GREEN-PRODUCING COCCI IN MEASLES.

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In 1917 and 1918 Tunnicliff announced that she was able to obtain cocci from the blood of cases of measles in the pre-eruptive and eruptive stages of the disease. It was claimed that anaerobic methods were necessary for their isolation, but after the initial growth appeared these organisms could be sub-cultured aerobically. Later, this worker (Tunnicliff, 1919) found a similar organism to be the predominating species in cultures obtained from the sputum of acutely ill cases. Caronia (1923) reported that he was able to isolate with great regularity a small diplococcus from the blood of measles cases by using the Tarozzi-Noguchi medium.

In 1925 Tunnicliff devised a skin test for susceptibility to measles by employing an anaerobic dextrose broth culture of the coccus killed by means of 0·5 per cent. phenol. Later Ferry and Fisher (1926) and Tunnicliff and Taylor (1926) showed that the diplococcus could produce an extracellular toxin which when used in low dilution for purposes of intradermal tests caused reactions which differentiated the susceptible from the immune. Again Tunnicliff and Hoyne (1926) and Hoyne and Gasul (1926) showed that the serum of goats immunised with broth cultures and filtrates obtained from the coccus could be used for the protection of susceptible children.

Hibbard and Duval (1926) were also able to isolate a gram-positive coccus in blood cultures incubated anaerobically. Similarly Ferry (1927) claimed to have isolated a gram-positive coccus from the blood of early cases by using aerobic methods. This organism produced an exo-toxin which when used in a dilution of 1 in 400 to 1 in 600 gave a skin reaction in susceptible children; he also claimed that an antitoxin prepared for these organisms protected against an attack of measles. Cary and Day (1927) reported that they were able to isolate a green-producing diplococcus, similar to the Tunnicliff organism, from the pharyngeal secretions in 93 or 98 per cent. of cases of measles. In 5 out of 15 instances this green-producing streptococcus was isolated by blood culture on Hiss's serum brain medium and Hibler's medium, while in 50 per cent. of cases a similar organism could be isolated from the conjunctiva. They believed that the green-producing streptococcus isolated during the acute stage of measles could be differentiated from the green-producing streptococcus found on the mucous membranes of the respiratory passages of non-measles cases. Zlatogoroff and his co-workers (1928) obtained from the blood of measles cases an organism similar to that isolated by Caronia in 1923.

Park, Williams and Wilson (1927) have cast doubts on the relationship of the green-producing cocci to measles. They isolated similar organisms from

84 per cent. of 57 cases. Fermentation and agglutination tests with these strains showed a great diversity of types, and they were further unable to confirm that a suitable dilution of exo-toxin when used in intracutaneous tests differentiated the immune from the non-immune. Long and Cornwall (1927) failed to isolate the green-producing streptococci from 47 blood cultures in Hibler's medium from 26 cases. Mair (1928) reasoning that the virus of measles was invariably transmitted from an acute case to a susceptible by being spray borne, allowed 50 cases in the early stage of the disease to cough on blood agar plates. He was unable to obtain any colonies of *Streptococcus viridans*, and further there was no constancy in the character of the colonies which appeared on the plates.

Reports on the value of passive immunisation with sera from horses immunised with Tunnicliff's and with Ferry and Fisher's organisms submitted by Gunn (1928 *a* and *b*) and Harries (1928) have shown that these sera have not been found of value.

Scope of Research.

In the first instance it was resolved to examine by blood culture all available cases of measles to see whether any micro-organism could be found. Next, the pharyngeal secretions of a limited number of cases were cultured and various gram-positive green-producing cocci were examined in detail as to their cultural and biochemical characteristics.

Next, since it has been previously repeatedly shown that in related cases of scarlet fever and diphtheria the serological characteristics of the strains isolated from them are identical, the serological characteristics of the isolated cocci were studied in order to see if this phenomenon occurred in measles.

Next, a comparison was made between the cultural and serological characteristics of the cocci isolated from measles during the acute and convalescent stages of the disease, and also with the cocci isolated from the pharyngeal secretions of children who gave no history of having had the disease. The capacity of strains from acute cases to produce exo-toxin was tested.

Finally, although haemolytic streptococci are infrequently present in the blood in cases of scarlet fever, an increase in the agglutinin titre of a patient's serum for the causative strain can frequently be shown to occur; it was argued that a similar increase should be demonstrable for these green-producing cocci if they were definitely associated in the pathogenesis of the disease. Accordingly the sera from cases were examined with this object.

Methods.

Blood culture. As soon as a case of measles was definitely diagnosed either in the pre-eruptive or eruptive stage of the disease, 25 c.c. of blood were withdrawn and immediately inoculated into various types of media. In the first 8 cases 2·5 c.c. of blood were added to two tubes containing 50 c.c. of Hibler's brain liver tissue medium, to two flasks containing 50 c.c. of nutrient broth

and to two tubes containing 10 c.c. of Wenyon-Noguchi medium, the remainder of the blood being placed in a sterile test tube. Three cultures were then incubated aerobically and three anaerobically in a McIntosh and Fildes anaerobic jar. The tubes were retained in the incubator for 10 days before being discarded. Later, since no organisms were obtained anaerobically, 5 c.c. of blood were added to each type of medium and the tubes and flasks were incubated under aerobic conditions only.

The collection of strains of green-producing cocci from measles cases. In the pre-eruptive or early eruptive stages the pharyngeal secretions were obtained personally by means of sterile swabs. The swabs were taken directly to the laboratory and the secretions were inoculated into tubes containing sheep's blood broth. Sheep's blood agar plates were then inoculated from the broth tubes and incubated for 24 hours. From the blood agar plates inoculated from an individual case, 9 green-producing colonies were selected and replated. After incubation 9 colonies were again selected and inoculated into blood broth tubes. Cultures having been established in the blood broth tubes, ordinary broth tubes were inoculated. After incubation the cultures were centrifuged, resuspended in saline and tested for bile solubility using various dilutions of sterile ox bile. Six bile-insoluble strains were retained for the study of their cultural, morphological, biochemical, antigenic, and toxin-producing characteristics.

The morphological characteristics of each strain were investigated from the 24-hour blood broth culture; the behaviour of each strain on chocolate blood agar medium was determined together with the type of colony formation, and fermentation tests were carried out on dextrose-free broth media containing the carbohydrates, saccharose, lactose, salicin, inulin, mannite and raffinose.

Nine agglutinating sera were prepared from various strains and agglutination tests were carried out with these various sera and the strains isolated. The isolated strains were grown in 50 c.c. proteose peptone broth for 24 hours. The culture was then centrifuged to remove coarse particles and was tested against the serum for agglutination by the macroscopic method, incubating the tubes at 55° C. for 2 hours. In the majority of instances shaking and centrifuging produced stable suspensions, but with certain strains which in culture consisted of long chains it was found impossible to obtain a primary agglutination test. In all cases, however, the deposits from the 50 c.c. culture flasks were used for the absorption of agglutinins.

Various strains were tested for the exo-toxin produced by growing them in 1 per cent. blood broth media, and in proteose peptone broth medium for 4 days.

RESULTS OF THE INVESTIGATION.

(1) *Blood cultures.*

By the methods already described blood cultures were obtained from 15 cases of measles either in the pre-eruptive stage of the disease or in the first

day of the rash, before the temperature had begun to fall. In the first 8 cases the cultures were grown anaerobically as well as aerobically, but the results being negative the anaerobic methods were discontinued, and the latter 7 blood cultures were incubated aerobically only. One blood culture on the liver brain medium showed a green-producing streptococcus and another culture was contaminated with a *Staphylococcus albus*. The results, therefore, were not indicative.

(2) *Investigation of strains from the pharyngeal secretions of acute cases.*

Three strains of the green-producing cocci were obtained from Dr Ruth Tunnicliff, Chicago, and 3 from Prof. Duval, New Orleans, but unfortunately, 1 of the strains from Prof. Duval was subsequently lost. Cultural and biochemical examinations of these strains were first carried out, and it was seen that on a blood agar plate inoculated with pharyngeal secretions it would be impossible to distinguish between colonies of normal inhabitants and colonies of the so-called measles-producing cocci. In consequence, to give every opportunity for finding these organisms, 6 strains of green-producing cocci were obtained from each of 20 cases of measles.

The first two (1–2) cases of measles cultured occurred in a scarlet fever ward simultaneously, and from these, 5 further cases (3–7) were infected, and these also were cultured. Case 8 was an isolated case, having no relation to any of

Table I. *Typical examples of cultural characteristics of strains.*

Case	Strain	Morphology	Characteristics of colonies on chocolate blood agar: Colour	Type	Saccharose	Lactose	Salicin	Inulin	Mannite	Raffinose
9	A	mc.	Dark green	R	A	A	0	A	0	0
	B	d.sc.	Dark green	S	A	A	A	0	0	A
	C	mc.	Dark green	S	A	A	0	0	0	A
	D	mc.	White	R	A	A	A	0	0	A
	E	d.sc.	Green	S	A	A	0	0	0	0
	F	lc.	Pale green	S	A	A	0	0	0	A
13	A	d.mc.	Yellow	R	A	A	A	0	0	A
	B	d.sc.	Dark green	R	A	A	0	0	0	A
	C	d.sc.	Dark green	R	A	A	A	0	0	0
	D	lc.	Yellow	R	A	A	0	0	0	0
	E	lc.	Yellow	S	A	A	A	0	0	0
	F	sc.	Yellow	S	A	A	0	0	0	0
14	A	lc.	Yellow	R	A	A	A	0	0	0
	B	d.sc.	Yellow	S	A	A	0	0	0	0
	C	sc.	Yellow	S	A	A	0	0	0	A
	D	sc.	Yellow	S	A	A	A	0	0	0
	E	sc.	Yellow	S	A	A	0	0	0	A
	F	lc.	Yellow	R	A	A	A	0	0	0
15	A	d.sc.	Green	R	A	A	A	0	0	0
	B	mc.	Green	S	A	A	A	0	0	A
	C	d.sc.	Green	S	A	A	A	0	0	0
	D	d.sc.	Green	S	A	0	A	0	0	0
	E	d.sc.	Green	S	A	A	A	0	0	A
	F	lc.	Yellow	R	A	A	0	0	0	0

d. = diplococci. mc. = moderate chains. S = smooth colony. sc. = short chains. lc. = long chains. R = rough colony.

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the others, but Case 9 developed in another scarlet fever ward and from this case three more children were infected (10–12). Another cross-infected ward furnished cases (13–18) all within 3 days of one another. Cases 19 and 20 were isolated examples of the disease.

The bile-insoluble strains were classified morphologically into diplococci, short chains of 1–10 cocci, moderate chains of 10–20 cocci, and long chains consisting mostly of more than 20 cocci. A comparison of the morphological characteristics, the colouration of the colonies on chocolate agar, and of the fermentation reaction of 120 strains from 20 acute cases of measles showed that there was no constancy. All kinds of combinations of the various characteristics and reactions occurred, and typical examples are given in Table I. Many of the short-chained cocci appeared to be similar to Tunnicliff's cocci.

The fermentation reactions of 120 strains showed that all strains with 5 exceptions produced acid in saccharose; all strains with 7 exceptions produced acid in lactose; 64 strains produced acid in salicin; 22 strains acid in inulin; 46 strains acid in raffinose, and no single strain produced acid in

Table II. *Agglutination reactions of strains from acutely ill measles cases.*

Strain	Agglutination titres with nine sera								
	1	2	3	4	5	6	7	8	9
Tunnicliff 1	1600	0	0	0	0	0	0	0	1600
„ 2	0	1600	0	0	0	0	0	0	1600
„ 3	0	0	1600	0	0	0	0	0	0
Duval 1	1600	0	0	1600	100	0	0	0	1600
„ 2	1600	0	0	1600	100	0	0	0	1600
Measles 4 E	1600	0	0	0	0	0	0	0	0
„ 6 D	0	0	0	0	0	0	0	0	100
„ 8 B	400	0	0	1600	100	0	0	0	0
„ 8 C	400	0	0	1600	400	0	0	0	0
„ 8 E	400	0	0	1600	200	0	0	100	0
„ 9 A	0	0	0	100	800	0	1600	100	800
„ 9 B	0	0	0	0	0	0	0	200	0
„ 9 E	1600	0	0	1600	1600	0	0	0	1600
„ 9 F	0	0	0	0	0	0	0	200	0
„ 10 B	0	0	0	0	0	0	0	100	0
„ 12 A	0	0	0	0	0	1600	0	1600	0
„ 12 B	0	0	0	200	0	0	0	800	0
„ 12 C	0	0	0	0	0	0	0	400	0
„ 12 D	800	0	0	800	200	0	0	0	0
„ 12 E	800	0	0	0	0	0	100	0	200
„ 12 F	0	0	0	800	0	0	0	200	200
„ 13 C	1600	0	0	1600	1600	100	1600	1600	1600
„ 14 B	0	0	0	0	0	1600	100	0	0
„ 14 E	0	0	0	0	0	400	0	0	0
„ 15 A	0	0	0	0	1600	0	1600	400	1600
„ 15 B	1600	0	0	800	1600	0	100	1600	400
„ 15 C	1600	0	100	1600	1600	800	0	1600	1600
„ 15 D	1600	0	100	1600	1600	800	0	1600	1600
„ 16 A	0	0	0	0	0	0	0	800	200
„ 16 B	0	0	0	0	0	0	0	0	0
„ 16 C	0	0	0	0	0	800	0	1600	200
„ 17 B	0	0	0	0	0	0	0	0	0
„ 18 A	1600	0	800	0	800	0	0	0	1600
„ 18 C	1600	0	1600	1600	1600	0	0	0	1600
„ 18 F	0	0	0	0	400	0	0	0	0
„ 20 A	1600	0	0	1600	200	400	0	1600	1600

mannite. Five strains produced no acid in any of the carbohydrate media and 1 strain was able only to produce acid in the medium containing inulin.

Agglutination tests were carried out on 67 strains only, since it was found impossible to obtain stable emulsion with 53 strains of long-chained cocci. All (120) strains were used in absorption tests with the 9 sera. In Table II the agglutination reactions are set forth. The serum prepared from Tunnicliff's No. 1 strain agglutinated both the Duval strains and 9 strains from measles cases to full titre 1 in 1600, 2 strains to 1 in 800, and 3 strains to 1 in 400. The sera for Tunnicliff's No. 2 and No. 3 strains did not show cross agglutination with any of the strains obtained from measles cases. Serum 4 was prepared from measles strain 8 C which was agglutinated to a titre of 1 in 400 by Tunnicliff's No. 1 strain. This serum did not cross agglutinate any of the Tunnicliff strains but agglutinated to full titre the Duval strains and 8 measles strains. Five other measles strains were partially agglutinated. Serum 5 prepared from measles strain 9 E, which was agglutinated to full titre by the serum for Tunnicliff No. 1 strain, did not agglutinate any of the Tunnicliff strains, it agglutinated the 2 Duval strains to 1 in 100 and 13 measles strains to various dilutions ranging from 1 in 100 to 1 in 1600. Serum 6 was prepared from strain 14 B, it agglutinated 7 measles strains besides the homologous one to various titres. Sera 7 and 8 were prepared for the A and B strains of Case 15. Serum 7 agglutinated 5 measles strains and serum 8, 16 measles strains in various dilutions. Serum 9 was prepared from strain 8 I obtained from a case of measles. The results obtained with this serum are discussed later when the strains from the acute and convalescent cases are compared.

Table III. *Absorption of agglutinins by strains from acute cases.*

		Sera and homologous strains								
Strains		1 T/1	2 T/2	3 T/3	4 8 C	5 9 E	6 14 B	7 15 A	8 15 B	9 8 I
Tunnicliff	1	X	0	0	0	0	0	0	0	0
,,	2	0	X	0	0	0	0	0	0	0
,,	3	0	0	X	0	0	0	0	0	0
Duval	1	X	0	0	0	0	0	0	0	0
,,	2	X	0	0	0	0	0	0	0	0
Measles	8 B	0	0	0	X	0	0	0	0	0
,,	8 C	0	0	0	X	0	0	0	0	0
,,	8 E	0	0	0	X	0	0	0	0	0
,,	9 E	0	0	0	0	X	0	0	0	0
,,	12 A	0	0	0	0	0	0	0	X	0
,,	12 B	0	0	0	0	0	0	0	X	0
,,	12 C	0	0	0	0	0	0	0	X	0
,,	12 D	0	0	0	X	0	0	0	0	0
,,	12 E	0	0	0	0	0	0	0	X	0
,,	12 F	0	0	0	X	0	0	0	0	0
,,	14 A	X	0	0	0	0	0	0	0	0
,,	14 B	0	0	0	0	0	X	0	0	0
,,	14 F	X	0	0	0	0	0	0	0	0
,,	15 A	0	0	0	0	0	0	X	0	0
,,	15 B	0	0	0	0	0	0	0	X	0
,,	16 B	0	0	0	X	0	0	0	0	0
,,	17 B	0	0	0	X	0	0	0	0	0

0 = no absorption. X = complete absorption.

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The absorption tests are summarised in Table III. Only the instances in which complete absorption of a serum was obtained are recorded. Classifying the strains by absorption tests, 2 Duval strains and 2 strains obtained from measles cases absorbed the serum prepared for Tunnicliff's No. 1 strain. No serological counterparts of Tunnicliff's Nos. 2 and 3 strains were obtained. Six strains (apart from the homologous strain) from 3 measles cases absorbed the agglutinins from Serum 4. Strains 9 F, 14 B, 15 A and 15 B, the homologous strains of Sera 5, 6, 7 and 8, were the only representatives of their types.

The cultural characteristics of strains identified serologically by absorption tests are compared in Table IV. From this table it is seen that there is no

Table IV. *Cultural characteristics of strains compared with serological characteristics.*

Strains		Morphology	Characteristics of colonies on chocolate blood agar: Colour	Characteristics of colonies on chocolate blood agar: Type	Fermentation reaction: Saccharose	Lactose	Salicin	Inulin	Mannite	Raffinose	Serological types
Tunnicliff	1	d.sc.	Green	S	A	A	A	0	0	A	1
,,	2	d.sc.	Yellow	S	A	A	A	A	0	0	2
,,	3	d.sc.	Nil	S	A	A	A	A	0	0	3
Duval	1	d.sc.	Green	S	A	A	A	0	0	A	1
,,	2	d.sc.	Green	S	A	A	A	0	0	A	1
Measles	8 B	d.	White	S	A	A	A	A	0	A	4
,,	8 C	d.sc.	Green	S	A	A	A	0	0	A	4
,,	8 E	d.sc.	Green	S	A	A	A	A	0	A	4
,,	9 F	lc.	Green	S	A	A	0	0	0	0	5
,,	12 A	d.	Dark green	R	A	A	A	0	0	0	9
,,	12 B	d.sc.	Dark green	S	A	A	A	A	0	0	9
,,	12 C	d.sc.	Dark green	S	A	A	A	A	0	0	9
,,	12 D	d.sc.	Green	R	A	A	A	A	0	A	4
,,	12 E	d.sc.	Green	S	A	A	A	0	0	A	9
,,	12 F	d.sc.	Green	R	A	A	A	A	0	A	4
,,	14 A	lc.	Yellow	R	A	A	A	0	0	0	1
,,	14 B	d.sc.	Yellow	S	A	A	0	0	0	0	6
,,	14 F	lc.	Yellow	R	A	A	A	0	0	0	1
,,	15 A	d.sc.	Yellow	R	A	A	0	0	0	0	7
,,	15 B	mc.	Yellow	S	A	A	A	0	0	A	8
,,	16 B	d.	Yellow	S	A	A	A	0	0	A	4
,,	17 B	d.sc.	Green	S	A	A	A	0	0	A	4

d. = diplococci.
sc. = short chains.
S = smooth type.
mc. = moderate chains.
lc. = long chains.
R = rough type.

correlation between cultural and serological characteristics. Even Tunnicliff's 3 strains of cocci gave entirely different cultural characteristics on chocolate blood agar and fermentation reactions in the various sugar media.

(3) *A comparison of strains isolated during the acute and convalescent stages of the illness.*

Six strains were obtained from each of two cases 8 and 9 during the acute stage of the illness, and 6 strains were similarly obtained 2 weeks after defervescence. The morphological and cultural characteristics are set forth in Table V. It will be seen that no evidence was obtained of any change in the

Table V. *Cultural characteristics of strains isolated during the acute and convalescent stages of the disease.*

Strain	Morphology	Characteristics of colonies on chocolate blood agar: Colour	Type	Fermentation reactions: Saccharose	Lactose	Salicin	Inulin	Mannite	Raffinose
Case 8. Acute									
A	lc.	Green	S	A	A	A	A	0	A
B	d.	White	S	A	A	A	0	0	A
C	d.sc.	Green	S	A	A	A	0	0	A
D	d.sc.	Dark green	R	A	A	A	0	0	A
E	d.sc.	Green	S	A	A	A	0	0	A
F	d.sc.	Yellow	R	A	A	A	0	0	A
Case 8. Convalescent									
G	d.sc.	Yellow	S	A	A	0	0	0	A
H	d.sc.	Yellow	S	A	A	0	0	0	0
I	d.sc.	Yellow	S	A	A	A	0	0	A
J	d.sc.	Green	R	A	0	A	0	0	0
K	d.sc.	Green	S	A	A	A	0	0	0
L	d.sc.	Green	S	A	A	A	0	0	A
Case 12. Acute									
A	d.	Green	R	A	A	A	0	0	0
B	d.sc.	Green	S	A	A	A	A	0	0
C	d.sc.	Green	S	A	A	A	A	0	0
D	d.sc.	Green	R	A	A	A	0	0	A
E	d.sc.	Green	S	A	A	A	0	0	A
F	d.sc.	Green	R	A	A	A	A	0	A
Case 12. Convalescent									
G	d.sc.	Green	S	A	A	A	0	0	A
H	d.sc.	Yellow	R	A	A	A	0	0	A
I	d.sc.	Green	S	A	A	A	0	0	0
J	mc.	Green	S	A	A	A	0	0	0
K	d.sc.	Green	R	A	A	A	0	0	0
L	d.sc.	Green	S	A	A	A	0	0	0

Table VI. *Agglutination and agglutinin absorption reactions of strains isolated during the acute and convalescent stages of the illness.*

Strain	Sera: 1	2	3	4	5	6	7	8	9
Case 8. Acute									
A	0	0	0	0	0	0	0	0	0
B	400	0	0	*1600*	100	0	0	0	0
C	400	0	0	*1600*	400	0	0	0	0
D	0	0	0	0	0	0	0	0	0
E	400	0	0	*1600*	200	0	0	0	0
F	0	0	0	0	0	0	0	0	0
Case 8. Convalescent									
G	0	0	0	0	0	0	0	0	0
H	0	0	0	0	0	0	0	0	0
I	1600	0	0	1600	1600	0	800	1600	*1600*
J	400	0	0	1600	100	0	0	0	0
K	0	0	0	0	0	0	0	0	0
L	1600	0	0	1600	1600	0	400	1600	*1600*
Case 12. Acute									
A	0	0	0	0	0	1600	0	*1600*	0
B	0	0	0	200	0	0	0	*800*	0
C	0	0	0	0	0	0	0	*400*	0
D	800	0	0	800	200	0	0	0	0
E	800	0	0	0	0	0	0	*100*	200
F	0	0	0	800	0	0	0	200	200
Case 12. Convalescent									
G	1600	0	0	1600	0	0	0	400	1600
H	0	0	0	0	0	0	0	0	0
I	400	0	0	800	0	0	0	400	400
J	800	0	0	200	0	0	0	0	0
K	0	0	0	200	0	0	0	0	0
L	0	0	0	0	0	0	0	0	0

Titres in italic indicate that strains completely absorbed the respective sera.

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type of the flora of the green-producing cocci present in the convalescent stage as compared with the acute stage.

In Table VI the agglutination and absorption reaction of these 24 strains are given. Serum 4 was prepared from strain 8 C, and Serum 9 from strain 8 I. Thus 3 strains from Case 8 in the acute stage were found to be identical and 4 strains obtained from Case 12 in the acute stage absorbed the agglutinins from Serum 8, but no similar types were found among the 12 strains obtained during convalescence. Further, Serum 9 prepared from strain 8 I, a strain obtained during convalescence, showed marked cross agglutination (Table II) with Tunnicliff's, Duval's and the various measles strains. The Tunnicliff and Duval strains and 8 measles strains were agglutinated to titre (1 in 1600) and 5 other measles strains were agglutinated between 1 in 100 and 1 in 800.

(4) *Comparison of strains from non-measles cases.*

The pharyngeal secretions of 6 children who gave no history of having had measles were similarly cultured and 6 bile-insoluble green-producing strains from each case were examined in detail. Examples of the cultural characteristics are set forth in Table VII, while the results of the agglutination tests are shown in Table VIII.

It is evident from the cultural tests that the cocci from the normal cannot be distinguished from those from acute measles cases. These strains, however, did not show any cross agglutination with the sera prepared from the Tunnicliff strains, but cross agglutinated with the sera prepared from strains from acute measles. Not a single strain was capable of absorbing the agglutinins from any of the sera.

(5) *Toxin production by various strains from measles.*

In a previous paper (Smith and Fraser, 1928) it was shown that only a relatively weak toxin, as judged by skin reactions, could be obtained from the Tunnicliff strains. Twenty-four strains of the freshly isolated cocci from acute measles cases were cultured in 1 per cent. blood broth media, and proteose peptone media for 4 days at 37° C. The filtrates obtained from these cultures were tested on numerous children by means of intracutaneous tests.

Skin tests were made using a series of 5 filtrates and a control test was made with uninoculated culture medium diluted to the same extent as the filtrate. It was found that several filtrates were capable at first of producing reactions in a dilution of 1 in 100, but in a few days' time the substance inciting the skin reaction became reduced in potency. Reactions could be elicited on various individuals with 17 filtrates by using a dilution of 1 in 10. These reactions, however, showed no relationship to the clinical history. Individuals, who undoubtedly must have had measles, reacted positively, while those who had not had measles frequently gave negative tests. Further, 8 children who had not had measles, were inoculated subcutaneously with the "toxin" from Tunnicliff's No. 1 strain which was capable of inducing a skin reaction in a

1 in 10 dilution. Four children received 1 c.c. and four 2 c.c. subcutaneously. They showed only a slight local reaction and no general disturbance.

Table VII. *Cultural characteristics of strains from non-measles cases.*

Case	Strains	Morphology	Saccharose	Lactose	Salicin	Inulin	Mannite	Raffinose
1	A	d.sc.	A	A	A	0	0	A
	B	d.	A	A	A	0	0	A
	C	d.sc.	A	A	A	0	0	0
	D	lc.	A	0	0	0	0	A
	E	lc.	A	A	0	0	0	0
	F	d.sc.	A	A	A	0	0	0
2	A	lc.	A	A	A	0	0	A
	B	sc.	A	A	A	0	0	A
	C	lc.	A	A	A	A	0	A
	D	d.sc.	A	A	A	0	0	A
	E	d.	0	A	0	0	0	0
	F	lc.	A	A	A	0	0	A
3	A	sc.	A	A	A	A	0	A
	B	d.sc.	A	A	A	0	0	A
	C	d.sc.	A	A	A	0	0	A
	D	d.sc.	A	A	0	0	0	0
	E	d.sc.	A	A	A	0	0	0
	F	d.	A	A	A	0	0	A

d. = diplococci. mc. = moderate chains.
sc. = short chains. lc. = long chains.

Table VIII. *Agglutination of strains from non-measles cases by sera prepared for strains from measles cases.*

		Sera								
Case	Strain	1	2	3	4	5	6	7	8	9
1	A	0	0	0	1600	0	0	0	1600	0
	B	0	0	0	0	0	0	0	1600	0
	C	0	0	0	0	0	100	100	1600	0
2	A	0	0	0	0	0	0	0	200	0
	B	0	0	0	0	0	0	0	200	0
	D	0	0	0	0	0	0	0	1600	0
	F	0	0	0	0	0	0	0	100	0
3	C	0	0	0	0	0	0	0	400	0
4	A	0	0	0	100	200	0	0	400	0
	B	0	0	0	100	200	0	0	400	0
	C	0	0	0	200	200	0	100	400	0
	D	0	0	0	200	1600	0	0	1600	0
	E	0	0	0	200	1600	0	0	1600	0
	F	0	0	0	400	1600	0	0	1600	100

Cases 5, 6. None of 6 strains showed any agglutination.

(6) *Agglutination of strains with sera from acute and convalescent cases.*

Serum was obtained from cases at the commencement of the illness and 3 weeks later a further sample was obtained from each case. The two sera from each case were then tested against the same emulsions of the Tunnicliff strains and the 6 strains isolated from the patient at the commencement of the illness. The results of this investigation are set forth in Table IX. The sera from 2 cases only agglutinated certain strains isolated from the patients. The sera obtained during the acute and convalescent stages of Case 12 agglutinated strain 12 B in a 1 in 20 dilution, and strain 12 F in a 1 in 80 dilution. Both samples of serum from Case 20 agglutinated Strain E to a 1 in 20 dilution.

Table IX. *Agglutination of Tunnicliff's strains and of strains from patients by sera obtained during the acute and convalescent stages of the illness.*

Case 12. Agglutination with serum obtained during acute stage.

Strain		Dilution of serum 1/5	1/10	1/20	1/40	1/80	1/160	Control
Tunnicliff	1	0	0	0	0	0	0	0
„	2	0	0	0	0	0	0	J
„	3	0	0	0	0	0	0	0
Patients' strains								
	A	0	0	0	0	0	0	0
	B	+ +	+ +	+	0	0	0	0
	C	0	0	0	0	0	0	0
	D	0	0	0	0	0	0	0
	E	+	0	0	0	0	0	0
	F	+ + +	+ + +	+ +	+ +	+	0	0

Case 20. Agglutination with serum obtained during acute stage.

Strain		Dilution of serum 1/5	1/10	1/20	1/40	1/80	1/160	Control
Tunnicliff	1	0	0	0	0	0	0	0
„	2	0	0	0	0	0	0	0
„	3	0	0	0	0	0	0	0
Patients' strains								
	A	0	0	0	0	0	0	0
	B	0	0	0	0	0	0	0
	C	0	0	0	0	0	0	0
	D	0	0	0	0	0	0	0
	E	+ + +	+ + +	+ +	0	0	0	0
	F	0	0	0	0	0	0	0

Agglutination with convalescent sera from both cases gave results identical to those obtained with first specimens.

DISCUSSION.

It was well understood that the selection of the various strains of cocci on the basis of bile solubility would conceivably allow the retention of certain strains which should have been classed as pneumococci, and it was to overcome this possibility that 6 strains were selected from each patient. It may be said at once that no cultural or biochemical test indicated a prevailing distinguishable type of green-producing coccus in the secretions from the measles cases, and similarly no type of coccus was found in the measles which could be said to be absent in the non-measles cases.

The serological tests were not carried out with a view of producing an orderly classification of the various strains, but were done in order to see whether, serologically, identical cocci could be obtained from cases which arose from a common source. These tests indicated that serologically the cocci were extremely heterogeneous. Even the strains from one individual frequently showed the utmost diversity. On the other hand, although very few strains from measles cases were classified by the absorption tests, quite a number of strains showed a considerable amount of cross agglutination to various titres with the various sera.

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Clinical and epidemiological observations indicate that a fresh measles case never arises as a result of a carrier condition in another individual. The cultural and the serological tests carried out on strains of cocci isolated during the acute and convalescent stages of the disease have indicated that morphologically and culturally the cocci do not show any marked change. The agglutination tests showed a considerable relationship between the strains, but absorption tests showed that no strains actually identical with the acute strains were obtained from the convalescent cases. Similarly the strains from normal throats when tested with the sera for the Tunnicliff strains showed no cross agglutination, but these strains cross agglutinated with the sera for strains from acute cases.

The tests for toxin production by the various strains showed that, compared with haemolytic streptococci, these green-producing cocci only produced feeble "toxins" which gave rise to skin reactions, difficult to interpret and unrelated to the immunity history of the patient.

Again it has been shown that in scarlet fever (Smith, 1927), although there is no actual blood invasion by the streptococcus, 40 per cent. of cases show, during convalescence, an increase in the agglutinin content of the serum for the strain of *S. haemolyticus* isolated from the throat at the commencement of the illness, and since the reports of various workers have shown that in measles a considerable percentage of cases yield green-producing cocci in blood cultures, it might be expected that a greater number of measles than of scarlet fever cases would show an increase in the agglutinin content of the serum during convalescence. In the series of sera tested against the various strains of cocci no increase in the agglutinin content of the serum has been demonstrable.

Conclusions.

In a previous paper (Smith and Fraser, 1928) it has been shown that skin tests made with the toxin obtained from Tunnicliff's strain of green-producing streptococcus on normal individuals, and on cases prior to and after an attack of measles, did not give any definite positive evidence of the etiological relationship of this organism to measles. Further, an attack of measles did not produce any apparent increase in the antitoxic content of the sera from patients for this toxin.

Now, strains of green-producing cocci obtained from the pharyngeal secretions of acute and convalescent cases of measles, and from non-measles cases have been examined by morphological, cultural and serological methods. Toxin production by various strains and the agglutination of strains by sera from acute and convalescent cases have been studied.

No proof has been obtained that these green-producing cocci bear any etiological relationship to measles. In fact it is believed that these green-producing cocci found in the pharyngeal secretions of acute cases are part of the normal bacterial flora of the upper respiratory tract.

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