THE RELATIVE FREQUENCY OF VARIOUS TYPES OF STREPTOCOCCI IN SCARLATINA.

By H. CUMPSTON, M.B., B.S., D.P.H.,
Assistant Medical Officer Western Fever Hospital.

(*From the Lister Institute of Preventive Medicine.*)

The Streptococci obtained in Scarlet Fever.

It has long been well established that during the height of scarlet fever streptococci flourish and become greatly predominant in the pharyngeal and buccal mucus. To quote only a few of the many recorded observations.

Lemoine (1895) in the mucus from 117 throats in scarlet fever found streptococci alone 93 times and with other organisms in 14.

Baginsky and Sommerfeldt (1900) in 701 cases found that streptococci were absent only five times.

The latest results are those obtained by Ruediger (1906). He inoculated (with broth in which the swab used to collect the material had been washed) blood-agar tubes, from which he made plates. On the medium the streptococci grew as grey colonies surrounded by a clear area of haemolysis. Pneumococci grew as green colonies and could thus be distinguished from streptococci. The colonies obtained were picked off and confirmed by further morphological and cultural investigation. Of 75 throats thus examined during scarlet fever, 2 showed only streptococci, 31 showed 60—95% of streptococcal colonies, 21 showed 15—40% streptococci, and 20 showed streptococci and pneumococci.

Ruediger makes this statement. "As a rule the streptococcus colonies greatly predominate over the other colonies when the inflammation of the throat in scarlet fever is pronounced, and they rapidly decrease in number with the subsidence of the throat

symptoms....When the throat symptoms are mild the proportion of streptococcal colonies to other colonies is quite small."

Ruediger further gives the results of his examination of 51 normal throats. "Streptococci were present in 30 out of 51 cases examined but were never present in large numbers and were entirely absent in 21 cases." The fact of this almost invariable preponderance of streptococci in the throats of early cases of scarlet fever agrees with the writer's experience. In over 250 plates made and examined the streptococci were present in nearly every case in much greater numbers than all the other types of colonies together.

Assuming the fact of an increase in the number of streptococci taking place in the throat during the first few days of scarlet fever as having been demonstrated, the object of this enquiry was an attempt to discover whether these streptococci showed any uniformity in type.

For this purpose the organic substances introduced by Gordon (1905) were employed and the streptococci were grouped according to the results produced by their growth on these media. The formula of the media used was peptone water 75%, beef broth (made from bovine heart muscle) 25%, and in this was dissolved 1% of the test substance.

The streptococci examined included those obtained from abscesses, from the nasal discharge and from the tonsils during scarlet fever. The pus was obtained from the abscesses by inserting a sterile hypodermic needle after sterilizing the skin and removing a few drops of pus, which were grown for 18 hours in broth and then plated off on agar in three dilutions of the broth.

In order to reduce to a minimum the amount of contamination by the ordinary salivary organisms when obtaining specimens from the throat the mouth was first swabbed as dry as possible with cotton-wool. All débris and secretion were then rubbed off the surface of the tonsil with a sterile cotton-wool swab until the mucous membrane just began to bleed. Then a second sterile swab was at once used and soaked in the tonsillar secretion. This swab was then lightly rubbed over the surface of an agar plate. A glass rod bent at right angles was then sterilized and rubbed over this plate, then on to a second plate and straight on to a third. The result on the third plate was a very limited number of colonies (in most cases entirely streptococcal) which could be picked off and examined. From three to five single colonies were taken from each plate and grown separately in broth for 48 hours at 37° C. At the end of 48 hours films were made and examined under the microscope. Any that did not show a uniform morphological type, or, at least, nearly uniform,

were discarded. Then from the broth culture the various chemical media were inoculated. In all 19 strains were examined from abscesses, 80 strains from throats, 1 strain from a case of scarlet fever post-mortem, and 1 from a case of rhinorrhoea within the first day after the onset of the discharge.

These 101 different specimens of streptococci gave the following results with the above tests:

(*a*) The whole series failed to reduce neutral-red after three days' growth at 37° C. under strictly anaerobic conditions.

(*b*) Every member of the series grew on gelatin in two days at 22° C.—none liquefied the gelatin, but the amount of growth shown in two days varied within moderate limits.

(*c*) As a general rule—with only a few exceptions—the streptococci fermented saccharose and lactose.

The remaining reactions need to be considered more in detail.

To take first those streptococci obtained from abscesses. These represent probably the streptococci which invade the general circulation and which, any serum which aims at the improvement of "septic" symptoms in scarlet fever, must attack.

The 19 strains present four distinct types.

No. of specimens	Neutral red	Saccharose	Lactose	Raffinose	Inulin	Salicin	Mannite	Milk	Gelatin at 22° C.
13	–	+	+	–	–	+	–	A	+
4	–	+	–	–	–	+	–	–	+
1	–	+	+	–	–	+	+	A	+
1	–	+	+	–	–	+	–	A. C.	+

A. C. means the production of acidity and clotting in litmus milk.

A means acidity only.

+ = the production of acidity except in the case of gelatin where it means simply "growth."

It will be seen then that the predominant type of streptococcus found in the abscesses examined was one which fermented saccharose, lactose and salicin, which produced acidity but no clotting in milk and which left the other media unaffected. In all the media growth was quite vigorous. These abscesses occurred between the 8th and 34th days of disease. The fact that 19 specimens obtained from abscesses could be divided into four distinct types suggests that if favourable results are to be obtained from serum therapy it will be by the use of a polyvalent serum.

Rhinorrhoea.

One strain fermented lactose, saccharose, salicin and mannite and produced only acidity in milk.

Streptococci from Throats.

The following table includes all those strains of streptococci obtained by the method described from the throat in scarlet fever.

TABLE A.

Neutral red	Saccharose	Lactose	Raffinose	Inulin	Salicin	Mannite	Milk	Gelatin	No. of specimens
−	+	+	−	−	+	−	A	+	40
−	+	+	−	−	+	+	A	+	13
−	+	+	F	−	+	−	A	+	2
−	+	+	+	+	+	−	A	+	1
−	−	+	−	−	+	+	A	+	1
Fermented Glucose only									2
−	+	+	−	+	−	−	A	+	1
−	+	+	−	−	−	−	A	+	1

TABLE B.

−	+	+	−	−	+	−	A. C.	+	6
−	+	+	−	−	−	−	A. C.	+	5
−	+	+	+	−	−	−	A. C.	+	4
−	+	+	+	+	+	+	A. C.	+	1
−	+	+	+	+	+	−	A. C.	+	1
−	+	+	−	F	+	−	A. C.	+	2

In Table A are included all those that do not clot milk but produce only acidity. In Table B are included those that both produce acidity and clot the milk. In both tables the letter F is intended to indicate that a faintly acid reaction was produced after five days.

(These 80 streptococci from the throats were isolated from 25 patients between the second and ninth days of disease with one exception, which was obtained on the 26th day of the disease. The colonies were picked off quite indiscriminately from the original plates.)

Streptococcus obtained Post-mortem.

This strain was obtained from a case of scarlet fever which was typically of the "Toxic" type. Death occurred on the third day of disease, a rigor having taken place about six hours before death.

Cultures were made from the blood removed from the left ventricle in a sterile pipette, from the splenic pulp and from the liver. Two sets of blood cultures were taken from the heart—both remained sterile. The culture from the liver showed a mixed staphylococcus and *Bacillus coli* culture. The spleen showed a pure streptococcal culture. This streptococcal culture was plated out on agar and single colonies examined. Morphologically it was of "medius" type—it grew in broth with a sandy sediment (type B)[1]—grew fairly well on gelatin at 22° C. in two days, produced acidity but no clotting in milk, and fermented saccharose, lactose, salicin and mannite, but showed no reduction of neutral-red.

Its pathogenicity was tested on mice and it produced a local abscess at the site of injection in four days and death of the mouse on the seventh day. The streptococci could not be recovered from the heart or liver, but only from the abscess of the mouse.

To compare, then, the results obtained by these fermentation tests on the various streptococci. First let us take the two types shown below, which between them include the great majority of the specimens examined. It will be seen that they are closely similar, the differential test being the power of fermenting mannite.

	No. of specimens	Neutral red	Saccharose	Lactose	Raffinose	Inulin	Salicin	Mannite	Milk	Gelatin
Abscess	13	−	+	+	−	−	+	−	A	+
Throat	40	−	+	+	−	−	+	−	A	+
Abscess	1	−	+	+	−	−	+	+	A	+
Throat	13	−	+	+	−	−	+	+	A	+
Rhinorrhoea	1	−	+	+	−	−	+	+	A	+
Cadaver	1	−	+	+	−	−	+	+	A	+

The fact that not one of these 101 cases reduced neutral-red is striking, several controls were made with streptococci from normal saliva on the same neutral-red media and they produced a reduction.

Gordon (1905) describes five samples of streptococci obtained by him from scarlet fever cases—three of these gave positive reactions to saccharose, lactose and salicin only, and two gave positive reactions to the same three reagents as well as to mannite. Thus all the five specimens belong to the same two groups as the majority of the strains in this series.

Andrewes and Horder (1906) have collected 1200 specimens of streptococci subjected to these differential tests. Thirty-three of these

[1] *Vide infra.*

https://doi.org/10.1017/S002217240003357X Published online by Cambridge University Press

strains were obtained from scarlet fever cases, and these fall under the classification used by those authors as follows:

S. pyogenes	12
S. salivarius	1
S. anginosus	20

The *S. pyogenes* group corresponds with that into which the large majority of the cases in this series fall. Of the 20 strains included in the *S. anginosus* group, nine do not reduce neutral-red and the reactions given by these nine correspond closely with the reactions given by members of the present series.

A careful repetition of the nine tests, in many of the cases after long intervals—in some cases three months—corroborated the assertion of these other writers that each strain of streptococcus preserves its characteristics unchanged.

Culture appearances in Broth.

In each case a note was kept of the way the streptococcus grew in broth.

The types of growth are divided into four.

Type A. Uniform turbidity with little deposit.
B. Clear broth with sandy deposit.
C. Clear broth with small flocculent masses adhering to the bottom and sides of the tube.
D. Clear broth with larger floccular masses on the bottom of the tube.

The following tables show the chemical reactions given by these different types.

It will be seen from these tables that there is no close connection between the type of growth in broth (the only method of separating streptococci before the introduction of these "nine tests") and the reactions given with the tests under discussion. It is interesting to

From Abscesses.

	Neutral red	Saccharose	Lactose	Raffinose	Inulin	Salicin	Mannite	Milk	Gelatin	No. of specimens
Type B	−	+	+	−	−	+	−	A. C.	+	1
	−	+	+	−	−	+	−	A	+	8
	−	+	−	−	−	+	−	−	+	4
Type C	−	+	+	−	−	+	−	A	+	5
	−	+	+	−	−	+	+	A	+	1

https://doi.org/10.1017/S002217240003357X Published online by Cambridge University Press

From other sources.

Type A.

Neutral red	Saccharose	Lactose	Raffinose	Inulin	Salicin	Mannite	Gelatin	Milk	No. of specimens
–	+	+	–	–	+	–	+	A. C.	2
–	+	+	–	–	–	–	+	A. C.	4
–	+	+	+	–	–	–	+	A. C.	3
–	+	+	+	+	+	+	+	A. C.	1
–	+	+	–	–	+	–	+	A	2
–	+	+	F	–	+	–	+	A	1

Type B.

–	+	+	–	–	–	–	+	A. C.	1
Glucose only							+		2
–	+	+	–	–	+	–	+	A	4
–	+	+	–	–	+	+	+	A	1

Type C.

–	+	+	–	–	+	–	+	A. C.	4
–	+	+	–	F	+	–	+	A. C.	2
–	+	+	–	–	+	–	+	A	30
–	+	+	–	–	+	+	+	A	14
–	+	+	F	–	+	–	+	A	2

Five other varieties with one member each.

Type D.

–	+	+	+	+	+	+	+	A. C.	1
–	+	+	–	–	+	–	+	A	4

note, however, that in the group, Type A, which so far as morphology is concerned coincides with *Streptococcus brevis*, 10 of the 13 members clotted milk, while in the group, Type C, morphologically *Streptococcus longus*, 51 of the 57 did not clot milk.

That is to say the majority of the streptococci found in scarlet fever throats correspond with the *S. longus* type.

This series of streptococci then shows:

(1) That 50% of the throat streptococci examined give identical results on these selected media.

(2) That 68·4% of the streptococci obtained from the abscesses give identical results.

(3) That the majority of throat streptococci in scarlet fever are of the same type as the majority of streptococci obtained from abscesses in various parts of the body.

(4) That if we include the specimens that "fermented" mannite, then we find that 69% of the streptococci obtained from throats,

abscesses, nose and cadaver in scarlet fever are of identical type and correspond with the specimens isolated by Dr Gordon from throats, abscesses, cadavers, ears and cervical glands in scarlet fever.

REFERENCES.

ANDREWES, F. W. and HORDER, T. J. (1906). A study of the streptococci pathogenic for man. *Lancet*, vol. II. p. 708.

BAGINSKY and SOMMERFELDT (1900), Ueber einen constanten Bacterienbefund bei Sharlach. *Berlin. klin. Wochenschr.*, 1900, p. 588.

GORDON (1905), Report of some Characters by which various Streptococci may be differentiated and identified. *33rd Annual Report of Medical Officer to Local Government Board*, p. 388.

LEMOINE (1895), Étude bacteriologique de 117 cas d'angines scarlatineuses. *Bull. de la Soc. Méd. des Hôpitaux*, 1895, p. 847.

RUEDIGER, G. F. (1906), Streptococci from Scarlatinal and Normal Throats. *Journ. Infectious Diseases*, vol. III. p. 755.