for replication of the agent but not crucial in the function of the cell.

Summary. A factor (BIF) has been demonstrated in a corynebacterium of unidentified species which interferes with the multiplication of 2 Group A Arbor viruses, Sindbis and Chikungunya, in cultures of human amnion and chick embryo cells. Cytopathogenicity of seven unrelated viruses was not inhibited by the factor. Evidence is presented that interference of the Arbor viruses depends upon inhibition of replication after virus has entered the cell. Evidence suggesting that the factor is a protein is presented.

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Effect of Periodate on Cell Wall Antigens of Streptococci.* (27792)

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The streptococci are divided into 17 serological groups on the basis of specific carbohydrate antigens present, with the exception of group D, in the cell wall(1). In the case of group A, the antigen is known to be composed of rhamnose and N-acetyl glucosamine (2), and in group C, rhamnose and N-acetyl galactosamine(3). Among the other groups of streptococci, however, the composition of the antigen appears to be more complex(1). In group K for example, glucose, galactose, glucosamine and galactosamine are present; rhamnose may or may not be present depending upon the strain(4). The same compounds have been found in the group antigen of group O and in the cell wall carbohydrate of a serologically unclassified streptococcus(4).

The sugars and amino sugars given above plus, in a few cases, arabinose and/or mannose, are most likely components of the streptococcal cell wall grouping antigens of all the serological groups(1). In addition, a number of type antigens of the streptococci are located in the cell wall and are carbohydrate in nature. As a consequence, many of the known grouping and typing antigens of the streptococci, and many others yet to be detected, depend for their specificity upon chemical structures composed of these substances.

To obtain further information on the properties of these cell wall antigens, a study has been made of the effect of periodate oxidation on the antigens as determined by ability of the antigen to combine with antibody. The results indicate that resistance or sensitivity to oxidation by periodate are properties which might aid in identification of antigenic determinants, as well as providing information on

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the chemical linkages involved.

Culture of streptococci. Streptococci were grown in Todd-Hewitt broth (Difco) supplemented with a glucose-salts mixture(5). After 18-24 hours growth at 37°, formalin was added to a final concentration of 2%, the cultures left overnight at room temperature, centrifuged, washed 3 times in distilled water and lyophilized.

Preparation of cell wall fractions. Streptococci were resuspended in distilled water to a concentration of 10 mg/ml. Five ml of this suspension and 4 g ballotini beads/cup were shaken in a Mickle disintegrator for 20 minutes at room temperature. The cell wall suspensions were treated with trypsin, ribonuclease and pepsin(6), washed and lyophilized. The average yield of cell walls was about 15% of original dry weight of the cells.

Cell wall agglutination tests. A series of doubling dilutions of serum in 0.9% saline was made, starting at 1/10, and to 0.5 ml of each serum dilution was added 0.5 ml of antigen suspension, giving a final dilution series 1/20, 1/40, 1/80, 1/160, etc. Antigens consisted of suspensions of purified cell walls at a concentration of 0.1 mg/ml, so that the final antigen concentration in the test was 0.05 mg/ml. This was a rather faintly turbid suspension. Antigens were usually kept as concentrated suspensions containing 1 mg/ml in distilled water with 0.3% sodium azide as preservative, and were diluted 1/10 in 0.9% saline for use. When making concentrated antigens from lyophilized cell wall preparations it was necessary to redisperse the cell wall fragments by a brief shaking in the Mickle disintegrator (5 minutes using 0.5 g beads/5 ml antigen).

Periodate treatment. Cell wall suspensions at a concentration of 1 mg/ml in M/10 phosphate buffer pH 7.0 were added to an equal volume of M/50 periodic acid at room temperature. After mixing, a sample (usually 2 ml) was removed at once and pipetted into a tube containing 0.5 ml 10% glucose: this was the '0-time' sample. Other samples were removed at intervals and pipetted into similar tubes containing glucose to destroy periodate. The samples were centrifuged and washed twice in distilled water to remove glucose and reaction products, then resuspended in saline, shaken in the Mickle disintegrator for 5 minutes and diluted to 0.1 mg/ml for use as antigens.

Antisera. The following sera were obtained from the USPHS: groups A, B, C, F, L, M and O; group C serum was also obtained from Lederle Laboratories. The following sera were prepared by the authors(1) against the following strains: group A variant–strain K43; group D–strains 8175, 8176, 8213, 8177, 8307; group E–strain K129; group G–strain D166B; group H–strain Hockley; group K–strain D34E; group N–strain C559; group Q–strain E6844.

Results. In Table I the reactions of treated and untreated cell walls from strains of 14 serological groups of streptococci are compared. From these results it appears that the antigenic determinants responsible for the agglutination of cell wall suspensions are usually either resistant or sensitive to periodate. Thus, the determinants in cell walls from all 4 strains of group A, 8175 and 8176 of group D, group L and group C appear to be resistant, while those from group A variant, group B, 8213, 8177 and 8307 of group D, and groups F,K,M,O and Q appear to be very sensitive, since their cell walls react scarcely at all with the homologous serum after periodate treatment.

Three strains out of the 22 tested gave rather equivocal results. In the case of the group E strain K131 (which was tested against serum to group E strain K129), agglutination is still strong, but the titer is reduced from 1/640 to 1/80. Strains Hockley (group H) and C559 (group N) show an over-all reduction in the strength of the reaction, although weak agglutination is detected to almost as high a titer as with untreated cell walls. These two strains were tested against homologous antiserum.

In cases where partial destruction of serological activity occurs in 2 hours it seems likely either that 2 separate specificities, one resistant and one sensitive to periodate, are contributing to the final result, or that it is due to a slower over-all rate of reaction.

It is of interest that the group A variant strain (K43) is sensitive to periodate while

the normal group A strains are resistant. The variant streptococcus during mouse passage loses its ability to synthesize the group A antigen, whereas the type antigens remain unchanged(7). The variant strain is found, however, to synthesize a carbohydrate antigen composed qualitatively but not quantitatively of the same chemical components as

TABLE I. Effect of Periodate on Cell Wall Antigens of Streptococci.

Strain	Group	Agglutination test dilutions 20	40	80	160	320	640	1280	2560	C
TIM	A	++	++	++	++	+	±	—		—
		++	++	++	++	+	±	—		—
Blackmore	A	++	++	++	++	++	tr	—	—	—
		++	++	++	++	++	tr	—	—	—
12RN	A	++	++	++	++	++	+	—	—	—
		++	++	++	++	++	+	—	—	—
Kilborne	A	++	++	++	++	++	++	±	—	—
		++	++	++	++	++	++	±	—	—
K43	A	++	++	++	++	++	++	±	—	—
	variant	tr	tr	—	—	—	—	—	—	—
Brownette	B	++	++	++	++	++	++	±	—	—
		±	tr	—	—	—	—	—	—	—
26RP66	C	++	++	++	++	+				—
		++	++	++	+					—
liquefaciens (8175)	D	++	++	++	++	++	++	—		—
		++	++	++	++	++	—	—		—
zymogenes (8176)	D	++	++	++	++	++	++	++	+	—
		++	++	++	++	++	++	+	±	—
faecalis (8213)	D	++	++	++	++	++	+			—
		tr	tr	—	—	—	—			—
bovis (8177)	D	++	++	++	++	+	—			—
		—	—	—	—	—	—			—
durans (8307)	D	++	++	++	++	±	±	—		—
		—	—	—	—					—
K131	E	++	++	++	++	++	+	—		—
		++	++	+	—	—	—	—		—
C628	F		++	++	+	tr	—			—
			tr	—	—	—	—			—
Valente	G	++	++	+	+	±				—
		tr	tr	tr	—	—				—
Hockley	H	++	++	++	++	+				—
		±	±	+	±	—				—
D34E	K	++	++	++	++	++	++	+		—
		tr	tr	tr	—	—	—	—		—
D167A	L	++	++	++	++	++	tr	—		—
		++	++	++	++	+	—	—		—
54/136	M	++	+	tr	—	—	—	—		—
		—	—	—	—	—	—	—		—
C559	N	++	++	++	++	+				—
		+	+	+	+	tr				—
B361	O	++	++	±	—	—				—
		—	—	—	—	—				—
E6844	Q	++	++	++	++	+				—
		—	—	—	—	—				—

2+ = complete agglutination; + = partial agglutination; ± = slight; tr = trace.
C = control.

First line of results in each case is zero time sample, second line is after 2 hr treatment with periodate (strain K131 was treated for 140 min).

the group specific antigen(8). The difference in reaction of these antigens to periodate oxidation is an indication of the importance of chemical linkage for determination of antigenic specificity.

Discussion. Treatment of carbohydrate antigens with periodic acid is a very useful procedure for determining the effect of oxidation on ability of antigen to react with antibody. The action of periodate is to destroy the C-C bond in α-glycols, α-hydroxyaldehydes, α-ketols, α-diketones and α-ketoaldehydes. The cell wall carbohydrate antigens of the streptococci which resist periodate must thereby possess linkages which are not attacked in the oxidative process. The antigens, other than D, responsible for agglutination are probably the grouping antigens of the streptococci. The effect of periodate, when considered in conjunction with the chemical composition of the antigen(4), provides additional evidence of the finite relationship between antigenic specificity and chemical structure.

Group D is known to contain 24 type antigens which can be detected by agglutination(9). The serological classification into types is not closely correlated with physiological characteristics. For example, strains of *S. faecalis, S. liquefaciens* and *S. zymogenes* are found in types 1, 3, 5 and 9. Similarly, *S. bovis* and *S. durans* strains each belong to more than one serological type(9). The type possessed by the D strains of Table I has not been determined due to lack of antisera, so that it is not certain how many different type antigens are present. The reaction to periodate, however, indicates that at least 4 different antigens are present. Strains 8175 and 8176 could possess a common antigen. The antigen of 8213 must be distinct from the former because of its sensitivity to periodate. Strains of *S. bovis* and *S. durans* do not possess common type antigens(9), thus the periodate sensitive antigens of 8177 and 8307 should be distinct also. The periodate reaction may be helpful in further studies on the classification of this group of streptococci.

It is clear from Table I that various cell wall grouping antigens of the streptococci exhibit marked differences in their behavior to periodate. Although strains D34E (K) and B361 (O) are both sensitive to periodate, the group antigen of the former contains rhamnose whereas the antigen of the latter does not contain rhamnose(4). It is thus likely that the periodate sensitive bond(s) in each antigen does not involve rhamnose. This agrees with the finding that the group antigen of K strains may or may not possess rhamnose(1,4).

Summary. The effects of periodate oxidation on cell wall polysaccharide antigens in strains of streptococci from 14 serological groups have been determined. In 11 of the 23 strains tested the serological reactivity was destroyed by 0.01 M periodate in 2 hours, while in 5 strains the serological reactions showed little or no change after periodate treatment. Three strains showed partial destruction under the conditions employed. In the cell walls of group D cells both resistant and sensitive antigens were found, whereas in group A the group antigen of all strains was resistant to periodate. The antigen of a group A strain, which no longer can synthesize specific group A carbohydrate antigen, was destroyed by the oxidation. The cell wall carbohydrate antigens of single strains examined in the remaining 9 serological groups were made up of both resistant and sensitive types.

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