GROUP A STREPTOCOCCUS POLYSACCHARIDE: STUDIES ON ITS PREPARATION, CHEMICAL COMPOSITION, AND CELLULAR LOCALIZATION AFTER INTRAVENOUS INJECTION INTO MICE*

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PLATE 1

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Previous studies of the group A streptococcus polysaccharide have been principally concerned with the use of this hapten in diagnostic bacteriology as a means of identifying streptococci pathogenic for man (1–4). However, several biological characteristics of the polysaccharide have been described. The group A polysaccharide is non-toxic for animals (3) and is capable of stimulating antibody formation only when combined with streptococcal cellular protein (5). It is one of the more stable components of the streptococcal cell (6) although variants have been produced with little or no group A polysaccharide after growth in the presence of a high oxygen tension (7) and following serial mouse passage (6). Loss of polysaccharide following mouse passage did not appear to alter the virulence of the variant strain (6). Antibodies to the group polysaccharide have been found to occur with antibodies to other cell components in the blood of patients following group A streptococcal infections and in the blood of patients with rheumatic fever (8–11). A study of the fate of the immunologically active components of the group A streptococcus in the host—in this instance, the group A polysaccharide—might provide information of use in elucidating the relationship of streptococcal infection to the rheumatic process.

The study of the tissue localization of the isolated polysaccharide following intravenous injection in mice was the primary object of the present investigation. The second object was to devise a mild method for the isolation of the group A polysaccharide. Previous preparations of this polysaccharide have been extracted by vigorous chemical procedures, usually with dilute hydrochloric acid at 100°C. (12) or formamide at 150°C. (13). The possibility existed that such treatment may have resulted in depolymerization of the polysaccharide and that a mild method of extraction would result in the isolation of a more native preparation of the polysaccharide for use in the localization studies.

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The method developed for the isolation of the group A polysaccharide employed pepsin digestion of ground streptococcal cells. The polysaccharide produced by this method was compared with polysaccharide prepared by the formamide extraction method; no significant difference could be found between the two preparations. The carbohydrate composition of the polysaccharide was determined, using paper chromatography and quantitative carbohydrate methods. The method of isolation, the comparison of the two preparations, and the analysis of the composition of the polysaccharide are described in the first part of this paper. Then, the results of the study of the cellular localization of the polysaccharide following intravenous injection into mice are described. The presence of the polysaccharide hapten in tissues was detected by a histochemical method using fluorescein-labeled antibody as a specific stain (14).

Preparation of Group A Streptococcus Polysaccharide

Proteolytic enzymes have been successfully employed to release serologically active substances from bacteria in a number of instances (15–18). Preliminary experiments indicated that a small amount of group A polysaccharide could be liberated from streptococcal cells by treating suspensions of these organisms with proteolytic enzymes. Better yields were obtained when the enzymes were allowed to act on suspensions of streptococci that had been ground in a ball mill or in the Booth-Green mill (19). Pepsin and a mixture of trypsin and chymotrypsin showed similar activity in releasing polysaccharide from ground cells. However, pepsin was selected for use in the large scale digestion experiments because it has a low pH optimum that lessens the possibility of bacterial contamination during the extraction process; it can be inactivated by exposure to slightly alkaline pH; and subsequent purification of the polysaccharide can be carried out with greater facility after the action of this enzyme on streptococcal protein components.

In the large scale extraction experiments, suspensions of ground streptococci were digested for 72 to 120 hours with crystalline pepsin at pH 2; the ground cell residue was removed by centrifugation, and soluble protein and nucleoprotein material precipitated by neutralization of the digest supernatant. Further purification was carried out by two different methods. The first consisted of differential precipitation with organic solvents followed by further purification in the Tiselius electrophoresis cell (20, 21). In the second method ion exchange resins were used to remove foreign material (22). Impurities were adsorbed to cationic and anionic resins, leaving the polysaccharide in the supernatant from which it was precipitated with acetone. These methods are described in detail below.

Method 1.—300 gm. of type 17 group A streptococci that had been extracted with $NaHCO_3$ solution (containing 0.5 per cent phenol) at pH 8.0-8.5[1] were used as starting material. The streptococcal cells were washed with distilled water and then ground in the cold in a Booth-Green mill. The ground cell paste was suspended in 20 l. of HCl-KCl buffer at pH 2, γ 0.02, containing 3.4 gm. of crystalline pepsin (Armour and Co.). Digestion was allowed to proceed for 120 hours at 37°C. with constant stirring. During the period of enzyme action, the digestion mixture was surrounded by coils of dialysis tubing through which buffer continuously flowed, thereby removing the dialyzable end products of pepsin digestion.

The digestion mixture was then passed through the Sharples centrifuge, and the effluent was collected and stored overnight in the cold. A precipitate that formed during this refrigeration was removed by a second centrifugation. The clear solution was then adjusted to pH 6.5 with normal sodium hydroxide, and the precipitate that formed was removed by Sharples centrifugation. The precipitate gave positive tests for pentose and desoxypentose nucleic acids and a positive biuret test. Free group A polysaccharide was not precipitated with this nucleoprotein material. However, group A polysaccharide could be obtained from this precipitate by extraction with formamide, according to the method of Fuller (13); possibly, this represents polysaccharide bound to protein that cannot be released by the action of pepsin.

After removal of the latter precipitate, the supernatant fluid containing the free polysaccharide liberated by pepsin digestion was concentrated approximately 20 times *in vacuo* and dialyzed against acetate buffer of pH 5.5, γ 0.04. A precipitate that formed during dialysis was removed by centrifugation. Acetone was then added to the dialyzed polysaccharide solution to a concentration of 45 per cent in the cold; the precipitate that formed was removed after centrifugation in the cold. To separate the polysaccharide from the 45 per cent acetone supernatant, one volume of the latter was added to eight volumes of a mixture of equal parts of acetone and 95 per cent ethanol. The polysaccharide precipitate was collected after centrifugation and redissolved in 250 cc. of 0.15 M sodium chloride buffered at pH 7. It was then concentrated four times *in vacuo* and dialyzed against acetate–sodium chloride buffer at pH 6.4, γ 0.12. Reprecipitation with acetone removed only a trace of contaminating material. The polysaccharide was reprecipitated from the 45 per cent acetone supernatant by adding aliquots of this solution to 10 volumes of the acetone–95 per cent ethanol mixture. The precipitate was dissolved in 0.15 M sodium chloride buffered at pH 7, concentrated two times *in vacuo*, and dialyzed against acetate buffer at pH 5.6, γ 0.02.

Further purification of this preparation of group A polysaccharide was carried out in the Tiselius electrophoresis cell (20). Three aliquots of the polysaccharide solution were subjected to electrophoresis at pH 6.8, γ 0.05 for 2½ hours; at pH 6.8, γ 0.05, for 4 hours; and at pH 7.7, γ 0.05, for 8 hours, respectively. Contaminating material moved away from the immobile polysaccharide, and when observation indicated maximum—but not absolute—separation of the mobile components from the immobile polysaccharide, the cell compartments were closed. This isolated the polysaccharide in the upper compartment of the descending (negative) arm from which it was pipetted. It was then dialyzed against distilled water and lyophilized.

Method 2.—Cells of the NY 5 strain of group A streptococcus were used in this digestion experiment. These streptococci had not been previously extracted with $NaHCO_3$. The cells were ground in a ball mill and were then digested for 72 hours with crystalline pepsin under the same conditions as in the preceding experiment. Following digestion, the cell debris was removed by centrifugation and the supernatant chilled and adjusted to pH 6.4. The

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[1] These streptococci were obtained from Sharp and Dohme, Inc., through the courtesy of Dr. C. P. Hegarty. They had been extracted with $NaHCO_3$ to obtain other cell material in the Sharp and Dohme laboratories but were found to be satisfactory for use in the extraction of the group A polysaccharide.

precipitate that formed was removed by centrifugation, and the resulting supernatant fluid that contained the polysaccharide was concentrated *in vacuo* to one half of its original volume.

Further purification was performed with the ion-exchange resins. In a typical experiment, 100 cc. of the crude polysaccharide solution that contained 39 mg. of nitrogen, was added to 150 gm. of amberlite IRA-400 in the chloride form, shaken for 15 minutes, and decanted from the resin. The resin was washed three times with small amounts of distilled water and the washings were combined with the original effluent. This solution, containing 20 mg. of nitrogen, was then concentrated *in vacuo* to 77 cc. and the pH adjusted to 4.5 with dilute acetic acid. It was then shaken with 35 gm. of amberlite IRC-50, buffered at pH 4.5 with 0.2 M acetate buffer, and decanted. The resin was washed, the washings were combined with the IRC-50 effluent, and the whole was concentrated *in vacuo* again. The polysaccharide was precipitated from the concentrated, resin-purified solution by the addition of five volumes of acetone in the cold, redissolved in distilled water, dialyzed against distilled water, and lyophilized.

No differences were found between the preparations of polysaccharide purified by electrophoresis and by ion exchange. The yields of polysaccharide by either method of purification were approximately 0.1 per cent of the dry weight of the starting material. The yield of partially purified polysaccharide obtained by extraction with formamide was reported to be approximately 1.0 per cent (13). The small yield obtained by pepsin digestion is probably the result of incomplete cell disruption by grinding and incomplete digestion of streptococcal cell protein by pepsin.

Properties of Group A Streptococcus Polysaccharide

Zittle and Harris purified group A streptococcus polysaccharide, extracted by the formamide method, by fractionation at varying pH and by the use of Lloyd's reagent (21). Their preparation had a nitrogen content of 1.72 per cent, a phosphorus content of 0.7 per cent, and gave a reducing value after hydrolysis of 87.2 per cent. It was reported to give a positive orcinol reaction for pentose and to contain an amino sugar. The amino sugar component was estimated to be about 20 per cent by weight. This formamide preparation was electrophoretically immobile and its relative viscosity was reported to be very low. It was estimated to have a molecular weight of approximately 8000 by analysis in the ultracentrifuge. It reacted in high dilution with group A antiserum. The results of these investigators confirmed and extended the information on the characteristics of the group A polysaccharide previously reported by Lancefield (12), Fuller (13), Kendall, Heidelberger, and Dawson (23), and Scherp (24).

Some of the chemical, physical-chemical, and immunological characteristics of the group A polysaccharide prepared by the pepsin digestion method are listed in Table I. These data are compared in this table with data obtained by Zittle and Harris for the group A polysaccharide prepared by formamide extraction (21). The comparison reveals no significant differences in the properties of the two preparations.

Analysis of the polysaccharide, prepared by pepsin digestion, in the ultracentrifuge showed it to be essentially homogeneous. The sedimentation constants of the pepsin digestion preparation and the formamide preparation, calculated from the ultracentrifugal data, are in close agreement. The diffusion constant of the polysaccharide prepared by pepsin digestion, required for the

TABLE I

Comparison of the Properties of Group A Streptococcus Polysaccharide Prepared by the Pepsin Digestion Method and by Formamide Extraction

Property	Pepsin digestion preparation	Formamide extraction preparation*
Reaction with group A streptococcal antiserum	In a dilution greater than 1:1,000,000	In a dilution greater than 1:2,000,000
Electrophoretic mobility	Negligible‡ at γ 0.05, pH 6.8, 7.7	Negligible at γ 0.02, pH 7.0, 9.0
Sedimentation constant	s_{20} 1.17‡§	s_{20} 1.35
Optical rotation: $[\alpha]_D^{24}$	−75°	−71.5°
Reducing value after hydrolysis calculated as glucose	83 per cent‖	87.2 per cent¶
Nitrogen content	2.6 per cent	1.72 per cent

* Data from the paper by Zittle and Harris (21).

‡ Electrophoresis and ultracentrifuge analysis were performed by Charles G. Gordon and Thomas E. Thompson in the University Laboratory of Physical Chemistry Related to Medicine and Public Health at Harvard University through the courtesy of Dr. J. L. Oncley.

§ The conditions of the ultracentrifuge run were: speed, 900 R.P.S.; temperature, t = 23.0 before run, t = 25.1 after run; cell = 10 mm. depth; time of run, 150 minutes. A 1 per cent solution of polysaccharide in PO_4-NaCl buffer at pH 6.8, γ 0.15 was examined in this run.

‖ Hydrolysis was carried out in 2 N HCl for 2 hours at 100°C. in a sealed tube; the reducing value was determined by the method of Park and Johnson as described by Umbreit *et al.* (25).

¶ Sample hydrolyzed in closed tube with 1.0 ml. of concentrated HCl and 5.0 ml. of water for 2 hours at 100°C.; reducing value was determined by the Hagedorn-Jensen method.

calculation of the molecular weight, was not obtained. However, it seems likely from a consideration of the sedimentation constants that the material obtained by pepsin digestion was not a more highly polymerized compound. Its molecular weight would appear to fall within the same range as the value of 8000 reported for the formamide preparation.

The identity of these polysaccharide preparations is further substantiated by studies of their carbohydrate composition.

Carbohydrate Components of the Group A Streptococcus Polysaccharide

The monosaccharide units that make up the group A streptococcus polysaccharide were determined by paper chromatography of hydrolysates (26) of formamide preparations and preparations obtained by pepsin digestion. After the sugar components were identified, quantitative methods were used to determine the ratio of their occurrence in the polysaccharide.

Methods.—The most efficient hydrolysis of this polysaccharide was found to result from exposure to 2 N HCl for 2 hours at 100°C. in a sealed tube. These conditions gave maximum reducing values. All hydrolysates used in chromatography and quantitative sugar determinations were prepared under these conditions.

Paper chromatograms were made using the method of capillary ascent described by Williams and Kirby (27). Polysaccharide hydrolysates were evaporated to dryness *in vacuo* to remove HCl, redissolved in distilled water, and aliquots were placed along one margin of the filter paper in the usual manner (27). The polysaccharide hydrolysates and known sugars were run on the same paper, and their mobilities were compared. The papers were irrigated in three solvents: (*a*) a mixture of secondary butanol, diethylene glycol, and water in the proportion 4:1:1 (28); (*b*) the same components in a 3:1:1 ratio; (*c*) ethyl acetate, pyridine, and water in the proportion 2:1:2 (29). "Trailing" of the amino sugars in the butanol-diethylene glycol-water solvents was prevented by chromatographing in an atmosphere of ammonia (26). The chromatograms, after irrigation, were heated in an oven to evaporate the solvent. They were developed by spraying with *m*-phenylenediamine in 76 per cent ethanol and heating to 105°C., and then examined under ultraviolet light as described by Chargaff *et al.* (30). If the chromatograms were dried without heating, and then sprayed with *m*-phenylenediamine, the amino sugars appeared as faint blue-yellow fluorescent spots when viewed in ultraviolet light. However, the spots were not clear-cut and development was irregular. Spots of these sugars were, therefore, made visible by spraying with acetylacetone and *p*-dimethylaminobenzaldehyde according to the Partridge modification (26) of the method of Elson and Morgan. The ethyl acetate–pyridine–water solvent interfered with the development of the simple sugars by the *m*-phenylenediamine method; therefore, only the amino sugars could be studied on chromatograms irrigated in this solvent.

Chromatograms of the polysaccharide hydrolysates showed two components of markedly different mobilities. The slow moving component gave the characteristic pink color of an amino sugar with the acetylacetone–*p*-dimethylaminobenzaldehyde reagents, and it had the same mobility as glucosamine in all three solvents. The fast moving component was found to have the same mobility as rhamnose in both of the secondary butanol–diethylene glycol–water solvents. Mixtures of rhamnose and polysaccharide hydrolysate were chromatographed, and the fast component of the hydrolysate and rhamnose formed a single spot with the characteristic mobility of rhamnose. Chromatograms of mixtures of glucosamine and polysaccharide hydrolysate were also made and were developed with the reagents for amino sugars. In these, the movement of the amino sugar component of the hydrolysate exactly coincided with the glucosamine control. Infrequently, hydrolysates, particularly those in which the acid concentration was greater than 2 N or those that had been heated for longer than 2 hours,

showed a very faint spot with the mobility of glucose. This glucose was apparently derived from glucosamine during hydrolysis, since hydrolysis of a sample of glucosamine for 2 hours at 100°C. in 2 N HCl produced a similar spot of roughly comparable intensity.

These chromatographic studies indicated that the monosaccharide components of the group A streptococcus polysaccharide are rhamnose and glucosamine. Further experiments were carried out to determine the relative amounts of rhamnose and glucosamine in this polysaccharide.

TABLE II

Composition of Group A Streptococcus Polysaccharide

Preparation	Rhamnose	Glucosamine	Rhamnose	Glucosamine	Reducing* value	Calculation‡ of theoretical reducing value	Deviation of theoretical reducing value from actual value
	per cent§	*per cent*§	*mol. fraction*	*mol. fraction*			*per cent*
Hydrolysate No. 2 (formamide preparation)	63.0	30.0	0.70	0.30	0.291	0.267	−6.5
Hydrolysate No. 3 (pepsin digestion preparation)	61.3	23.2	0.74	0.26	0.279	0.277	−0.6
Hydrolysate No. 4 (pepsin digestion preparation)	58.2	25.2	0.72	0.28	0.285	0.289	+1.4
Average	60.8	26.1	0.72	0.28			

* Spectrophotometer density reading.

‡ Calculated as the sum of the molar fraction contributions of rhamnose and glucosamine × molar extinction reducing value of their respective standards.

§ Calculated as weight rhamnose or glucosamine/weight polysaccharide before hydrolysis, uncorrected for dehydration theorized in the polymerization of sugar units in polysaccharide formation.

Methods.—Hydrolysates of polysaccharide used in these determinations have been described above. Glucosamine was determined according to the method of Kabat and Mayer (31) with the exception that 1.0 N Na_2CO_3, instead of 0.5 N, was used as a solvent for acetylacetone.[2] Rhamnose was determined by the cysteine-sulfuric acid method described by Dische and Shettles (33). The procedure designated CyR 10, employing a 10 minute heating period, was used. Hydrolysates of both formamide and pepsin digestion preparations were studied, and the reducing value of each of these hydrolysates was determined.

The results of several of these determinations are presented in Table II.

These determinations indicate the average rhamnose content to be 61 per cent and the average glucosamine content to be 26 per cent of the polysaccharide

[2] According to the data reported by Blix (32).

by weight. The variation in these values may represent the error inherent in the hydrolysis procedure as well as the error in the methods for the determination of rhamnose and glucosamine. Therefore, reducing values are compared in Table II with the calculated reducing values in an attempt to indicate the error due to hydrolysis.

Dische and Shettles, in describing the cysteine–sulfuric acid method for methylpentoses stated that this determination might be carried out on unhydrolyzed polysaccharides (33). In one experiment using a formamide preparation of group A polysaccharide, the rhamnose content of the unhydrolyzed material was found to be 59.5 per cent as compared with a value of 63.0 per cent on the hydrolyzed polysaccharide.

Calculation of the rhamnose and glucosamine content of the polysaccharide hydrolysates as mole fractions shows average values of 0.72 and 0.28, respectively. Rhamnose and glucosamine, therefore, would appear to be present in the polysaccharide in the approximate ratio of five moles of rhamnose to two moles of glucosamine.

Localization of Group A Streptococcus Polysaccharide in Tissue Cells

The tissue distribution of the group A streptococcus polysaccharide was determined in a number of mice sacrificed at intervals following the intravenous injection of this substance. The localization of the polysaccharide in the tissue cells was determined by the method of Coons and Kaplan (14), employing antibody labeled with fluorescein isocyanate as a tissue-staining reagent selective for group A polysaccharide by virtue of its unchanged immunologic specificity. This method has been used to study the localization of the type II and type III pneumococcus polysaccharides and the type B Friedländer bacillus polysaccharide in the tissues of mice following intravenous injection (34, 35).

Methods.—Antistreptococcal serum that reacted with dilutions of group A polysaccharide greater than 1:1,000,000 was obtained by immunizing rabbits with formalinized suspensions of group A streptococci. The globulin fraction of this serum was obtained by ethanol fractionation in the cold (36). To prepare the fluorescein-labeled antibody solution, the globulin fraction was conjugated with fluorescein isocyanate and absorbed with mouse liver powder according to the methods previously described (14).

Mice were injected intravenously with single doses of approximately 10 mg. of the respective polysaccharide preparations dissolved in sterile normal saline. The mice were sacrificed at various intervals by decapitation and autopsied. Blocks of tissue from the heart, lung, liver, spleen, kidney, adrenal gland, axillary node, skeletal muscle, and thymus were taken for study.

The picric acid–formalin–ethanol fixation method of Rossman, used in other studies of polysaccharides by the fluorescein antibody method (34, 35), was unsatisfactory here. Frozen sections prepared in the cryostat (14) were also unsuitable. Apparently, because of its small molecular size and marked water solubility, group A polysaccharide can diffuse out of tissues fixed by these methods. However, tissues fixed for 18 hours in cold acetone, containing suspended anhydrous $CaSO_4$ as a dehydrating agent, were found to retain this polysaccharide. The cold acetone-fixed tissues were imbedded in paraffin and sectioned in the

usual manner. Five-micron sections were floated on 80 per cent alcohol[3], mounted on gelatinized slides, dried 6 to 12 hours at 37°C., and stored in the refrigerator until used.

Before being stained with labeled antibody, the sections were deparaffinized in two changes of xylene and the xylene removed by gentle agitation in acetone. The acetone was removed by drying at 37°C. for 30 to 60 minutes. The sections were exposed to the fluorescein-labeled antibody solution for 30 minutes according to the method previously described (14). They were then examined under the fluorescence microscope. Controls consisted of tissues of uninjected mice treated in the same manner.

In a mouse sacrificed 7 minutes after intravenous injection, group A streptococcus polysaccharide was found only in the tubular epithelial cells of the kidney (Figs. 1 and 2). It appeared in sections of these organs as brightly fluorescent material varying in intensity from a faint trace in one or two cells in a cross-section of a tubule to a homogeneous, non-granular brilliant fluorescence filling most of the cells. No specifically stained polysaccharide could be detected within the nuclei of the tubular cells. Some of the polysaccharide-containing cells appeared to have the brush border characteristic of the cells of the proximal segment of the tubule. Fluorescent-staining material was found in cellular casts in the lumens of the tubules.

In a mouse sacrificed 10 minutes after injection, the same localization of polysaccharide was observed. The amount of fluorescent-staining polysaccharide found in the tubular epithelial cells of a mouse sacrificed after 15 minutes was greatly decreased. Precipitin tests with antistreptococcal serum were done on the blood and urine obtained from this mouse at the time of autopsy. The blood gave a positive reaction through a dilution of 1:500, whereas the urine gave a strongly positive reaction at a dilution of 1:50,000. No polysaccharide could be detected with fluorescent antibody in the renal tubular cells of a mouse sacrificed 30 minutes after injection.

The fluorescent antibody-stained sections of the other organs of these mice revealed no definite cellular localization of polysaccharide. In the mice sacrificed at 7, 10, and 15 minutes, a rare specifically stained cell was seen in the lung parenchyma, in the red pulp of the spleen, or in the renal glomerular capillaries. In these cells, the fluorescent material seemed to be localized in the peripheral cytoplasm of the cell or to be coated on the cell surface. These cells appeared to be blood cells lying in capillaries or tissue spaces.

No differences were noted in the localization of the polysaccharide prepared by the pepsin digestion method and that prepared by formamide extraction. No evidence of any toxic effects of these polysaccharide preparations were noted in the mice.

DISCUSSION

In molecular size, charge, and chemical composition, preparations of group A streptococcus polysaccharide isolated by the pepsin digestion method closely

[3] This is necessary to prevent diffusion of the polysaccharide from the paraffin sections.

resembled preparations extracted by the more vigorous formamide method. The isolation of preparations of this polysaccharide of such close similarity by two different methods offers some basis for the belief that this substance may exist naturally in this form. Other bacterial polysaccharides of approximately this molecular size have been described. One of these is the polysaccharide hapten released into the culture medium by tubercle bacilli (MW 9000 (20)). Another is the polysaccharide hapten of *Salmonella typhosa* (MW about 10,000 (37)), which exists conjugated to protein and lipid in the bacterial cell. Heidelberger and Kendall (5) isolated protein fractions from group A streptococci that contained chemically combined, non-nucleic acid carbohydrate. They identified this chemically bound carbohydrate as group A polysaccharide by the precipitin test and suggested that it might exist conjugated to protein under natural conditions.

The cellular localization of injected group A polysaccharide, traced with fluorescent-labeled antibody, was limited to the tubular epithelium of the kidney. The transitory appearance of the polysaccharide in the tubular epithelial cells indicates that it is passing through these cells (Figs. 1 and 2); whether it is being excreted or resorbed cannot be definitely determined. Without doubt, a substance of the molecular size of this polysaccharide is filtered by the glomerulus, and the high concentration of the polysaccharide found in the urine 15 minutes after injection is evidence that this substance was being rapidly excreted by the kidney.

Group A polysaccharide resembles the polysaccharide inulin, which is rapidly excreted after injection (38) without being stored in the tissues (39, 40). These polysaccharides are chemically neutral and have similar molecular weights—8000 and 5000 (38), respectively. Yet, in one aspect of their behavior *in vivo*, these polysaccharides are markedly different. Inulin is excreted by the kidney solely by glomerular filtration (38), whereas the passage of group A polysaccharide through the kidney appears to involve tubular activity as well as glomerular filtration. Heparin, an acidic polysaccharide with a molecular weight of about 20,000 (41), bears some resemblance to the group A polysaccharide in its mode of excretion. It differs from the group A polysaccharide in that it is stored in the tissues after injection. In tissue sections of kidneys from animals given heparin, this polysaccharide can be seen in various stages of its passage through the glomerulus and through the renal tubular epithelium (42); it is present in highest concentration in the tubular cells during the first hour after injection (42). Clearance studies show that about 25 per cent of an injected dose of heparin is excreted by the kidneys during this interval (43, 44). As in the case of the group A polysaccharide, the passage of heparin through the renal tubular cells cannot be definitely demonstrated to be excretion or resorption (42–45). Heparin shows a pattern of tissue distribution

(42) remarkably similar to that of the types II and III pneumococcus polysaccharides (34) and the type B Friedländer bacillus polysaccharide (35). Heparin, however, persists in tissues for a much shorter period of time (42). The pneumococcal and the Friedländer bacillus polysaccharides were widely distributed in many different tissues and organs, particularly in the cells of the reticulo-endothelial system where they persisted for many months (34, 35). The distribution and storage of these acidic, macromolecular[4] bacterial polysaccharides is in distinct contrast to the limited localization and rapid excretion of the neutral polysaccharides of small molecular size. The marked differences in the *in vivo* behavior of these polysaccharides may be a function of their molecular size and charge (34, 39, 47).

In conclusion, although the complete mechanism of the excretion of the group A streptococcus polysaccharide cannot be determined from these experiments, the demonstration of this polysaccharide in high titer in the urine and its absence from the blood and tissues 30 minutes after injection is good evidence that the end result of this renal activity is the elimination of this substance. If, during the course of a natural streptococcus infection, group A polysaccharide is released from the streptococcal cell so that it exists in the free state in the tissues of the host, it would appear from these studies that it would be rapidly excreted by the kidney.

SUMMARY

A method was developed for the extraction of the group A streptococcus polysaccharide employing pepsin digestion of ground streptococcal cells. This method did not result in the isolation of polysaccharide with chemical and physical chemical properties different from those exhibited by preparations extracted with hot formamide.

Studies of the chemical composition of this polysaccharide demonstrated it to be composed chiefly of rhamnose and glucosamine monosaccharide units in the approximate ratio of five moles of rhamnose to two moles of glucosamine.

The fate of the polysaccharide after intravenous injection into mice was studied using the fluorescent antibody technique. It was found to be rapidly eliminated by the kidney. The presence of the polysaccharide in the renal tubular epithelial cells during the excretory phase was the only evidence of its cellular localization that could be detected under the conditions of these experiments.

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[4] The molecular weights of the type II and the type III pneumococcus polysaccharides are reported as 500,000 and 140,000, respectively (46).

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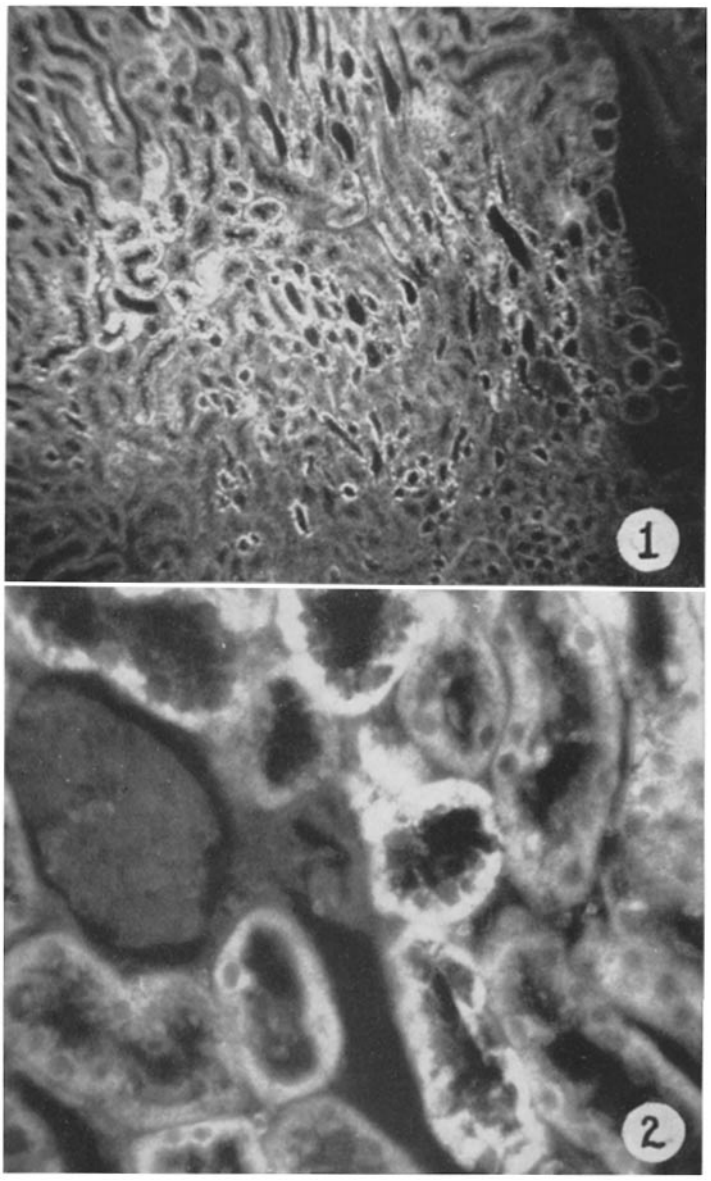
EXPLANATION OF PLATE 1

These figures are photomicrographs of sections of the kidney of a mouse killed 7 minutes after receiving 10 mg. of group A streptococcus polysaccharide intravenously. The sections were treated with fluorescein-labeled antibody (14) and photographed under the fluorescence microscope. The lightest areas represent the yellow-green fluorescence of the fluorescein–antibody–group A polysaccharide precipitate. The histological topography can be seen faintly as a result of the normal blue autofluorescence of the tissues.

FIG. 1. Kidney of mouse 2; fixed in acetone-$CaSO_4$. Polysaccharide-fluorescein-antibody precipitate can be seen in the epithelium of a number of tubules. × 140.

FIG. 2. Same section as in Fig. 1. The pattern of the deposition of the polysaccharide-fluorescein-antibody precipitate in the tubular epithelial cells can be seen in detail. × 560.



(Schmidt: Group A streptococcus polysaccharide)