

## EDEMA-PRODUCING ACTIVITY OF GROUP A STREPTOCOCCAL POLYSACCHARIDE AND ITS POSSIBLE ROLE IN THE PATHOGENESIS OF CELL WALL-INDUCED POLYARTHRITIS\*

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The group-specific polysaccharide (PS)<sup>1</sup> isolated from group A streptococcal cell wall consists of a polyrhamnose backbone (alternating  $\alpha$ -1,2 and  $\alpha$ -1,3 linkages) which has  $\beta$ -1, 3-linked *N*-acetyl-D-glucosamine (NADG) residues attached to the available 3 position in the backbone (1-4). Fung et al. showed that the PS molecule is heterogeneous because of varying amounts of NADG attached to the rhamnose backbone (5). The antibody raised against PS is reported to cross-react with a glycoprotein of connective tissue (6) and with antigens of thymus and skin epithelial cells (7). In various animal models of chronic inflammation induced by group A streptococcal cell walls (8-13), the PS played an important role in protecting the toxic peptidoglycan (PG) moiety from tissue lysozyme and other degradative enzymes (10, 14). Although the PS has been well characterized chemically and immunologically, there have not been any well-documented reports of a biological activity of this polymer.

In an earlier study (15), we observed that a spectrum of various sizes of PG-PS fragments was obtained by treatment of group A streptococcal cell walls with mutanolysin or phage-associated lysin. Some of these fragments induced edema in rats, and the severity of edema was related to the molecular weight of the PS polymer. The present study presents evidence that the active moiety responsible for the edematous responses is the PS and that this property is dependent upon the NADG epitope. Data are also presented that suggest that the edema-producing PS might play an important role in the pathogenesis of experimental cell wall-induced polyarthritis in rats (9, 16, 17).

### Materials and Methods

*Preparation of Cell Wall and Cell Wall Fragments.* Purified cell walls were prepared by disruption of group A, type 3, strain D-58 streptococci in a Braun shaker (B. Braun Instruments, San Francisco, CA). Cell wall fragments were generated from the cell walls by sonic treatment (17) or by treatment with mutanolysin (15). The fragments obtained by treatment with mutanolysin included: high molecular weight (HMW) PG-PS (~500,000 mol wt), low molecular weight (LMW) PG-PS (~30,000 mol wt), and PG fragments; the PG-PS fragments obtained

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<sup>1</sup> *Abbreviations used in this paper:* gbw, grams of body weight; HMW, high molecular weight; HSA, human serum albumin; LMW, low molecular weight; MDP, muramyl dipeptide; NADG, *N*-acetyl-D-glucosamine; PBS, phosphate-buffered saline; PG, peptidoglycan; PS, polysaccharide.

by sonic treatment were separated by differential centrifugation and included the 100S60 ( $\sim 5.3 \times 10^6$  mol wt) and 100P60 ( $\sim 5.0 \times 10^7$  mol wt). In certain experiments, the 100P60 was further fractionated by filtration through a column of Bio-Gel A-50m, and the fragments eluted in the void volume fractions were collected by centrifugation (100,000 g, 60 min, 4°C) and referred to as a filtered 100P60 preparation.

*Preparation of Group-specific Polysaccharide.* Group-specific PS was isolated from the cell walls of group A (type 3, strain D-58) and group A-variant (strain A486) streptococci according to the method described by Krause (18) with some modifications. A cell wall preparation was suspended in formamide (5 mg/ml) and extraction was performed at 150°C for 20 min. After being cooled overnight at 4°C, the extracted preparation was centrifuged (36,000 g, 30 min, 4°C), the supernatant was mixed with 2 vol of acid-alcohol (95% ethanol and 5% 1 N HCL), and the mixture was centrifuged (12,000 g, 30 min, 4°C). The PS in the alcoholic supernatant was precipitated by adding 5 vol of acid-acetone (99% acetone and 1% HCL) and a few crystals of sodium acetate to the supernatant. This was held at 4°C for 72 h. The acid-acetone precipitable carbohydrate was dissolved in distilled water, clarified by centrifugation, and treated with the acid-alcohol and acid-acetone once more. The PS obtained from the second acid-acetone treatment was suspended in distilled water, dialyzed against distilled water for 48 h at 4°C, and passed through Dowex-50  $\times$  8 (200–400 mesh) cation exchange resin (Bio-Rad Laboratories, Richmond, CA) before lyophilization. The lyophilized PS was suspended in an appropriate amount of distilled water and was treated again with acid-alcohol and acid-acetone before use.

An aliquot of the group A PS was also treated with an *N*-acetyl-D-glucosaminidase to remove NADG from the rhamnose backbone. The enzyme was prepared from a soil bacillus (strain 2 aSm) as described by McCarty (4). The enzymatic digestion was performed by incubating a solution of the PS (60 mg in 22 ml of a buffer containing 0.5 M sodium citrate and 0.15 M NaCl, pH 6.0) with 8 ml of the partially purified enzyme (in 0.15 M NaCl). The mixture was incubated at 25°C for 72 h and then heated at 100°C for 5 min to stop the reaction. The enzyme-treated PS was clarified by centrifugation (25,000 g, 20 min, 4°C) and filtered through a column of Sephadex G-100. The fractions which contained rhamnose were pooled, dialyzed against deionized distilled water, and lyophilized.

*Animals.* We used outbred female Sprague-Dawley rats (Zivic-Miller Laboratory, Allison Park, PA) that weighed 120–160 g at the beginning of the experiments.

*Iodination of Human Serum Albumin.* Human serum albumin (HSA) (Cutter Laboratories, Inc., Berkeley, CA) was iodinated with  $^{125}\text{I}$  (Amersham Corp., Arlington Heights, IL) by solid-state lactoperoxidase as described by David and Reisfeld (19). The  $^{125}\text{I}$ -HSA had a specific activity of 94  $\mu\text{Ci}/\text{mg}$  HSA, and was diluted with phosphate-buffered saline (PBS) before use.

*Methods of Inducing and Scoring Edematous Response and Arthritis.* Three routes of injection were used to induce edema in the rats, intravenous (i.v.), intraperitoneal (i.p.), and intra-articular (i.a.). When edema was induced by i.v. or i.p. injections, the samples were dissolved in and diluted with sterile PBS (0.04 M phosphate, 0.15 M NaCl; pH 7.2) and 0.4 ml samples were injected. Intra-articular injections were performed by injecting 10  $\mu\text{l}$  of control (PBS) or test samples in PBS through the Achilles tendon and above the calcaneus into the region of the tibio-talar joint. In all experiments, each rat was injected with the PBS control in the left joint and the test samples in the right joint. The intensity of edema in each limb was estimated on a scale 0–4 by gross observations, or by measuring the amount of  $^{125}\text{I}$ -HSA accumulated in the limbs. An earlier report (15) showed that there was an excellent correlation between the clinical scoring of edema and the amounts of  $^{125}\text{I}$ -HSA accumulation in the limb ( $r = 0.97$ ,  $P < 0.001$ ).

The time course of edema after the i.a. injection was determined by measuring  $^{125}\text{I}$ -HSA accumulation in the limbs at various times after the limbs were injected with the HMW PG-PS. The  $^{125}\text{I}$ -HSA was injected (i.v.,  $1.45 \times 10^{-3}$   $\mu\text{Ci}/\text{g}$  body weight [gbw]) 1 h before the rats were scored for edema. The experiment was performed by injecting each of 24 rats (6 groups of 4 rats each) with 10  $\mu\text{l}$  of PBS into the left joint and 10  $\mu\text{g}$  of HMW PG-PS in 10  $\mu\text{l}$  PBS into the right joint. At 15, 30, and 60 min, and 4, 24, and 48 h after i.a. injection, one group of rats was scored for edema and immediately anesthetized by ether. The heart blood was withdrawn, and the left and the right limbs were removed. The amounts of  $^{125}\text{I}$ -HSA in the left and right

limbs and in the blood were determined with a Gamma counter (1197 series; Automatic Gamma Counting System, Searle Analytic Inc., Des Plaines, IL).

The  $^{125}\text{I}$ -HSA was also used to quantitate vascular permeability changes in various tissues after the rats were injected systemically with the PS. In these experiments, each member of a group of five rats was injected i.v. with  $^{125}\text{I}$ -HSA ( $1.18 \times 10^{-3} \mu\text{Ci/gbw}$ ); after 30 min, the rats were injected i.v. with either PBS or with a PS preparation ( $6 \mu\text{g/gbw}$ ). 30 min after the injection of PBS or PS, the rats were anesthetized by ether, the heart blood was collected, and various tissues were removed, including heart, lung, spleen, liver, thymus, kidney, skin (flank), muscle (trapezius), submandibular lymph node, mesenteric lymph nodes, ascending colon, as well as all four limbs. The amount of  $^{125}\text{I}$ -HSA in the tissue and in the blood were determined with the Gamma counter.

Arthritis was induced by a single i.p. injection of 100P60 preparations (in sterile PBS) at 12.75 or 10  $\mu\text{g}$  rhamnose per gbw. The rats were scored for arthritis as described (9).

*Isolation of Edema-producing Activity from Cell Wall Sonicate.* After sonication of cell walls for 70 min (Branson Sonifier, model 350; Branson Sonic Power Co., Danbury, CT), the heterogeneous collection of fragments was centrifuged at 100,000  $g$  for 60 min and the resulting supernatant fraction was labeled 100S60. The 100S60 fraction (300 mg) was dissolved in 10 ml of 0.01 M Tris/HCl (pH 7.4) and filtered through a column of Sepharose 6B equilibrated in the buffer. Cell wall fragments retained by the column were concentrated and washed with glass-distilled water in a stirred cell equipped with an Amicon UM05 membrane (Amicon Corp., Scientific Systems Div., Lexington, MA) and lyophilized. The materials in the lyophilized preparation (40 mg) were separated by DEAE-Sephacel ion-exchange chromatography into three fractions, designated FI, FII, and FIII. The FI referred to the materials that did not bind to the column. The FII and FIII were isolated from the rhamnose-containing fractions that bound to the column and which were eluted with a linear gradient of NaCl (0–0.15 M). They were separated by Sephadex G-100 gel filtration based on differences in their sizes. The FII was eluted in the void volume, whereas the FIII was retained by the gel. All fractions which contained rhamnose were tested for edema-producing activity by i.a. injections.

*Molecular Weight Determinations.* Molecular weight of the PS was estimated by gel filtration using Dextran T fractions of known molecular weight as markers as previously described (15).

*Chemical Analyses.* Muramic acid and glucose were assayed as their alditol acetates by gas-liquid chromatography (20). Samples were hydrolyzed under vacuum for 3 h at 100°C with 2 N sulfuric acid before derivatization. Rhamnose was assayed according to Dische and Shettles (21). Glucosamine was determined by a modified Elson-Morgan method as described by Blumenkrantz and Asboe-Hansen (22). Amino acid analyses were performed after the samples were hydrolyzed in 6 N HCl at 110°C in vacuo for 24 h. Hydrolyzed samples were lyophilized to dryness, redissolved in 0.2 M sodium citrate buffer (pH 2.2), and centrifuged to remove carnalized material. The supernatant was applied to a gradient high pressure liquid chromatograph system adapted to perform post-column derivatization amino acid analysis (23).

*Muramyl Dipeptide.* Muramyl dipeptide (MDP; *N*-acetyl-muramyl-L-alanyl-D-isoglutamine) and the analog (*N*-acetylmuramyl-D-alanyl-D-isoglutamine) were obtained from Sigma Chemical Co., St. Louis, MO. They were dissolved in sterile PBS, and the pH was adjusted to 7.4 with 1 N NaOH before use.

## Results

*Edema Induced by PS and PG-PS Fragments.* The time course of edema developing in the limbs of rats injected with PS or PG-PS depended on the route of injection. When injected i.v., the kinetics of the edematous response was similar for PS and HMW PG-PS. Edematous swelling appeared in 5–10 min in both front and both hind limbs, reached maximal intensity by 30 min, began to disappear by 1 h, and was undetectable by 10 h. The minimum amount of PS required to give obvious edema was 1.0  $\mu\text{g/gbw}$  (Table I). The PS isolated from group A-variant (strain A486) did not induce edema even when injected at 10  $\mu\text{g/gbw}$ , and the glucosaminidase-treated group A

TABLE I  
*Response of Rats to i.v. Injection of PS*

PS	Dose ( $\mu\text{g}/\text{gbw}$ )	Number positive/ number in- jected	Average score of positive rats at 30 min postinjection*
Group A, strain D58	1.00	1/5	1.0
	2.50	5/5	$1.80 \pm 1.30$
	5.00	5/5	$10.60 \pm 3.29$
	10.00	4/4	$14.75 \pm 1.66$
Group A-variant strain A486	4.86	0/5	
	10.00	0/4	
Enzyme-treated group A, strain D58	5.00	0/5	
	10.00	1/4	2.0
PBS control	0.4 ml	0/5	

\* A positive response was indicated by the appearance of edema in the front and hind limbs. A scale of 0-4 was used to estimate the intensity of edema of each limb; therefore, the maximum total score per rat was 16 (mean  $\pm$  1 SD).

PS was much less active in inducing edema than was the untreated preparation (Table I).

Edematous responses could also be induced locally in the limbs when the edema-producing PG-PS or PS preparations were injected i.a. (Table II). Edema was not accompanied by detectable erythema and microscopic examination of tissue sections gave no evidence of erythrocyte or leukocyte infiltration into edematous joints. The time course of edematous responses in the limbs of rats (determined by measurements of  $^{125}\text{I}$ -HSA accumulation at various times) was similar to those seen after i.v. injection, with the exception that the increase in vascular permeability persisted at a low level for at least 24 h (Fig. 1). The dose-response curve of edema after i.a. injection of HMW PG-PS is shown in Fig. 2.

Intraperitoneal injection of the group A PS at  $15 \mu\text{g}/\text{gbw}$  resulted in the appearance of edema at 60 min. The edematous response reached maximal intensity at 2 h, began to decline, and usually was not observable at 15 h.

*Analysis of PS Preparations by Gel Filtration.* The PS from group A streptococcal cell wall was retained by Sephadex G-100 and had a size slightly larger than that of the PS isolated from the group A-variant (Fig. 3 A-B). Treatment of group A PS with glucosaminidase for 72 h resulted in a partial removal of glucosamine from the rhamnose backbone. Enzymatic digestion of the PS was indicated by the appearance of a second peak of glucosamine which eluted close to the bed volume ( $V_t$ ) of the Sephadex G-100 column (Fig. 4). In addition, the enzyme-treated PS had an average size slightly smaller than that of the untreated group A PS.

*Isolation of Edema-producing Activity from Cell Wall Sonicate.* Edema-producing cell wall fragments in the 100S60 fraction of the cell wall sonicate were retained by Sepharose 6B and were very heterogenous in size. Some of these fragments (FI) did not bind to the column of DEAE-Sephacel, which was equilibrated with 0.01 M Tris/HCl (pH 7.35), whereas other fragments (FII and FIII) bound to the column and were eluted by a linear 0-0.15 M NaCl gradient. The FII was excluded by the Sephadex G-100 column but the FIII was retained by the column and had a  $K_{av}$  of 0.29,  $\bar{M}_N = 26,000$ . All fractions induced edematous responses (Table II).

TABLE II  
Response of Rats to i.a. Injection of Various PG-PS, PS, and PG Fragments

Preparation	Dose $\mu\text{g}/\text{limb}$	Number positive/ number in- jected	Average score of positive rats at 30 min postinjection*
Fragments generated by mutanolysin			
HMW PG-PS	2.50	5/5	$0.88 \pm 0.25$
(~500,000 mol wt)	10.00	5/5	$2.25 \pm 0.29$
LMW PG-PS	10.00	5/5	$1.44 \pm 0.52$
(~30,000 mol wt)			
LMW PG‡	20.00	0/5	
Group A PS	0.25	2/5	0.50
	1.25	5/5	$1.05 \pm 0.67$
	5.00	5/5	$2.20 \pm 0.27$
Group A-variant PS	70.00	0/5	
Fragments isolated from 100S60			
FI	2.50	4/4	$0.63 \pm 0.14$
	10.00	4/4	$2.75 \pm 0.29$
FII	2.50	5/5	$0.60 \pm 0.55$
	10.00	5/5	$1.75 \pm 0.29$
FIII	2.50	5/5	$0.70 \pm 0.45$
	10.00	5/5	$2.50 \pm 0.41$
LMW fragments derived from 100P60 (re- tained by Bio-Gel A-50m)	10.00	5/5	$1.20 \pm 0.57$
MDP	300.00	0/5	
MDP analog	300.00	0/5	
Lipopolysaccharide ( <i>Salmonella typhimurium</i> )	0.10	0/5	
	1.00	0/5	
	10.00	0/5	
PBS	10 $\mu\text{l}$	0/5	

\* A positive response was the appearance of edema in the right limb (sample-injected limb). A scale of 0-4 was used to estimate the intensity of edema of the limb (Mean  $\pm$  1 SD).

‡ Retained by Sephadex G 25 (15).

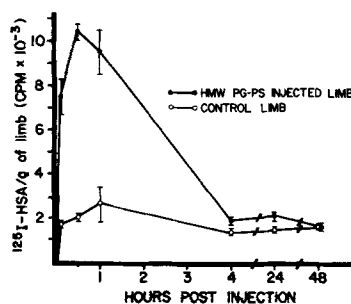


FIG. 1. Time-course of edema after i.a. injection of HMW PG-PS. (●) <sup>125</sup>I-HSA in the HMW PG-PS injected limb; (○) PBS-injected limb. Each dot represents the mean of four rats and the bracket represents the standard error of the mean. There were no significant differences in the level of <sup>125</sup>I-HSA in the blood (cpm/ml) of the 24 rats.

Chemical Analyses (Table III). The PS preparations used in our studies contained no detectable amounts of muramic acid (<0.3% wt/wt), contained <0.3% alanine, glutamic acid, and lysine and contained only small amounts of other amino acids

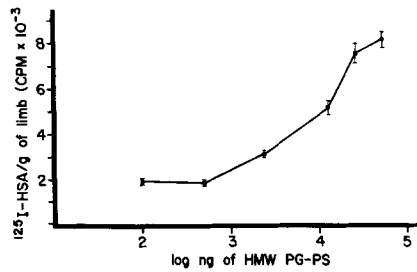


FIG. 2. Dose response curve of edema induced by i.a. injection of the HMW PG-PS. Each group of four rats was injected with HMW PG-PS (0.1–50  $\mu\text{g}/\text{limb}$ ). Each dot represents the mean  $\pm$  SEM.

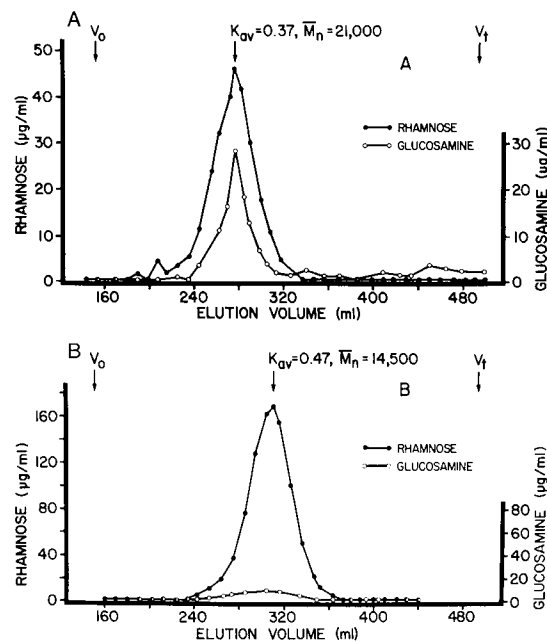


FIG. 3. Sephadex G-100 elution profiles of polysaccharides isolated from (A) group A strain D58 and (B) group A-variant strain A486 streptococci. A sample (6 mg of group A polysaccharide or 10 mg of group A-variant polysaccharide) was dissolved in 1 ml of 0.01 M Tris/HCl (pH 7.4), applied to a column of Sephadex G-100 (2.6  $\times$  94 cm), and eluted with Tris/HCl at 4°C at a flow rate of 18 ml/h. Fractions (4.6 ml) were assayed for rhamnose (●) and glucosamine (○).

(<0.05% wt/wt). The enzyme-treated PS had significantly lower glucosamine content than did the untreated PS. The composition of the various edema-producing fragments in the 100S60 indicated the heterogeneity of these fragments. The FI consisted of the PS and possibly some membranous contaminants, whereas the FII and FIII consisted primarily of PG-PS fragments. The ratio of the PS to the PG components of the FIII was much higher than that of the FII.

*Vascular Permeability Changes after i.v. Injection of Edema-producing PS.* 30 min after i.v. injection of group A PS (6  $\mu\text{g}/\text{gbw}$ ), the amount of  $^{125}\text{I-HSA}$  in the blood decreased by 34% when compared with the control group (Table IV). The permeability increase of all four limbs was highly significant ( $P < 0.001$ ), and the increase in the muscle and submandibular lymph node was of questionable significance ( $P < 0.05$ ). However,

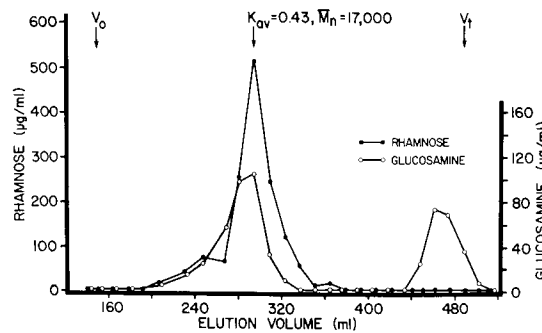


FIG. 4. Sephadex G-100 gel filtration of the glucosaminidase-group A polysaccharide mixture. The incubation mixture (25 ml) was applied to the Sephadex G-100 column (4°C) as described in Fig. 3. The elution was performed at a flow rate of 27.6 ml/h, and fractions (6.9 ml) were assayed for rhamnose (●) and glucosamine (○).

TABLE III  
Composition of PG-PS and PS Fragments

Component	Fragments isolated from 100S60						Polysaccharide		
	FI		FII		FIII		Group A (Percent wt/wt)	Enzyme-treated group A (Percent wt/wt)	Group A-variant (Percent wt/wt)
	Percent wt/wt	Molar ratio to lysine	Percent wt/wt	Molar ratio to lysine	Percent wt/wt	Molar ratio to lysine			
Rhamnose	5.20	10.12	11.20	4.59	20.58	31.93	55.72	55.00	92.98
Glucosamine	3.50	6.25	6.33	2.38	12.50	17.77	24.53	14.50	6.25
Muramic acid	ND*		1.83	0.49	0.39	0.40	ND	ND	ND
Alanine	0.63	2.27	4.72	3.56	0.87	2.47	0.27	0.11	
Glutamic acid	0.42	0.91	2.22	1.01	0.84	1.46	0.12	0.11	
Lysine	0.46	1.00	2.17	1.00	0.57	1.00	0.11	0.05	
Glucose	18.20		3.80		1.82				
Total non-PG-associated amino acids	1.53		1.51		3.34		0.05	0.28	

\* Not detected.

when the experiment was repeated, the increase in vascular permeability in the muscle and submandibular lymph node was not observed. On the other hand, there was a slight decrease in the vascular permeability in the mesenteric lymph nodes ( $P < 0.01$ ) and ascending colon ( $P < 0.05$ ).

*Edema-producing Activity and Arthritogenic Activity of 100P60.* Preliminary experiments indicated that the 100P60 fraction of the cell wall sonicate (unfiltered 100P60) contained small amounts of edema-producing PG-PS fragments (Table II). These fragments were retained by a Bio-Gel A-50 m column and, thus, could be separated from the 100P60 (excluded by the gel). The 100P60, which was free of edema-producing activity (filtered 100P60), was significantly less arthropathic than the unfiltered 100P60 when compared on the basis of equal amounts of rhamnose injected (Fig. 5). The differences in the severity of arthritis induced by these preparations were most easily observed during the first 14 d ( $P < 0.01$ ) and between day 52 and day 60 ( $P < 0.05$ ). The arthropathic activity of the filtered 100P60 was significantly increased when it was mixed with the edema-producing PS before injection (Fig. 6).

TABLE IV  
*Vascular Permeability Changes after i.v. Injection of Group A PS and PBS Control*

Tissue	<sup>125</sup> I-HSA (cpm/g)*		
	PBS	PS	
Blood	15,728 ± 1,815‡	10,427 ± 2,741	<i>P</i> < 0.01
Limbs			
Right front	1,134 ± 176	6,470 ± 1,620	<i>P</i> < 0.001
Left front	1,172 ± 296	6,056 ± 833	<i>P</i> < 0.001
Right hind	1,062 ± 200	3,120 ± 504	<i>P</i> < 0.001
Left hind	990 ± 190	3,580 ± 155	<i>P</i> < 0.001
Heart	4,710 ± 1,318	3,641 ± 792	NS§
Lung	3,976 ± 517	3,646 ± 941	NS
Spleen	1,978 ± 162	1,949 ± 412	NS
Liver	3,374 ± 438	3,248 ± 580	NS
Thymus	860 ± 378	788 ± 151	NS
Kidney	5,004 ± 678	4,506 ± 557	NS
Skin (flank)	971 ± 112	1,022 ± 175	NS
Muscle (trapezius)	451 ± 82	571 ± 162	<i>P</i> < 0.05
Submandibular lymph node	2,064 ± 280	3,981 ± 1,637	<i>P</i> < 0.05
Mesenteric lymph node	2,269 ± 161	1,724 ± 313	<i>P</i> < 0.01
Ascending colon	951 ± 136	782 ± 133	<i>P</i> < 0.05

\* Mean ± standard deviation (five rats), 30 min postinjection.

‡ cpm/ml.

§ Not significant.

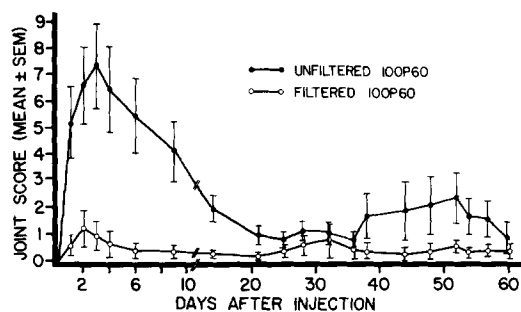


FIG. 5. Arthropathic activity of an unfiltered and a filtered 100P60 fraction of PG-PS. Each 100P60 preparation was injected i.p. into a group of 10 rats at 12.75  $\mu$ g rhamnose/gbw. The average score of arthritis from all 10 rats in the group is expressed as mean joint score  $\pm$  standard error of the mean. The level of significance between the two groups was calculated for each time point by Student's *t* test. (●) unfiltered 100P60; (○) filtered 100P60.

### Discussion

The group-specific PS isolated from group A streptococcal cell walls selectively induces a rapid but transient edematous response in the limbs of rats that is not detected in other tissues examined. The time-course of edema depends on the route of injection. To our knowledge, this is the first documented report of a biologic activity of the group A PS. The PS preparations used in these studies contained <1% (wt/wt) total amino acids and did not contain muramic acid detectable by GLC or amino acid analyzer. Therefore, contamination with PG (if any) and other peptides was minimal. Other evidence to support the idea that the activity is associated with the PS include the findings that isolated PG fragments did not induce edema and that



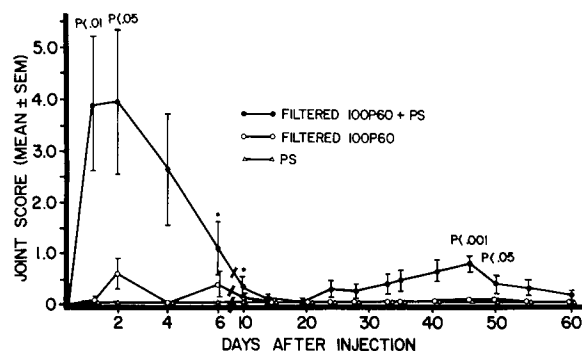


FIG. 6. Enhancement of arthropathic activity of the filtered 100P60 by polysaccharide. A sample containing the filtered 100P60 (10  $\mu\text{g}$  rhamnose/gbw) and group A PS (15  $\mu\text{g}$  dry wt/gbw) was injected i.p. into each of 10 rats. The arthritis (●) was scored and expressed as described in Fig. 5. Each of the 10 rats in the 100P60 control group (○) was injected with the filtered 100P60 in a dose of 10  $\mu\text{g}$  rhamnose/gbw, and each of the 10 rats in the PS control group (Δ) received the group A PS in a dose of 15  $\mu\text{g}$  dry weight/gbw. The level of significance was calculated only when both the experimental and control groups showed joint inflammation. At time points indicated by asterisks, the differences between the experimental and the control group were not significant.

the edema-producing activity was markedly reduced by glucosaminidase. This effect of glucosaminidase, combined with absence of activity of group A variant PS, indicates that the NADG epitope is a critical structure for this property.

Morisaki et al. (24) reported that mutanolysin digests of group A streptococcal cell wall (which was rich in group A carbohydrate) could activate murine polyclonal B cells. Because the mutanolysin digests consist of PG-PS complexes (15, 24), and isolated PG has been described as a polyclonal B cell activator (25), it is difficult to ascribe the mitogenic activity to the PS. Recently, it was suggested that the PS derived from *Streptococcus pyogenes* OK-432 augmented natural killer activity of human peripheral blood lymphocytes in vitro (26). The chemical compositions of the PS used in their study was not reported; thus, the possibility of PG and other cell wall and/or membranous contaminants in the PS preparation cannot be excluded.

Our findings suggest that the edema-producing PG-PS and PS polymers might play a role in the pathogenesis of cell wall-induced polyarthritis, although they are not themselves arthropathogenic. The PS caused a selective increase in vascular permeability in the limbs, and this vascular change might be the initial event that allows localization of the arthropathic cell wall fragments in the synovial tissue. Our speculation is based on the finding that the edema-producing PS increased both the incidence and the severity of arthritis induced by the 100P60 (Figs. 5, 6). Experiments are in progress to examine the effect of the PS on the distribution and degradation of arthropathic cell wall fragments in rat tissues. These experiments involve the use of solid-phase radioimmunoassays that were recently developed in this laboratory (27).

The edematous response is transient and limited to a serous exudate with no evidence of erythrocyte or leukocyte movement. Whether this effect on vascular endothelium of the joints is a direct action of PS or involves activation of inflammatory mediators is unknown at this time. The remarkable selectivity for the limbs suggests that a specific receptor for the PS may be located in the synovial or the periarticular vascular endothelium. Blood vessels of similar size and structure in different tissues

have been shown to vary also in their response to histamine-type permeability factors (28-31).

### Summary

Edematous responses were induced in the limbs of Sprague-Dawley rats by intravenous, intraperitoneal, or intra-articular injections of group-specific polysaccharide (PS) isolated from the cell walls of group A streptococci. After intravenous injection of the edema-producing PS, vascular permeability increase (measured by  $^{125}\text{I}$ -human serum albumin) was detected in the limbs, but not in the heart, lungs, spleen, liver, thymus, kidney, skin, skeletal muscle, submandibular lymph nodes, mesenteric lymph nodes, or ascending colon. This indicates a selective effect on vascular endothelium of the joints. Evidence to suggest that the edema-producing activity of the PS might play an important role in the pathogenesis of cell wall-induced polyarthritis included the following: (a) the presence of edema-producing activity in arthropathogenic cell wall preparations; (b) cell wall preparations without edema-producing activity were significantly less active in inducing arthritis than were those which contained edema-producing activity; and (c) the addition of edema-producing PS to cell wall preparations increased both the incidence and the severity of arthritis.

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