

# Somatic Antigens of *Streptococcus* Group E

## II. Separation and a Partial Physicochemical Characterization<sup>1</sup>

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Antigenic extracts of *Streptococcus* group E (SGE) were subjected to fractional ethanol precipitation, block (preparative) electrophoresis, and gel filtration for the purpose of separating the type antigen from the group antigen. Ethanol precipitation was ineffective in separating the substances. Block electrophoresis yielded serologically pure group antigen and a mixture of type and group antigen. Serologically pure type antigen was obtained by gel filtration. In some cases, pure group antigen was also recovered; in others, it was contaminated with type antigen. Gel filtration column effluents of antigenic extracts of SGE serotypes, I, II, III, IV, V and "untypable" isolates, collected from the region in which type antigen was eluted, were studied by paper chromatography and infrared spectrophotometry in an effort to develop a nonserological means of detecting type antigen. Hydrolysates of type antigens or suspect type antigens of serotypes I through V contained L-rhamnose, D-glucose, and several unidentified substances. D-Galactose also was present in hydrolysates of serotypes III and V. Untypable isolates and negative controls contained traces of D-glucose only. The data suggested that serotypes I through V contained a type antigen and that the untypable isolates were devoid of type antigen. Infrared absorbance spectra seemed to support the paper chromatography data.

The type-specific antigens of *Streptococcus* group E (SGE) are of interest because of their potential involvement in the immunogenesis and pathogenesis (1) of streptococcal lymphadenitis of swine (SLS) (3, 4, 15). Reports regarding the type-specific antigens of SGE are conflicting. It was reported initially (11) that SGE consisted of four serotypes (serotypes I, II, III, and IV). Results of a subsequent study substantiated the validity of types II and IV, but the existence of types I and III could not be confirmed (18).

SGE serotype IV was recovered from the majority of affected swine (1, 5, 18). Serotypes I, II, and a recently discovered serotype provisionally designated type V have been recovered from a few cases of SLS (1, 11). A substantial number of SGE isolates cannot be serotyped. Whether such untypable isolates represent serotypes for which typing antisera are unavailable or whether they are deficient in or devoid of type antigen is unknown.

The work reported here represents an effort to develop a nonserological means of detecting the presence of SGE type antigens. Methods of sep-

arating the type antigen from the group antigen were studied. The results provided a basis for producing serologically pure type antigens of reference strains of SGE serotypes II, IV, and V, of suspect type antigens of SGE serotypes I and III, and of untypable isolates. The products so obtained were analyzed by paper chromatography and infrared spectrophotometry.

### MATERIALS AND METHODS

**Bacteria.** SGE strains studied and their serotypes and origins were the same as previously reported (13) except that strain CA-10 was replaced with strain EM-5 (both serotype IV).

**Antigens.** Type and group antigens were extracted from SGE by HCl and formamide hydrolysis (13). Dried bacteria (1 g) were hydrolyzed by the HCl method at pH 1.8 for 10 min at 100 C, and the resulting antigen solution was diluted to 50 ml. Formamide hydrolysis consisted of heating 250 mg of bacteria in 15 ml of formamide for 30 min at 180 C and diluting the antigen to 5 ml.

**Antisera.** *Streptococcus* group C, and SGE group-specific antiserum, and *Streptococcus* group E type-specific antiserum against serotypes II, IV, and V were prepared as described previously (1, 13).

**Precipitin test.** The presence of group and type antigens was assayed by the ring precipitin test (1, 8).

**Fractional ethanol precipitation.** Sufficient cold

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absolute ethanol (4 C) was added fractionally to 50 ml of HCl-prepared antigen solution of SGE serotype IV to give, respectively, 0.25, 0.5, 1, 2, and 4 volumes of ethanol per volume of antigen solution. Each precipitate was collected by centrifugation, dissolved in 5 ml of physiologic saline solution, and tested serologically with homologous grouping and typing antiserum.

**Block (preparative) electrophoresis.** Antigens from SGE reference strains of serotypes II, IV, and V were extracted by formamide hydrolysis and were deproteinized according to the method of Sevag (14). A supporting medium (Pevikon C-870, Mercer Chemical Corp., New York, N.Y.), previously washed in 0.1 N NaOH, rinsed in deionized water, and equilibrated with barbital-HCl buffer (pH 8.2 and ionic strength 0.1), was used to pack a 21- by 33-cm cell (cell model E-8002B, Warner Chilcott Laboratories, Morris Plains, N.J.). The packed cell was divided into four longitudinal sections with Plexiglas strips, and a core 1 by 1 by 5 cm was removed (10 cm from the cathode) from each section. Each core was mixed with 2 ml of crude antigen extract and replaced in the cell. A 200-d-c volt potential was applied for 40 hr and the cell was cooled with running tap water. At the completion of electrophoresis, each section was cut transversely into blocks 1 by 2 by 5 cm. Antigen was eluted from each block by deionized water and collected by filtration, and the filtrates were freeze dried. The dried samples were dissolved in 5 ml of deionized water and analyzed serologically (with

homologous grouping and typing antisera) and chemically [for total carbohydrates by using the anthrone test (2)].

**Gel filtration chromatography.** Crude formamide-extracted antigens of SGE serotypes II, IV, and V were prepared and dextrose was added to each antigen solution to give a final concentration of 8%. The antigen-dextrose mixture was applied to gel columns previously equilibrated with 0.015 M phosphate-buffered saline (PBS) at pH 7.0. Gels used were cross-linked dextrans (Sephadex, Pharmacia Fine Chemicals, Piscataway, N.J.) with molecular-weight exclusion limits (for dextran) of 700, 1,500, 5,000, 10,000, 50,000, 100,000, and 200,000 (G-10, G-15, G-25, G-50, G-75, G-100, and G-200, respectively) and a polyacrylamide gel with an exclusion limit of 200,000 (Bio Gel P-300, Bio Rad Laboratory, New York, N.Y.). Flow rates of 8 to 14 ml per hr of PBS were established, and 5-ml fractions were collected. Each of the fractions was tested serologically with SGE group-specific antisera and selected fractions were tested with SGE type specific antisera. Serologically pure components were pooled, concentrated under vacuum, and dialyzed against glass-distilled water.

Type-specific antisera were not available for serotypes I and III and for untypable isolates. In those circumstances, the effluents from 20 tubes preceding the appearance of group antigen were collected and processed in the same way as serologically active fractions. Dialyzed fractions of type antigens and

TABLE 1. Results of electrophoresis of *Streptococcus* group E somatic antigens

Migration (cm) from origin <sup>b</sup>	Antigenic extract <sup>a</sup> of <i>Streptococcus</i> group E strains								
	Serotype II (strain K-131)			Serotype IV (strain EM-1)			Serotype V (strain TG-9)		
	Precipitin reaction <sup>c</sup>		(CH <sub>2</sub> O) <sub>n</sub> (μg/ml) <sup>d</sup>	Precipitin reaction <sup>c</sup>		(CH <sub>2</sub> O) <sub>n</sub> (μg/ml) <sup>d</sup>	Precipitin reaction <sup>c</sup>		(CH <sub>2</sub> O) <sub>n</sub> (μg/ml) <sup>d</sup>
	Group	Type		Group	Type		Group	Type	
-10	-	-	55	-	-	50	-	-	50
-8	-	-	60	-	-	81	-	-	62
-6	-	-	78	-	-	96	-	-	75
-4	-	-	100	+	-	96	+	-	68
-2	+	-	125	+	-	156	+	-	70
+2	+	-	148	+	-	250	+	-	75
+4	+	-	164	+	-	250	+	-	81
+6	+	-	200	+	-	250	+	-	96
+8	+	-	190	+	-	190	+	+	87
+10	+	-	102	+	-	74	+	+	75
+12	+	+	87	-	+	62	+	+	81
+14	+	+	62	-	+	62	+	+	68
+16	-	+	62	-	+	50	+	+	50
+18	-	+	200	-	-	62	+	+	50
+20	-	+	125	-	-	50	+	-	62
+22	-	-	74	-	-	75	-	-	50
+24	-	-	50	-	-	70	-	-	62

<sup>a</sup> Formamide hydrolysate prepared from whole bacteria cells.

<sup>b</sup> Negative sign denotes cathodal movement; positive sign denotes anodal movement.

<sup>c</sup> Determined by the tube-precipitin test, incubated for 15 min at room temperature.

<sup>d</sup> Total carbohydrate determined by the anthrone method, using dextrose as a standard.

suspect type antigens were freeze dried and stored in desiccator jars over phosphorous pentoxide at room temperature until used for paper chromatography and infrared spectrophotometry studies.

**Characterization of somatic antigens: paper chromatography.** A 2-mg amount of type antigen and suspect type antigen, prepared by gel filtration as described above, was hydrolyzed with 1 ml of 2 N H<sub>2</sub>SO<sub>4</sub> for 2 hr at 105 C. The acid was neutralized and the hydrolysate was filtered and adjusted to pH 4.0. The filtrates were dried under reduced pressure, dissolved in 0.2 ml of deionized water, and applied to Whatman no. 3 paper. D-Glucose, L-rhamnose, D-mannose, D-galactose, D-glucosamine, D-galactosamine, N-acetyl-D-glucosamine, and N-acetyl-D-galactosamine were used as standards. Papers were developed in a descending system using *n*-butanol, glacial acetic acid, and water [6:1:2 (6)] for 12 hr. Reducing sugars were detected with alkaline silver nitrate reagent (16), and amino sugars were detected with acetylacetone and *p*-dimethylamino-benzaldehyde (12).

**Infrared analysis.** A 1.5-mg amount of antigen, prepared as described above, was added to 200 mg of oven-dried (105 C) KBr, placed in a die previously heated to 105 C, and subjected to high pressure under partial vacuum. The KBr-antigen pellet was analyzed with an infrared spectrophotometer (Grating infrared spectrophotometer, model 521, Perkin, Elmer & Co., Norwalk, Conn.).

## RESULTS

**Antigen separation: fractional ethanol precipitation.** The precipitates recovered from treatment of antigens with the different volumes of ethanol all contained a mixture of group and type antigens.

**Block electrophoresis (Table 1).** Group antigen migrated toward both the anode and the cathode in a diffuse band, occupying an area from as much as -4 to +20 cm from the point of sample application. The type antigen moved toward the anode only and was more compact. In most cases, it was contaminated by the leading edge of the group antigen. The anthrone test for carbohydrates did not seem to be of value for detection of antigen; i.e., values were too equivocal to be of use.

**Gel filtration chromatography.** Cross-linked dextran gels with exclusion limits of 100,000 and 200,000 and polyacrylamide gel with an exclusion limit of 200,000 were satisfactory for separation of the type antigen from the group antigen (Table 2). In some cases, pure group antigen was also recovered; in others, the trailing edge of the type antigen overlapped the group antigen. Incomplete separation occurred with gels with exclusion limits of 10,000 and 50,000, and no separation occurred with gels with exclusion limits less than 10,000.

**Physicochemical characterization of antigens: paper chromatography (Table 3).** Chromatograms

TABLE 2. Results of gel filtration of *Streptococcus* group E somatic antigens

Tube no. <sup>b</sup>	Antigenic extract <sup>a</sup> of <i>Streptococcus</i> group E					
	Serotype II (strain OSC 1) precipitin reaction <sup>c</sup>		Serotype IV (strain EM-1) precipitin reaction <sup>c</sup>		Serotype V (strain TG-9) precipitin reaction <sup>c</sup>	
	Group	Type	Group	Type	Group	Type
1-3	-	+	-	+	-	+
4-6	-	+	-	+	-	+
7-9	-	+	-	+	-	+
10	-	+	-	+	-	+
11	-	+	-	+	+	+
12	-	+	-	+	+	+
13	-	+	-	+	+	+
14	-	+	+	+	+	+
15	-	+	+	+	+	+
16	-	+	+	+	+	+
17	-	+	+	+	+	+
18	-	+	+	+	+	+
19	+	+	+	+	+	+
20-25	+	+	+	+	+	+
26	+	+	+	+	+	+
27	+	+	+	+	+	+
28	+	+	+	-	+	+
29	+	+	+	-	+	+
30	+	+	+	-	+	-
31	+	+	+	-	-	-
32	+	+	+	-	-	-
33	+	+	+	-	-	-
34	+	+	+	-	-	-
35	+	+	+	-	-	-
36	+	+	+	-	-	-
37	-	-	+	-	-	-
38	-	-	+	-	-	-
39	-	-	+	-	-	-
40	-	-	+	-	-	-
41	-	-	+	-	-	-
42	-	-	+	-	-	-
43	-	-	-	-	-	-
44	-	-	-	-	-	-
45	-	-	-	-	-	-
46-50	-	-	-	-	-	-

<sup>a</sup> Antigenic extract was prepared by formamide hydrolysis.

<sup>b</sup> Tube number of 5-ml sample of effluents obtained from a chromatographic column of Bio-Gel P-300 (100 to 200 mesh).

<sup>c</sup> Tube-precipitin test, incubated for 15 min at room temperature.

of hydrolysates of type antigens and suspect type antigens of SGE serotypes I, II, III, IV, and V revealed the presence of D-glucose, L-rhamnose, and several unidentified components. Serotypes III and V also contained D-galactose. The untypable isolates and the negative control contained traces of D-glucose only. Some of the

TABLE 3. Results of paper chromatographic analysis of *Streptococcus* group E type antigens and suspect type antigens

Antigens						
Hydrolysate of strain <sup>a</sup>	Serotype	Identified components			Unidentified components	
		D-Glucose	L-Rhamnose	D-Galactose	No. present	<i>R<sub>F</sub></i> values <sup>b</sup>
K-129	I	Yes	Yes	No	0	
C24A	I	Yes	Yes	No	1	0.32
K-131	II	Yes	Yes	No	1	0.31
OSC-1	II	Yes	Yes	No	2	0.16, 0.29
4678	III	Yes	Yes	Yes	0	
EM-1	IV	Yes	Yes	No	2	0.13, 0.26
EM-5	IV	Yes	Yes	No	1	0.26
TG-9	V	Yes	Yes	Yes	2	0.14, 0.26
TG-19	V	Yes	Yes	Yes	1	0.26
NNJ-10	Unknown	Yes	No	No	0	
NNJ-21	Unknown	Yes	No	No	0	
Control <sup>c</sup>		Yes	No	No	0	

<sup>a</sup> Prepared by hydrolysis with 2 N H<sub>2</sub>SO<sub>4</sub> for 2 hr at 105 C.

<sup>b</sup> Obtained from chromatograms developed at room temperature with *n*-butanol, glacial acetic acid, and water (6:1:2). Standards (*R<sub>F</sub>* values in parentheses): D-glucose (0.19), L-rhamnose (0.40), D-galactose (0.18), D-mannose (0.24), D-glucosamine (0.14), D-galactosamine (0.13), *n*-acetyl-D-glucosamine (0.29), and *n*-acetyl-D-galactosamine (0.29).

<sup>c</sup> Buffer used in gel filtration.

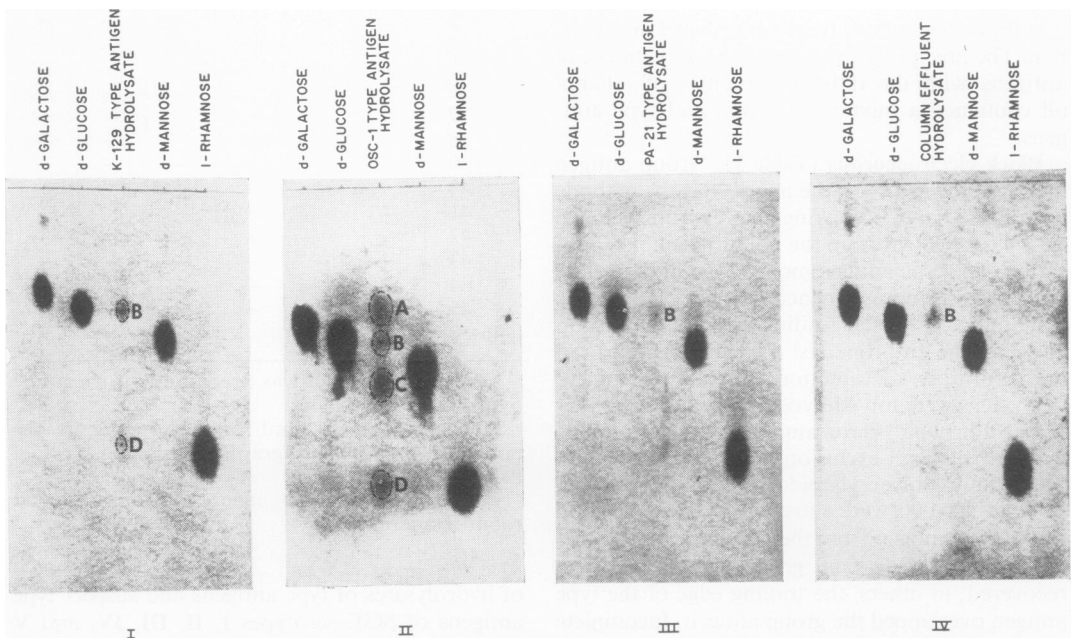


FIG. 1. Paper chromatographs of *Streptococcus* group E type antigens and suspect type antigens. (I) Chromatographic evaluation of suspect type antigen of serotype I strain K-129; (II) an evaluation of serologically identified type antigen of serotype II strain OSC-1; (III) an evaluation of suspect type antigen of untypable strain PA-21; (IV) an evaluation of column effluent collected after antigens were eluted (negative control); A and C, unidentified components; B, D-glucose; D, L-rhamnose.

chromatograms used to formulate Table 3 are illustrated in Fig. 1.

**Infrared spectrophotometry.** Absorbance spectra of serotypes I, II, an untypable isolate, and a negative control are illustrated in Fig. 2. Absorbance at 3.0, 3.5, 6.0, and 7.5  $\mu\text{m}$  were interpreted to indicate, respectively, OH stretching, CH stretch-

ing, OH overtone, and CH overtone. Absorbance in the "fingerprint" region (7 to 30  $\mu\text{m}$ ) of type antigens from serotypes I, II, III, IV, and V were very similar (resembling those illustrated for serotypes I and II), suggesting the presence of similar substances. The spectra of the untypable isolates resembled the pattern of the neg-

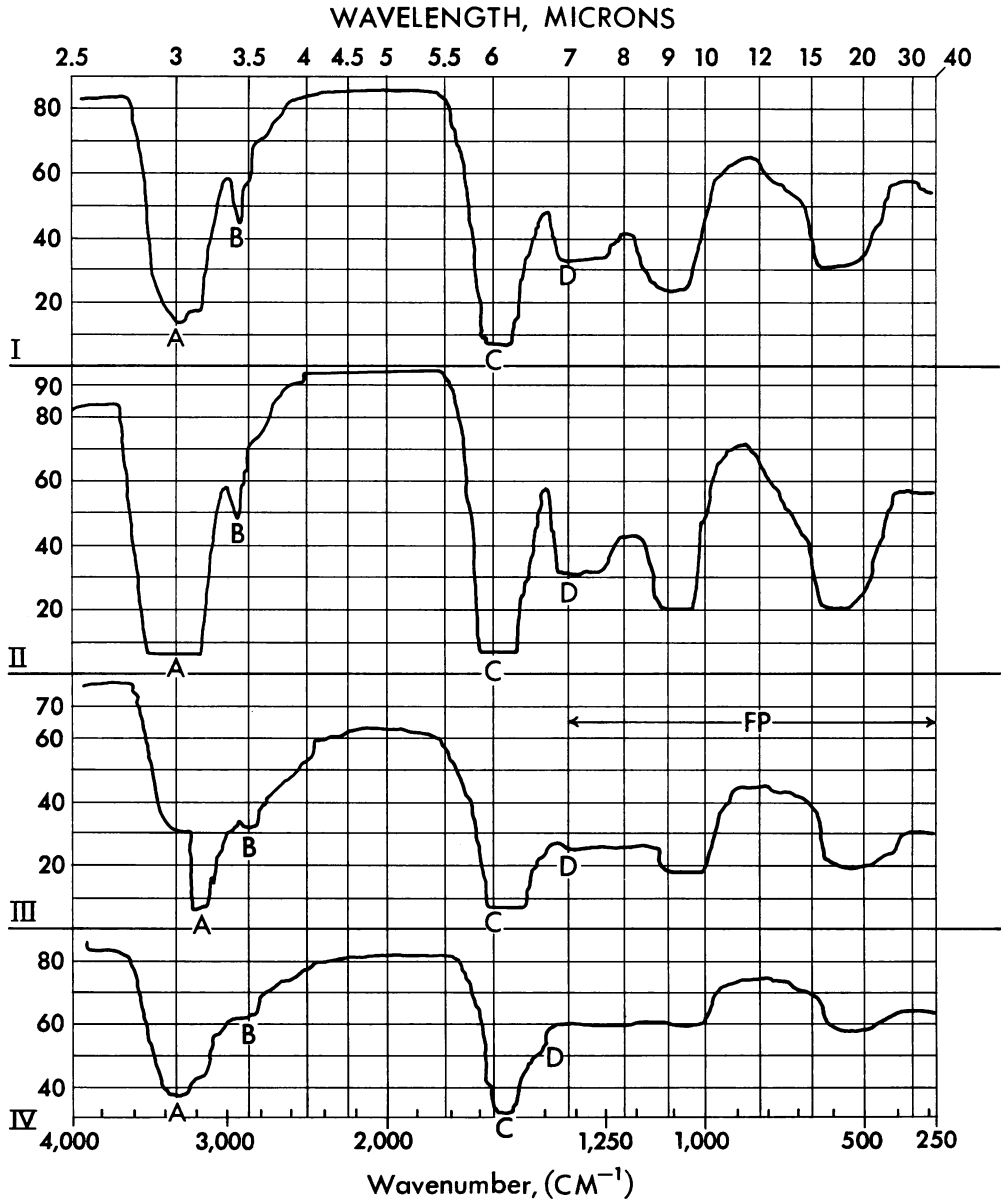


FIG. 2. Infrared absorbance spectra of *Streptococcus* group E type antigens and suspect type antigens. (I) Spectra of suspect type antigen of serotype I strain K-129; (II) spectra of serologically identified type antigen of serotype II strain OSC-1; (III) spectra of suspect type antigen of untypable strain PA-21; (IV) spectra of column effluent collected after antigens were eluted (negative control); A and C, OH stretching and OH overtone; B and D, CH stretching and CH overtone; FP, fingerprint region.

ative control; i.e., there was relatively little absorbance in the fingerprint region, suggesting that the untypable isolates were devoid of type antigen.

### DISCUSSION

Fractional ethanol precipitation was used successfully in separating the group and type antigens of *Streptococcus* group B (7, 17). A similar procedure, used in this work with SGE, was unsuccessful; i.e., each of the precipitates (from different ethanol concentrations) contained both type and group antigen. Results suggested that the type and group antigens of SGE were isoelectrically similar.

Block electrophoresis was satisfactory for recovering serologically pure group antigen. The relatively slow and diffuse migration of the group antigen suggested that it had a lower and more variable net charge than the type antigen. Type antigen migrated relatively rapidly and formed a fairly compact band; however, it was contaminated by the leading edge of the group antigen. The anthrone test was used to detect carbohydrates. Peak amounts of carbohydrates correlated roughly with peak serological activity, but fairly substantial amounts of carbohydrates were present in fractions devoid of serological activity.

Gel filtration, employing cross-linked dextran and polyacrylamide gels with exclusion values of 100,000 and 200,000, were satisfactory for recovering serologically pure type antigen. In some cases, pure group antigen was also recovered; in others, it was contaminated by type substance. Type antigen appeared in the effluent shortly after the void volume, indicating that it was of high molecular weight, whereas the group antigen appeared later. Furthermore, the antigens were collected over a 20-tube area, suggesting heterogeneity of molecular weight (polydisperse). Gels with exclusion limits of <100,000 were unsatisfactory for separating the type from the group antigen. This observation is in conflict with published data, suggesting that the SGE group antigen had a molecular weight of 8,000 to 10,000 (H. D. Slade et al., *Bacteriol. Proc.*, p. 46, 1965). The difference might be due to the degree of hydrolysis employed in extracting the antigens. It is likely that prolonged hydrolysis would result in increased depolymerization.

Serologically pure SGE type antigens (serotypes II, IV, and V) and suspect type antigens (serotypes I and III and untypable isolates) were subjected to paper chromatography and infrared spectrophotometry in an effort to develop a non-serological means of detecting type antigen. If such a method could be developed, it would be useful in determining the existence of type antigens in SGE isolates for which typing antisera

were unavailable. The presence of L-rhamnose and D-glucose, as well as several unidentified components, in hydrolysates of SGE serotypes I and III suggested that a type antigen existed in those isolates. Detection of D-galactose in the hydrolysate of serotype III seemed to offer additional evidence of type antigen. The two untypable isolates resembled the negative control in that traces of D-glucose constituted the only substance observed, and it is likely that the two isolates were devoid of type antigen.

H. D. Slade et al. (*Bacteriol. Proc.*, p. 46, 1965) associated rhamnose and dextrose with the group-specific antigen. Our results indicated that these sugars are also components of the type-specific antigen. These observations suggest that there may be relatively minor chemical differences between the type- and the group-specific antigens of SGE.

Infrared spectrophotometry tended to substantiate results of paper chromatography. Although distinctive spectra occurred in the fingerprint region (7 to 30  $\mu$ m), absorption of the SGE antigens in that region was disappointing. Specific functional groups could not be identified as in studies with pneumococci (10) and klebsiella (9). However, absorption patterns of SGE serotypes I and III were similar to those of serologically identified type antigens of types II, IV, and V. Absorption of suspect antigens of the untypable isolates resembled the spectrum of the negative control; i.e., they seemed devoid of type antigen.

It was concluded that paper chromatography was a potentially useful method for detecting components of SGE type antigen. Results of this study suggested that SGE serotypes I and III contained type antigen. Whether the type antigen was distinct from those of other SGE serotypes was not determined.

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