HUMAN SCARLATINAL STREPTOCOCCI IN MONKEYS

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Hemolytic streptococci have been isolated on numerous occasions from spontaneously infected animals, and apparently in some animals they constitute a portion of the normal flora. In 1933, Lancefield demonstrated that the animal strains could be distinguished from those of human origin by means of a precipitin test. This method gave results in accord with those obtained by the already available biochemical and cultural tests. Organisms causing human disease fell into one serological group, group A, while those isolated from animals were found to belong in other groups, notably B and C. Excluding human strains isolated from the udders of cows during epidemics of sore throat and scarlet fever, exceptions to this rule were very rare. Such was the experience of other investigators (Edwards, 1934; Brightman, 1935; Pilot et al., 1936 a and b; Evans, 1936; Coffey, 1937 and 1938).

In 1936, Seegal, Heller and Jablonowitz in a study of the pharyngeal flora of normal rhesus monkeys recovered hemolytic streptococci in large numbers from the throats of 28 out of 49 animals. Of these strains, 19 by tests were of the human variety. There was no evidence that these organisms were causing disease in the monkeys. The authors suggest that possibly the monkeys had acquired the organism from human contacts since they had been in captivity for some time, and that the animals in turn


2 Aided by a grant from the Fluid Research Fund of the Yale University School of Medicine.
might have been responsible for the occasional streptococcal infections which occurred among their handlers.

Pilot in 1937 recovered a mucoid hemolytic streptococcus from the blood and internal organs of an orangutan with a fatal septicemia. This organism, which culturally reacted as a human strain, was identified by Dr. Lancefield as a member of group A. It dissolved a human plasma-clot, and produced an erythrogenic toxin for man which was not identical with the Dick toxin.

During the winter of 1936–1937 three of the 203 rhesus monkeys, used in this laboratory in the study of poliomyelitis, developed streptococcal infections (two, facial erysipelas; one, peritonitis); a fourth was already infected (axillary abscess) on arrival from the laboratory animal supply house in New York City. In each instance a culture of the lesion yielded a pure growth of gram-positive cocci in chains, which formed typical beta-hemolytic colonies in blood agar plates, were bile-insoluble, and produced a strong soluble hemolysin for rabbit erythrocytes. All strains were found to belong serologically in the human pathogenic group, group A. This isolation of group A hemolytic streptococci from non-experimentally infected animals induced us to study the strains by the methods available at the present time.

SOURCE OF STRAINS

The four strains of hemolytic streptococci are designated by the numbers of the rhesus monkeys from which they were isolated. The monkeys weighed between two and three kilograms. All were purchased from the same laboratory animal supply house. Their clinical course is shown in charts 1, 2, and 3.

Monkey no. 633 arrived at this laboratory on December 29, 1936. On January 15, 1937, the right side of her face became red and swollen, and by the following day the lesion had spread across the nose in typical butterfly distribution to involve the left side of the face. Her temperature on that day was 105.2°F. Aspiration of a small vesicle at the center of the lesion on January 19 showed hemolytic streptococci by smear and culture. There was no further extension of the infection, and two weeks later the lesion had entirely disappeared.
Chart 1. Clinical Chart of Monkey No. 633 with Facial Erysipelas

Chart 2. Clinical Charts of Monkey No. 645 with Peritonitis, and Monkey No. 676 with an Axillary Abscess
Monkey no. 645 was received at this laboratory on January 15, 1937, and was placed in a cage adjoining that of monkey no. 633. On January 19, she received 2.0 ml. of 10 per cent Dial (diallylmalonylurea) intraperitoneally in preparation for experimental studies with the virus of poliomyelitis. She succumbed three days later, January 22, and at autopsy the peritoneum was found to be acutely inflamed, and there were about 100.0 ml. of thin cloudy fluid in the peritoneal cavity. Smears of the exudate re-

vealed large numbers of gram-positive cocci in chains. Hemolytic streptococci were grown in pure culture from the peritoneal exudate and from the heart’s blood.

Monkey no. 676 was suffering from a large right axillary abscess when she arrived at this laboratory on February 16, 1937. Aspiration of the abscess on March 5 yielded hemolytic streptococci by smear and culture. Shortly afterwards, the abscess ruptured spontaneously and continued to discharge purulent material until the monkey was sacrificed on March 15, 1937.
Monkey no. 483 had been observed in this laboratory since April 23, 1936 and was in good health. From the time of arrival, monkey no. 676 was kept in the same cage with this animal. On March 23, 1937, monkey no. 483 developed a typical facial erysipelas which persisted for two weeks. Culture of material obtained on March 29 by aspiration of the edge of the lesion showed hemolytic streptococci.

METHODS OF STUDY

The following accepted methods were employed in the study of the four strains isolated from infected monkeys:
1. Serological grouping

Precipitin tests (Lancefield, 1933) were performed using hot hydrochloric-acid extracts of the organisms and group-specific antisera produced by the inoculation of rabbits with hemolytic streptococci known to belong in groups A, B, C, F, G, and H. The extract of each strain was tested against antisera for each of the six groups. Controls of antiserum without antigen were included. Antisera were tested for potency and specificity prior to use.

2. Fibrinolytic activity

The strains were tested for ability to lyse human and rhesus monkey plasma-clots (Tillett and Garner, 1933). Three well children and three healthy monkeys served as a source of blood plasma. A strain of group A hemolytic streptococcus, Ro, isolated from the blood of a child with a fatal septicemia was used as a control.

3. Final pH

The organisms were incubated for 4 days in 1.0 per cent glucose broth (Avery and Cullen, 1919). The pH attained was determined colorimetrically, using brom-cresol green as the indicator.

4. Hydrolysis of sodium hippurate

Strains were grown for 4 days in infusion broth containing 1.0 per cent sodium hippurate, and to the clear supernatant medium was added 0.25 per cent concentrated hydrochloric acid in 12.0 per cent ferric chloride (Ayers and Rupp, 1922). Known positive and negative cultures, and uninoculated sodium hippurate broth were the controls.

5. Reduction of methylene blue milk

Tubes of sterile milk containing enough 1.0 per cent methylene blue to make a final concentration of 1:5000 were inoculated and
incubated (Avery, 1929). Readings were made at daily intervals up to one week. Control tubes of plain milk and of uninoculated methylene blue milk were used.

6. Fermentation of sugars

Tubes of 1.0 per cent lactose, mannitol, salicin, trehalose and sorbitol with Andrade’s indicator were inoculated (Holman, 1916; Edwards, 1932). The tubes were observed daily and final readings made at the end of one week of incubation.

7. Growth on blood agar containing bile

The ability of the strains to grow on rabbit’s blood agar plates containing 10.0 and 40.0 per cent ox bile was tested (Belenky and Popowa, 1929). Control plates of rabbit’s blood agar without added bile were inoculated at the same time. Readings were made daily up to 4 days.

8. Mouse virulence

Serial dilutions of an 18-hour broth culture of each strain were injected intraperitoneally in 1.0 ml. amounts into white mice. The highest dilution which killed a mouse within 48 hours was determined, and the minimal lethal dose expressed in milliliters of undiluted culture. Mice which succumbed were autopsied, and their peritoneum and heart’s blood were smeared and cultured. The virulence of the strains was tested twice.

9. Erythrogenic toxin production

Each strain was grown for 48 hours in beef-heart infusion broth with 0.05 per cent glucose, and the sterile filtrate obtained by passage through a Chamberland candle was diluted serially in multiples of ten up to 1:100,000. Three children, who were found to react positively to Dick toxin, were injected intradermally in the forearm with 0.1 ml. of filtrate beginning with the highest dilution. One skin test dose of toxin was defined as the highest dilution of filtrate which would produce at 20 to 24 hours an area of skin erythema at least as large as that produced...
by 0.1 ml. of Dick toxin. Control observations were carried out with filtrate heated to 100.0°C. for 2 hours and with the filtrate from uninoculated broth.

Three Dick-negative children were inoculated as above with 0.1 ml. of toxic filtrate in decreasing dilutions through 1:10. Neutralization tests were performed by mixing 2.0 ml. of toxic filtrate containing 10 skin test doses of toxin per 0.1 ml. with an equal volume of serum from each of three children who were Dick negative. The mixtures were incubated for 30 minutes in a water bath at 37.5°C., kept at room temperature for 2 hours, and inoculated intracutaneously into the three Dick-positive children. The controls consisted of toxin plus Dick-positive serum, Dick-positive serum, and heated toxin.

### RESULTS

The serological, biochemical and cultural characteristics of the four strains of hemolytic streptococci from infected rhesus monkeys, as shown in tables 1 and 2, were those of human pathogens. All strains fell clearly into Lancefield's group A. There was no evidence of reaction with antisera prepared against the other groups.

- The criterion for a positive Dick test was a local area of skin erythema, at 20 to 24 hours after injection, which measured 1.0 cm. or more in any diameter.

### TABLE 1

<table>
<thead>
<tr>
<th>MONKEY STRAIN</th>
<th>DISEASE</th>
<th>FIBRINOLYTIC</th>
<th>PRECIPITIN TEST</th>
</tr>
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<tbody>
<tr>
<td></td>
<td></td>
<td>HUMAN Clot</td>
<td>MONKEY Clot</td>
</tr>
<tr>
<td></td>
<td></td>
<td>STRAIN</td>
<td>STRAIN</td>
</tr>
<tr>
<td>633</td>
<td>Facial erysipelas</td>
<td>3+</td>
<td>4+</td>
</tr>
<tr>
<td>645</td>
<td>Streptococcal peritonitis</td>
<td>4+</td>
<td>4+</td>
</tr>
<tr>
<td>676</td>
<td>Axillary abscess</td>
<td>2+</td>
<td>4+</td>
</tr>
<tr>
<td>483</td>
<td>Facial erysipelas</td>
<td>4+</td>
<td>4+</td>
</tr>
</tbody>
</table>

Fibrinolytic test results are graded as follows: 4+, complete lysis of plasma-clot in 0-60 minutes; 3+, complete lysis in 1-3 hours; 2+, complete lysis in 3-8 hours; 1+, complete lysis in 8-24 hours; 0, no lysis in 24 hours.
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All produced an antihuman fibrinolysin, two being as active as our stock human strain in dissolving the plasma-clots of normal children. This stock strain, which has been used in this laboratory as a source of fibrinolysin for about three years, was chosen because of its consistently high fibrinolytic activity. Tillett and Garner (1933) have shown that the lysis of fibrin is a property possessed by human hemolytic streptococci. Organisms causing severe infections in man are more active in this respect than those from mild illnesses. This direct correlation between virulence and fibrinolytic activity has been frequently observed (Hadfield, Magee and Perry, 1934; Madison, 1934; Tillett, 1935; Dack, Woolpert and Hoyne, 1935; Fraser and Madison, 1935). Neither

<table>
<thead>
<tr>
<th>TABLE 2</th>
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<tbody>
<tr>
<td>Characteristics of hemolytic streptococci from rhesus monkeys (continued)</td>
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<table>
<thead>
<tr>
<th>MONKEY STRAIN</th>
<th>pH</th>
<th>SODIUM HIPPURATE</th>
<th>MALT MUSCLE MILK</th>
<th>Trehalose</th>
<th>Lactose</th>
<th>Mannitol</th>
<th>Salicin</th>
<th>GROWTH ON BILE BLOOD AGAR</th>
<th>MOUSE VIRULENCE</th>
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<tbody>
<tr>
<td>633</td>
<td>5.4</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>-</td>
<td>Very poor</td>
<td>0* .001</td>
</tr>
<tr>
<td>645</td>
<td>5.4</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>-</td>
<td>Very poor</td>
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<tr>
<td>676</td>
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<td>+</td>
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<td>-</td>
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<td>0 .0001</td>
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<tr>
<td>483</td>
<td>5.4</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>-</td>
<td>0</td>
<td>0 .0001</td>
</tr>
</tbody>
</table>

* 0 indicates no growth.

the strains from monkeys nor the stock human strain was able to act on normal rhesus plasma-clots.

The final pH attained by the strains in glucose broth was 5.4. There was no hydrolysis of sodium hippurate or reduction of methylene blue milk. The organisms fermented lactose, salicin and trehalose, but not mannitol or sorbitol. Growth on rabbit's blood agar with 10.0 per cent bile was very poor (2 strains) or absent (2 strains), and there was no growth on 40.0 per cent bile blood agar.

The virulence of the strains for mice was determined as a matter of interest and as an additional differential test. Strains from animal sources are generally much more highly virulent for
mice than human strains. These strains were of moderate virulence. The minimal lethal dose for two strains was 0.001 ml. (about 300,000 organisms), and for the remaining two, 0.0001 ml. (about 30,000 organisms).

TABLE 3

<table>
<thead>
<tr>
<th>SUBJECT NUMBER</th>
<th>DICK TEST</th>
<th>FILTERED TOXIN (HIGHEST DIL. GIVING + SKIN REACTION)*</th>
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<tbody>
<tr>
<td></td>
<td>Number 633</td>
<td>Number 645</td>
</tr>
<tr>
<td>1</td>
<td>+</td>
<td>1:100</td>
</tr>
<tr>
<td>2</td>
<td>+</td>
<td>1:100</td>
</tr>
<tr>
<td>3</td>
<td>+</td>
<td>1:100</td>
</tr>
<tr>
<td>4</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td>6</td>
<td>-</td>
<td></td>
</tr>
</tbody>
</table>

Controls of broth and of heated toxin were negative.

* Dilutions used ran from 1:10 to 1:100,000.

TABLE 4

<table>
<thead>
<tr>
<th>SUBJECT NUMBER</th>
<th>NUMBER 633 TOXIN PLUS</th>
<th>NUMBER 645 TOXIN PLUS</th>
<th>NUMBER 676 TOXIN PLUS</th>
<th>NUMBER 483 TOXIN PLUS</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Dick + serum</td>
<td>Dick - serum</td>
<td>Dick + serum</td>
<td>Dick - serum</td>
</tr>
<tr>
<td>1</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>2</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>3</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>-</td>
</tr>
</tbody>
</table>

Controls of heated toxin and of Dick + serum were negative.

Dick + serum denotes blood serum from Dick positive children. Dick - serum denotes blood serum from Dick negative children. Subjects 1, 2 and 3 were Dick positive as shown in table 3.

All strains produced a human erythrogenic toxin, as shown in table 3. Two of the strains were excellent toxin producers. The skin test dose with these two strains was 0.1 ml. of a 1:10,000 dilution of filtrate, and 0.1 ml. of a 1:100 dilution with the other two strains. The three Dick-negative children failed to react to the toxin in a 1:10 dilution. The four toxins were neutralizable with scarlatinal antitoxin as is shown in table 4.
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With the available tests these strains of hemolytic streptococci from infected rhesus monkeys were indistinguishable from those found in severe human infections.

SUMMARY

Hemolytic streptococci were recovered from four rhesus monkeys. Three of the monkeys had spontaneous infections (two, facial erysipelas; one, an axillary abscess). The fourth, who developed a fatal peritonitis following the intraperitoneal administration of Dial, was apparently accidentally infected. The organisms were seen in stained smears of the pus from the peritoneum and abscess, and in all cases a culture of the lesion yielded a pure growth of hemolytic streptococci. These four strains were indistinguishable from those found in scarlet fever and other severe human streptococcal diseases.

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