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Indifferent and Haemolytic Streptococci Possessing Group-Antigen F

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SUMMARY

'Indifferent' strains of streptococci—i.e. strains which give no haemolysis or greening on blood-agar plates—frequently occur in cultures from dental root-canals. A serological study of over 200 strains of these streptococci showed that about half of them belonged to Lancefield groups F, G or C. It was shown by cross-absorption that the group-antigen of indifferent streptococci of group F is identical with the group-antigen of haemolytic strains of group F. Apart from the group-antigen, five independent carbohydrate type-antigens, localized in the cell wall, were demonstrated in group F strains. These type-antigens were found in groups other than F, e.g. the type-antigen I was observed in haemolytic and indifferent strains of group G; the type-antigen III occurred in indifferent strains of group C. Several strains with a type-antigen but without group-antigens were observed. The presence of carbohydrate type-antigens in formamide extracts can cause confusing cross-reactions in the grouping procedure, unless strains without type-antigen are used for the preparation of sera.

INTRODUCTION

The frequent occurrence of 'indifferent streptococci' in cultures from dental root-canals was described by Winkler & Van Amerongen (1959). The adjective indifferent is used for a strain which gave no haemolysis and no greening on blood-agar plates. In a new series of root-canal cultures, taken with the same technique, 822 cultures out of 2730 were positive. From the positive cultures in the present series, 212 strains of indifferent streptococci (25, 8 %) were isolated against 27 strains of haemolytic streptococci (3, 3 %) (see Table 1). It was observed by the same authors and by Ottens (1961) that about 50 % of the indifferent strains could be grouped serologically and belonged to the Lancefield groups F, G and C (Table 2). The haemolytic strains belonged mainly to groups F and G. No haemolytic strains of group C were observed in the present series; some haemolytic strains of this group were, however, isolated by Winkler & Van Amerongen. The occurrence, in the same habitat, of haemolytic and indifferent strains of the same serological group, posed the question of the relationship between the haemolytic and the indifferent streptococci. Only minor differences in biochemical properties were observed between haemolytic and indifferent strains in the same serological group (see Table 3; and Ottens 1961), though the indifferent strains of group F and C generally fermented lactose which the haemolytic strains did not.

A further study of the serological properties of the indifferent and haemolytic strains of group F is reported in this paper.

Table 1. *Bacteria isolated from 822 positive dental root-canal cultures*

	Total number	% of all positive cultures	% of all isolations
Streptococcus faecalis	158	19·2	15·2
Indifferent streptococci	172	20·9	16·6
Indifferent streptococci (anaerobic)	40	4·9	3·9
Haemolytic streptococci	27	3·3	2·6
Streptococcus mitis	200	24·3	19·3
Streptococcus salivarius	31	3·8	3·0
Lactobacillus sp.	96	11·7	9·3
Staphylococci	156	19·0	15·0
Diphtheroids	49	6·0	4·7
Others	107	13·0	10·3

Total isolations 1036; total positive cultures 822; total cultures 2730.

Table 2. *Serological properties of indifferent and haemolytic streptococci from root-canals*

	Tested	Non-groupable	Group F	Group G	Group C
Haemolytic	35	5	16	14	—
Indifferent	159	77	51	15	16

METHODS

Root-canal cultures were taken as described by Winkler & Van Amerongen (1959). At first the isolated strains were cultured in brain heart infusion broth and stored in the refrigerator. These strains were recultured at regular intervals; later, all strains were lyophilized. They were designated by numbers preceded by H for haemolytic strains and I for indifferent strains.

Protein-free extracts were prepared with formamide at 160–180° according to Fuller (1938). Precipitation reactions were done in capillary tubes, pipetting the extract on to the serum. Gel-diffusion experiments were performed with the micromethod of Scheidegger (1955).

Sera. Reference group F sera were obtained from the Central Dutch Public Health Laboratories and from Burroughs, Wellcome and Co. Group- and type-specific sera were prepared as follows. Well-grown cultures in brain-heart infusion broth (Difco) with 0·1 % (w/v) glucose were centrifuged. The sediment was heated for 1 hr., at 60°, washed twice in saline and suspended in saline at 1/50 of the original volume. This stock antigen was kept at 4° and diluted 1/10 with saline before use.

Rabbits were injected on 12 subsequent days with three 0·5 ml. and nine 1 ml. doses of diluted antigen. Sera were tested 1 week after the last injection (primary sera). Animals with positive primary sera, after an interval of 6–18 weeks, received two booster injections with 0·5 ml. of undiluted antigen, spaced 1 week. Blood was collected 1 week after the last injection (booster sera). It has been shown (Ottens, 1961) that the specificity of the primary and booster sera do not differ.

Cross-absorption tests. The many cross-absorption tests required prompted us to develop a micro-method. The sediment of a well-grown culture, after heating

1 hr. at 60° and washing with saline, was centrifuged in a haematocrit for 1 hr. at 4000 rev./min. The capillary of the haematocrit tube had a calibrated volume of 100 mm.^{3} which can be read down to about 1 mm.^{3}. After centrifugation the volume of the sediment was read and the overlying supernatant fluid removed with a capillary pipette. An equal volume of serum was then added to the sediment and mixed with a wire. The mixture was incubated in a waterbath for 30 min. at 37°. After centrifugation the clear supernatant serum could be used for three or four precipitation reactions. In general a volume-ratio of cocci:serum of 1:1 was sufficient for complete absorption. The minimal quantity of serum which can be absorbed by this technique is about 20 mm.^{3}. All sera were also absorbed with the homologous strain as a control. With weak sera control absorptions with a non-related strain should be performed.

RESULTS

General properties of the indifferent strains of group F

Morphologically short chains of gram-positive cocci are observed with a size slightly smaller than *Streptococcus mitis*. The colonies on blood agar are white and small but never so small as the 'minute' colonies of haemolytic streptococci of group F. No greening or haemolysis was observed on blood agar plates. No fibrinolysin, collagenase or hyaluronidase is produced (Ottens, 1961). Bile (10 and 40 %, v/v) and

Table 3. *Fermentative properties of streptococci of Lancefield groups F, G and C*

Group	Indifferent or haemolytic	Origin*	Lactose	Mannitol	Trehalose	Raffinose	Inulin	Starch	Aesculin
F	Indiff.	RC	48/49†	2/49	47/49	2/49	5/49	3/49	49/49
F	Haem.	RC	0/8	0/8	6/8	0/8	0/8	0/8	7/8
F	Haem.	D	3/22	0/22	14/22	1/22	1/22	0/22	17/22
G	Indiff.	RC	9/9	0/9	8/9	0/9	0/9	0/9	9/9
G	Haem.	RC	9/9	0/9	9/9	1/9	0/9	0/9	9/9
G	Haem.	D	9/10	0/10	10/10	0/10	0/10	0/10	9/10
C	Indiff.	RC	10/10	0/10	5/10	0/10	0/10	0/10	10/10
C	Haem.	D	0/5	0/5	5/5	0/5	0/5	0/5	2/5

* RC = strains from root canals; D = strains isolated from other materials (mainly throat swabs).
† Numerator = number of strains positive after 5 days/denominator = number of strains tested;

methylene blue 0·1 % (w/v) suppressed growth, but optochin did not. All strains were sensitive to penicillin, streptomycin, chloramphenicol, tetracyclines and erythromycin. Some fermentation reactions are given in Table 3. Salicin was always hydrolysed; arabinose and sorbitol were not attacked; hippurate was not hydrolysed. No dextrans or levans were formed from 5 % (w/v) sucrose. Most strains produced ammonia from arginine. Some strains were micro-aerophilic on primary isolation. No pathogenicity for mice was observed. Indifferent strains were less sensitive to crystal violet than was *S. mitis* and could be isolated from saliva on blood-agar plates containing 1/750 crystal violet.

Serological identity of group-antigens of haemolytic and indifferent streptococci of group F

The positive precipitation reactions of formamide extracts of indifferent streptococci with the usual Lancefield group F sera (prepared with haemolytic strains) might be due to a cross-reaction with a related but not identical antigen. To examine the identity or otherwise of the group-antigens in haemolytic and indifferent strains, cross-absorption tests were performed with grouping sera prepared against both types of strain. Sera were prepared, as described under Methods. The precipitation reactions of some strains with these sera and with the reference sera are given in Table 4.

Table 4. *Precipitation reactions of sera obtained by immunization with group F streptococci*

All the formamide extracts gave precipitation with the group F precipitating sera from the Dutch Public Health Laboratory and from Burroughs, Wellcome and Co. None of the antisera reacted with formamide extracts of authentic group A, B or C streptococci.

Formamide extract of strain	Type	Origin	Sera: S 1 (Sera prepared with strain: La 60 R)	S 61 (I 57)	S 21 (I 15)	S 58 (H 146)	S 2 (La 60 R)	S 59 (H 145)	S 53 (I 14)	S 70 (I 107)	S 64 (I 62)	S 63 (I 62)	S 69 (I 250)
I 15	I	RC	+	+	+	+	−	−	−	−	−	+	+
H 146	I	RC	+	+	+	+	−	−	−	−	−	+	+
I 65	I	RC	+	+	+	+	−	−	−	−	−	+	+
I 103	I	RC	+	+	+	+	−	−	−	−	−	0	0
I 111	I	RC	+	+	+	+	−	−	−	−	−	0	0
H 180	I	RC	+	+	+	+	−	−	−	−	−	+	0
H 183	I	RC	+	+	+	+	−	−	−	−	−	+	0
H 174	I	D	+	+	+	+	−	−	−	−	−	+	0
H 177	I	D	+	+	+	+	−	−	−	−	−	+	0
La 60 R	II	—	+	+	−	−	+	+	−	−	−	+	+
C 628	II	—	−	−	−	−	+	+	−	−	−	−	−
H 145	II	RC	+	+	−	−	+	+	−	−	−	+	+
H 192	II	RC	+	+	−	−	+	+	−	−	−	0	0
H 194	II	RC	+	+	−	−	+	+	−	−	−	+	+
H 206	II	RC	+	+	−	−	+	+	−	−	−	+	0
H 199	II	D	+	+	−	−	+	+	−	−	−	0	0
H 200	II	D	+	+	−	−	+	+	−	−	−	0	0
H 230	II	D	+	+	−	−	+	+	−	−	−	0	0
I 2	III	RC	+	−	−	−	−	−	+	−	−	+	+
I 14	III	RC	+	−	−	−	−	−	+	−	−	+	+
I 101	III	RC	+	−	−	−	−	−	+	−	−	+	+
I 195	III	RC	+	−	−	−	−	−	+	−	−	+	0
I 89	III	RC	+	−	−	−	−	−	+	−	−	0	0
I 199	III	RC	+	+	−	−	−	−	+	−	−	+	0
I 255	III	RC	+	−	−	−	−	−	+	−	−	+	0
I 62	IV	RC	+	−	−	−	−	−	−	−	+	+	+
I 107	V	RC	+	−	−	−	−	−	−	+	−	+	+
I 57	—	RC	+	−	−	−	−	−	−	−	−	+	+
I 112	—	RC	+	−	−	−	−	−	−	−	−	+	0
I 124	—	RC	+	−	−	−	−	−	−	−	−	+	0
I 250	—	RC	+	+	−	−	−	−	−	−	−	+	+

Origin RC = strains isolated from dental root-canals. D = strains isolated from other materials.
0 = not tested.

The usual difficulty of defining a group serum arose immediately. Starting from the two group F reference antisera we considered all streptococci which showed a positive reaction with these sera as group F streptococci. When one of our sera reacted with extracts of all available F streptococci we considered the serum as a group-specific serum (S 1, S 61). The negative reaction with strain C 628 will be discussed later. A serum which reacted with only some of the extracts was considered as a type-specific serum (S 21, S 58, S 2, S 59, S 53, S 70, S 64). The sera S 63 and S 69 were only tested with some of the extracts; since they gave positive reactions with representative extracts from strains in all groups, they were evidently group-specific sera.

It was observed that with the same streptococcus as an antigen one animal might produce a group-specific serum whereas another animal produced a type-specific serum (e.g. sera S 63 and S 64 in Table 4). It was furthermore observed, in accordance with Bliss (1937) and Seelemann & Obiger (1958), that streptococci which possessed a group-antigen as well as a type-antigen easily induced a type-specific serum and rarely a group-specific serum. The presence of the type-antigen seemed to inhibit the formation of group antibodies. In our case the best group-specific sera were indeed obtained with streptococci which lacked a type-antigen (strains I 57 and I 250 in Table 4).

Table 5. *Cross-absorptions showing the identity of the group F antigen in indifferent and haemolytic streptococci*

Serum	Prepared with strain	Absorbed with	Formamide extracts		
			La 60 R	I 57	I 62
S 1	La 60 R	—	+ +	+ +	+ +
S 1	La 60 R	I 57	−	−	−
S 61	I 57	—	+ + +	+ + +	+ + +
S 61	I 57	La 60 R	−	−	−
S 61	I 57	I 62	−	−	−
S 63	I 62	—	+	+	+ + +
S 63	I 62	I 57	−	−	+ +

+ + + = precipitation immediately positive; + + = precipitation positive in *c.* 30 sec.; + = precipitation positive in 1–3 min.; − = no precipitation within 5 min.

Once the group-specific sera prepared with haemolytic strains (La 60 R) and indifferent strains (I 57, I 62, I 250) were available, cross-absorption tests could be done. An example of such an experiment is given in Table 5. It can be seen that a group-specific serum prepared with the haemolytic strain Lancefield 60 R was completely exhausted after absorption with the indifferent strains I 57 and I 62, and vice versa. This serological identity was confirmed by gel diffusion experiments according to Ouchterlony (Ottens, 1961).

We conclude that, as far as serological evidence goes, the group F antigen in the indifferent streptococci was identical with that in haemolytic strains. These indifferent streptococci have indeed to be considered as group F streptococci.

Type-antigens of group F streptococci

The results of Table 4 suggested that some streptococci (indifferent as well as haemolytic strains) can give rise to antibodies which react only with the formamide extracts of some of the strains. This permits the subdivision of group F into five types of which I, II and III are frequent and IV and V are rare. Cross-absorption tests with the five type-sera showed that the type-antigens in the formamide extracts were independent (Table 6).

Table 6. *Cross-absorptions showing the serological independence of type-antigens*

The sera were absorbed with the indicated cells and tested with formamide extracts of the homologous strains. The results of the precipitation reactions of the absorbed sera with these extracts are indicated in the table as + or −.

	Sera				
	Type I	Type II	Type III	Type IV	Type V
	Sera prepared with strain				
Sera absorbed with cells of	H 146	H 145	I 14	I 62	I 107
I 15 (type I)	−	+	+	+	+
La 60 R (type II)	+	−	+	+	+
I 14 (type III)	+	+	−	+	+
I 62 (type IV)	+	+	+	−	+
I 107 (type V)	+	+	+	+	−

Type I and type II occurred frequently in haemolytic strains of group F streptococci. Type III is the most frequent type-antigen found in indifferent strains (Table 7). Type I is most probably identical with the type I described by Bliss (1937).

The type-antigens are most probably carbohydrates. This was already implied in their presence in formamide extracts prepared at 160°–180° and was confirmed by pepsin treatment of formamide extracts, which did not change the reactivity (Ottens, 1961). Recently Dr J. M. N. Willers (personal communication) in our laboratory has shown the presence of rhamnose, galactose and glucosamine in the type I antigen. The group- and type-antigens are probably localized in or on the cell wall. This is already suggested by the absorption of antibodies by whole cells. It was confirmed for the group-antigen with strain I 57 and for the type-antigen with strain I 8. Both strains were disintegrated with a Mickle disintegrator. The cell debris were sedimented and treated with trypsin. The resulting cell walls were used for formamide extraction. These extracts showed the same reactivity and specificity as did the extracts prepared from whole cocci. Though this experiment does not exclude the presence of the antigens within the cell, it seems to indicate that they are localized mainly in the cell wall.

Occurrence of type antigens in groups other than group F

When the available streptococci (haemolytic and indifferent strains) were tested systematically with the type-specific sera obtained with group F streptococci, it appeared that some of the type-antigens also occurred in groups G and C (Table 7). That a type-antigen in group F haemolytic streptococci also occurred in group G streptococci was observed by Bliss (1937). The identity of the type I antigen in

group F and G was proven by cross-absorption tests (Table 8) and by gel-diffusion. We observed, furthermore, that type-antigen III occurred in indifferent strains of group C streptococci; this was confirmed by cross-absorption and gel-diffusion. The distribution of type-antigens over the available strains is given in Table 7. These results prompted us to indicate strains by group- and type-antigens: F, I standing for an F strain with type I antigen, G, O standing for a G strain with no (known) type-antigen, etc.

Table 7. *Distribution of type-antigens in the Lancefield groups F, G and C*

Repeated culture from the same root-canal (patient) often yielded isolations of identical strains, which could not be considered as independent. The numbers in the table refer to independent isolations only. The numbers in brackets refer to all isolations.

		Total		Type I antigen	Type II antigen	Type III antigen	Type IV antigen	Type V antigen	No type antigen
Group F:	Origin								
Indiff. str.	RC	68 (85)	35 (44)	3 (4)	—	22 (25)	1	2	7 (12)
Haem. str.	RC		8 (10)	2 (3)	6 (7)	—	—	—	—
Haem. str.	Clin		25 (31)	8 (13)	11 (12)	—	—	—	6
Group G:									
Indiff. str.	RC	36 (44)	6 (12)	2 (6)	—	—	—	—	4 (6)
Haem. str.	RC		11 (12)	11 (12)	—	—	—	—	—
Haem. str.	Clin		19 (20)	8 (9)	—	—	—	—	11
Group C									
Indiff. str.	RC	29 (32)	10 (12)	—	—	5 (7)	—	—	5
Haem. str.	Clin		19 (20)	—	—	—	—	—	19 (20)
	Total	133 (161)		34 (47)	17 (19)	27 (32)	1	2	52 (60)

Origin RC = strains isolated from dental root-canals; Clin. = strains isolated from clinical material.

Table 8. *Absorption tests to prove the identity of the type I antigen in group F and group G streptococci*

	Serum						
	G	Type I	G	G	G	Type I	Type I
	Serum absorbed with strain of a						
	–	–	G, O	G, I	F, I	G, O	G, I
Formamide extracts (G, O)	+	–	–	–	+	.	.
Formamide extracts (G, I)	+	+	–	–	+	+	–
Formamide extracts (F, I)	–	+	.	.	.	+	–

(G, O) strain = group G streptococci; no (known) type-antigens. (G, I) strain = group G streptococci; with type I antigen. (F, I) strain = group F streptococci; with type I antigen.

The streptococcus strain C 628

Lancefield (1955) required that a group F serum prepared with her (haemolytic) strain 60 R should also react with extracts of strain C 628. However, all our group F sera lacked this property and could accordingly not be considered as acceptable group F sera. A further study of this puzzle seemed indicated. Table 4 shows that strain 60 R reacted with all group-sera and with the type II serum, suggesting that the strain contains both the group-antigen and the type II antigen; antibodies against one or both antigens might thus be expected in sera prepared with this

strain. Strain C 628, on the other hand, did react with the reference group sera and with the type II serum but not with our grouping sera of group F. This might be explained by supposing that strain C 628 contains the type II antigen but not the group-antigen. Cross-absorption tests seemed to corroborate this hypothesis (Table 9), since strain C 628 exhausted the type II serum but did not decrease the reactivity of our group serum, whereas strain 60 R exhausted both sera.

Table 9. *Absorption test showing the absence of group F antigen in strain* C 628

	Serum					
	F	F	F	T II	T II	T II
	Serum absorbed with strain					
	–	La 60 R	C 628	–	La 60 R	C 628
Formamide extracts:						
of strain La 60 R	+	–	+	+	–	–
of strain C 628	–	–	–	+	–	–
of strain F, O	+	–	+	–	–	–

Serum F = group F serum, prepared with strain I 57 (see Table 4); serum T II = antiserum containing type II antibodies only (see Table 4); strain F, O = contains group F antigen, type-antigens I–V not present (strain I 57).

Table 10. *Analysis of a reference group F serum R.I.V. showing that this serum contains antibodies against group F antigen* and *against type II antigen*

	Formamide extract of strain						
	La 60 R	C 628	I 57	I 15	I 14	I 62	I 107
	Containing the antigens						
	(F, II)	(O, II)	(F, O)	(F, I)	(F, III)	(F, IV)	(F, V)
Group F serum R.I.V.	++	++	++	++	++	++	++
Same after absorption with La 60 R	–	–	–	–	–	–	–
Same after absorption with C 628	+	–	+	+	+	+	+
Same after absorption with I 57	+	+	–	–	–	–	–

A further consequence of this explanation would be that the reference sera prepared according to Lancefield's requirement that they should react with extracts of strain C 628, could contain antigroup F (as well as anti-type II) antibodies. Both reference sera were consequently tested before and after absorption with La 60 R (F, II), C 628 (O, II) and I 57 (F, O) with the extracts of various strains. One such experiment is shown in Table 10; the other serum gave similar results. It is evident that absorption with strain C 628 eliminated only the antibodies against its own antigen (O, II), that with strain I 57 all group antibodies but not the type II antibodies were absorbed, whereas absorption with La 60 R eliminated both kinds of antibody. These results seem to indicate that strain C 628 should indeed be designated as a (O, II) strain containing only the type II antigen.

Lancefield obviously added the requirement of reactivity with strain C 628 to include the haemolytic strains with type II antigen (but without F antigen) in the

F group. Though this was practical at the time it may lead to confusion. This is illustrated by a third commercial serum which contained only antibodies against type II and fulfilled the Lancefield requirements of reacting with strains 60 R (F, II) and C 628 (O, II) but which did not react with (F, I) strains (false negative). If the group II antigen should occur outside group F false positives could also be obtained; we observed one such case. One of the three strains of group T (de Moor, 1960) contained the type II antigen and reacted consequently with the reference F sera.

Table 11. *Observed combinations of group- and type-antigens*

	Antigens				Antiserum								
Number	Group	Type		Origin	F	G	C	T	Type I	Type II	Type III	Type IV	Type V
I 57	F	—	RC	Indiff.	+	−	−	−	−	−	−	−	−
I 15	F	I	RC	Indiff.	+	−	−	−	+	−	−	−	−
I 14	F	III	RC	Indiff.	+	−	−	−	−	−	+	−	−
I 62	F	IV	RC	Indiff.	+	−	−	−	−	−	−	+	−
I 107	F	V	RC	Indiff.	+	−	−	−	−	−	−	−	+
H 146	F	I	RC	β-Haem.	+	−	−	−	+	−	−	−	−
H 145	F	II	RC	β-Haem.	+	−	−	−	−	+	−	−	−
3519	F	—	D	β-Haem.	+	−	−	−	−	−	−	−	−
3548	F	I	D	β-Haem.	+	−	−	−	+	−	−	−	−
3637	F	II	D	β-Haem.	+	−	−	−	−	+	−	−	−
I 18	G	—	RC	Indiff.	−	+	−	−	−	−	−	−	−
I 40	G	I	RC	Indiff.	−	+	−	−	+	−	−	−	−
H 68	G	I	RC	β-Haem.	−	+	−	−	+	−	−	−	−
H 4	G	—	D	β-Haem.	−	+	−	−	−	−	−	−	−
H 170	G	I	D	β-Haem.	−	+	−	−	+	−	−	−	−
I 35	C	—	RC	Indiff.	−	−	+	−	−	−	−	−	−
I 51	C	III	RC	Indiff.	−	−	+	−	−	−	+	−	−
H 5	C	—	D	β-Haem.	−	−	+	−	−	−	−	−	−
639	T	II	D	β-Haem.	−	−	−	+	−	+	−	−	−
I 8	—	I	RC	Indiff.	−	−	−	−	+	−	−	−	−
I 42	—	I	RC	Indiff.	−	−	−	−	+	−	−	−	−
I 42v	—	I	RC	α-Haem.	−	−	−	−	+	−	−	−	−
H 189	—	II	D	β-Haem.	—	—	—	—	−	+	−	−	−
3634	—	II	D	β-Haem.	−	−	−	−	−	+	−	−	−
3675	—	II	D	β-Haem.	−	−	−	−	−	+	−	−	−
3690	—	II	D	β-Haem.	−	−	−	−	−	+	−	−	−
C 628	—	II	L	β-Haem.	−	−	−	−	−	+	−	−	−
I 265	—	IV	RC	Indiff.	−	−	−	−	−	−	−	+	−

+ = positive precipitation; − = no precipitation; Origin RC = strains isolated from root-canals; D = strains isolated from clinical material; L = strain from Dr Lancefield.

Streptococci with type antigens without group antigens

Strain C 628 poses the problem whether this strain, which does not contain the group F antigen, should be considered as a group F streptococcus. Furthermore, it might be asked whether other strains occur which contain one of our type-antigens without the group-antigen. An analysis of twenty haemolytic strains which reacted with a reference group F serum produced three strains which contained only the type II antigen. Another of these haemolytic (O, II) strains was present in our own material. Several indifferent strains were found which contained type I antigen but no group-antigen and one which contained the type IV antigen only. Table 11

gives instances of the various combinations of group- and type-antigen that were observed. The strains without group-antigen are shown in the lower half of the table.

DISCUSSION

The properties of the streptococci studied in this paper illustrate the danger—so ably discussed by Sneath (1957)—of giving undue weight to any one character in the classification of bacteria. Since Schottmüller (1903) great importance has been attached to the character of β-haemolysis because so many pathogenic streptococci show haemolytic properties. The occurrence of α-haemolytic, β-haemolytic and indifferent streptococci within the same serological group, seems to decrease the weight to be given to the character of haemolysis. The value of the work of Lancefield was proved by its practical results with many groups of streptococci. It induced however, a tendency to rely overmuch on serological characters even outside these groups. The occurrence of the type-antigens of group F in strains with other group-antigens and the occurrence of strains with type-antigens but without group-antigens, illustrates the relativity of the notion of group-antigen, at least within the groups F, G and C. Omitting a fruitless discussion about the concepts of group-antigen and type-antigen and their possible equivalence for some groups, we propose that, for the streptococci of groups F, G and C the described antigens be included in the description of the strain, in our cases e.g. (F, O), (F, I), (G, I), (C, III), (O, II), etc. The realization that many formamide extracts contain other carbohydrate antigens than the group-antigen might help to prevent the occurrence of unexpected cross-reactions from confusing the issue. The advantage of using well-known strains, with as few antigens as possible, for the preparation of sera, is obvious. The use of an (F, O) strain (e.g. strain I 57) for the preparation of group F sera is advocated. The fact that an additional test with a type II serum is then necessary to find (O, II) strains is a complication which, however, in the end will decrease confusion. The distribution of biochemical and serological properties over the various indifferent streptococci studied in this paper obviously makes it senseless to give them species rank or to invent a name for this group. It is realized that, for example, the indifferent streptococci of group F are different from the haemolytic strains of group F (in haemolysis, lactose fermentation, colony-size). On the other hand the habitat and the serological data illustrate the close relationship between the two groups.

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