, have ranged from 1 to 5. In old lesions where there is secondary infection, it is very rare to find the parasite.

The 115 patients all showed either +, ++, or ++++ with the S fraction, and +++, ++++, or ++++ with the killed leptomonads, at the height of the reaction, 24 hours after injection. None of the 11 nonimmune individuals showed any reaction with S fraction or killed leptomonads. Nine were vaccinated against Oriental sore with inoculating living leptomonads, and all developed sores. Twenty-four immune individuals showed no immediate reaction, but in about half of the cases there was a slight reaction after 24 hours, while with the killed leptomonads there was slight immediate reaction which definitely became positive after 24 hours. Ten of these immune individuals were injected with living leptomonads, and severe allergic inflammatory reaction followed, but under no circumstance were we able to produce Oriental sore.

Five patients were also tested with S fraction which was heated at 100° C for 1 hour. There was some diminution in the intensity of the reaction as compared with the unheated but still it gave rise to positive reactions. One infant...
was extremely marasmic, and the skin tests were negative.

**DISCUSSION**

Little is known about the antigenic composition of the animal parasites, and practically nothing is known about the antigenic composition of leishmania.

The leptomonads have a flagellar antigen which gives large-flaking, loosely-knit clumps, and a somatic antigen which gives small-flaking, tightly-knit clumps.

The chemical fractionation of the leptomonads of *Leishmania tropica* reveals that there are at least two fractions: the S fraction, which is a polysaccharide and thermolabile, and the H fraction.

The nonimmune individual is not allergic to S or H fractions, or to the whole leptomonads.

Patients suffering from clinical Oriental sore react positively to both fractions as well as to the killed and living leptomonads, the reaction appearing in 15 to 30 minutes and reaching its height after 24 hours and fading away gradually in 4 or 5 days. The allergic state is established soon after the appearance of the boil. In devitalized individuals the allergic reaction may be lost.

In individuals who previously have had Oriental sore the allergic reaction with the S fraction is not constant. Usually it is negative, but sometimes a slight reaction is observed. On the other hand, the leptomonads give rise to a very definite reaction.

Super-infection may occur in Oriental sore because, as long as the patient has boils, natural or artificially produced, the introduction of living leptomonads gives rise to a new Oriental sore. However, after the healing of the boil (natural or artificial) it is not possible to produce a new boil. In other words, reinfection is not possible.

**SUMMARY AND CONCLUSIONS**

1. There are flagellar and somatic agglutinogens in the leptomonads of *Leishmania tropica*.

2. There are two fractions in *L. tropica* leptomonads: (a) a thermostable polysaccharide (S). (b) a protein (H).

3. A standard allergic skin test is described.

4. (a) The nonimmune individual is not allergic to either fraction.

(b) Oriental sore patients are allergic to the S fraction and to leptomonads.

(c) A previous infection with *L. tropica* produces an allergic state toward the leptomonads, while it may or may not produce a reactivity to the S fraction.

**REFERENCES**
