RELATION OF PARTICLE SIZE OF C POLYSACCHARIDE COMPLEXES OF GROUP A STREPTOCOCCI TO TOXIC EFFECTS ON CONNECTIVE TISSUE*

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A single intradermal injection of a sterile extract of sonic disrupted Group A streptococci has been shown to produce a chronic remittent and intermittent lesion of the dermal connective tissue of rabbits (1).

Histological studies reveal areas of fibrinoid necrosis and an associated granulomatous reaction (2). There is no evidence that hypersensitivity is responsible for the lesions, but rather the material appears to have a direct toxic effect on the connective tissue (2). The recurrence of acute lesions as late as 80 days after a single injection of this material indicates that it persists in tissue in an active form for a considerable time. The cellular component responsible for this lesion has been isolated by physical methods and shown to be a macromolecular complex of the group specific C polysaccharide and a peptide (3). Antibodies specifically directed against the polysaccharide will neutralize the toxic activity (4). A variety of other evidence confirms the importance of the C polysaccharide in this process (1, 3).

In the course of these studies it became apparent that although the C polysaccharide moiety is essential for this toxic manifestation, not all fragments containing this substance can produce this remarkable lesion. The capacity to initiate the reaction and bring about the acute exacerbations appears to be related to either the physical state or some chemical associations of the C polysaccharide. The present studies were undertaken to establish the minimal unit of the polysaccharide complex having complete toxic activity, and to clarify the relationship between toxicity and size or configuration of the polysaccharide complex. The experimental results allow some speculation on a mechanism of tissue damage not previously associated with microorganisms.

Materials and Methods

Collection of the Cells.—The organism used in these studies was a Group A, Type 1 streptococcus. Culture methods have been previously described (1, 3). Cells from an 18 hour Todd-

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Hewitt broth culture were collected by centrifugation, washed two times with cold saline, and resuspended in water or phosphate buffer, pH 7.0, $\Gamma/2 = 0.1$, depending upon the subsequent method employed.

Preparation of Cell Walls.—The washed cells from 2 to 3 liters of culture, suspended in water to a total volume of 10 ml., were added to 10 cc. of small glass beads.[1] This mixture was vibrated at maximum amplitude for 25 minutes in a Raytheon 9 kc. sonic oscillator. Beads and vibrated cell material were separated by allowing the beads to settle, removing the supernate, and washing the beads with small aliquots of water which were added to the supernate. Cells and cell walls were sedimented by centrifugation at 10,000 R.P.M. for 30 minutes in a Spinco No. 30 rotor. The supernate, which contained most of the soluble cytoplasmic components and very little cell wall material as indicted by rhamnose determination (see Table I), is referred to as supernate from initial disruption of cells. A rough separation of cell walls from whole cells was made by rinsing the top layer from the 10,000 R.P.M. sediment. The top layer was suspended and centrifuged two additional times, with a similar separation of the layers, to yield a pellet composed almost entirely of whole cells and a suspension of cell walls with few whole cells. This cell wall suspension was sonic-vibrated for 10 to 30 seconds to produce uniform dispersion and then sedimented through a sucrose gradient (5). The gradient was prepared by layering solutions of sucrose with decreasing densities in a 40 ml. centrifuge bottle and placing in the cold for 16 hours. The specific gravity of each successive layer of sucrose was: 1.30 (1.5 ml.), 1.25 (4 ml.), 1.20 (4 ml.), 1.15 (4 ml.), 1.10 (4 ml.), 1.05 (4 ml.), 1.025 (4 ml.). An aliquot of the cell wall suspension was then layered on top, and centrifuged at 2,700 R.P.M. for 1¾ hours in an International refrigerated centrifuge with a No. 269 rotor, employing controlled acceleration and deceleration according to the method of Anderson (6). Several distinctly visible bands developed, and samples of each of these were examined with the electron microscope and phase microscope. The top band containing "granules" was removed. The broad middle band containing cell walls was collected, sedimented at 36,000 *G* (17,500 R.P.M., Spinco No. 30 rotor), washed 3 times with water and 3 times in neutral phosphate buffer. The latter washing has been shown to remove ribonucleic acid (7). This procedure has been shown to yield a suspension of cell walls displaying a high degree of purity by various criteria (5).

Preparation of Extracts of Whole Cells.—Washed cells, suspended in pH 7.0 phosphate buffer, $\Gamma/2 = 0.1$, were exposed to maximum vibration in a Raytheon oscillator without glass beads for 1 hour. A crude extract was prepared by centrifugation of this suspension at 36,000 *G* for ½ hour and filtering through an 02 Selas filter (1). Centrifugation of this fraction at 75,000 *G* for 1 hour has been shown to sediment the fraction containing the most potent lesion-producing activity and permit removal of most of the solubilized protoplasmic constituents. This partially purified extract is designated according to the terminology previously used (3). The letter *p* refers to the sediment; the prefixed number, the sedimentation force in 1000 *G*; the suffixed number, the time in minutes of the centrifugation; and the number after the hyphen, the number of such centrifugations. Following treatment with the proteolytic enzymes trypsin, chymotrypsin, and papain, the suspension was further subdivided by centrifugation through a discontinuous sucrose gradient. Attempts at further deproteinization included shaking with chloroform, according to the method of Sevag (8), and shaking with $Zn(OH)_2$ (9).

Estimation of Lesion-Producing Activity.—Each preparation was injected intradermally into rabbits weighing approximately 2 kg., and the area around the injection site was observed for a minimum of 45 days. In order to provide an estimate of the lesion-producing activity of each preparation, an index was calculated by dividing the maximum area of the lesion in

[1] Flexolite No. 18, average diameter 70 microns. Flex-O-Lite Manufacturing Corp., St. Louis.

square millimeters by the number of days after injection required for the gross appearance of the nodular lesion. The use of the index as a measure of biological activity has been reported (3), and is based on the observation that dilutions of a single extract plotted against the indices approximate a straight line. The index relative to the nitrogen or rhamnose content was calculated to permit comparison of relative toxicity and purity of each fraction.

Chemical Analyses.—Rhamnose was determined by the method of Dische and Shettles (10). Nitrogen determination was based on a modified micro-Kjeldahl technique utilizing Nessler's reagent (11). The Elson-Morgan method, as modified by Johnston *et al.* (12) was used for hexosamine estimation. Phosphorus was determined by a modification of the Fiske and SubbaRow method using heat and increased acid concentration during color development (13).

RESULTS

Relationship between Particle Size and Toxicity: Studies with Cell Walls.—The group-specific C polysaccharide is an essential part of the toxic complex. Since it is also a major component of the cell wall, it follows that purified cell walls should provide an advantageous starting material for studying the nature of this toxic substance. In the process of collecting cell walls for this study, three basic fractions were obtained: the supernate after the initial cell disruption and centrifugation at 10,000 R.P.M., which contained most of the cytoplasmic material and little cell wall material; cell walls, which were free of whole cells, and appeared quite homogeneous by electron microscopy and electrophoresis (5); and a fraction of electron-dense "granules," which are composed of a significant amount of cell wall material (5).

Intradermal injection of a suspension of the large cell walls, as well as the "granule" preparation, does produce a dermal lesion of moderate dimensions as indicated in Fig. 1. However, considerably greater toxic activity is observed, on a weight basis, if the suspensions are first dispersed by sonic vibration. Therefore, a suspension of cell walls was prepared for injection by sonic vibration for 2½ hours, followed by centrifugation for 15 minutes at 10,000 R.P.M. in a Spinco SW 39 rotor to remove remaining large fragments. The supernate was injected intradermally into rabbits in a dosage of 100 μg. (dry weight). The supernate from the initial glass bead disruption of the cells, and a portion of the "granules" which had been dispersed by sonic vibration, were injected also in comparable doses.

The results are recorded in Table I, which shows that the capacity to produce the connective tissue lesion is derived only from cell walls or the chemically comparable but morphologically distinctive "granules." The supernatant from glass bead disruption failed to display lesion-producing activity at this dosage, which is consistent with its very low concentration of cell wall polysaccharide.

Particles of Varying Complexity Obtained from Cell Walls.—In an attempt to establish the basic toxic unit, the observations on the effect of dispersion relative to lesion-producing activity were extended. Cell walls were used as the starting material for preparing a series of fractions containing the C poly-

saccharide in varying degrees of complexity. This suspension of cell walls had been washed with neutral phosphate buffer to remove adhering ribonucleic acid.

The first reduction in cell wall mass was accomplished by treatment with papain, which removed 45 to 50 per cent of the nitrogen associated with the cell wall, without producing apparent morphological changes (5). Approximately 150 mg. (dry weight) of cell walls, suspended in 10 ml. phosphate buffer, pH 7.0, $\Gamma/2 = 0.01$, were incubated for 5 hours at 37°C. with 2 mg. reduced papain and a few drops of chloroform as a preservative. Following this treatment, cell walls were washed 3 times by repeated centrifugation at 36,000 *G* for 30 minutes.

TABLE I

Lesion-Producing Activity of Fractions Derived from the Group A Streptococcal Cell

Fraction	Dry weight injected	Rhamnose	N	Index*
	μg.	μg.	μg.	
Cell walls‡	100	36.3	7.5	128
"Granules"‡	100	32.5	11.5	72
Supernate after initial disruption of cells with glass beads and centrifugation at 10,000 R.P.M.	100	1.14	9.2	0

* Index refers to maximum area of lesion in millimeters divided by time in days required for gross appearance.

‡ Dispersed by sonic vibration, centrifuged at 10,000 R.P.M. 15 minutes in Spinco No. SW 39 rotor, and supernate injected.

To achieve solubilization of the cell wall, a suspension of the papain-treated walls, in water, was subjected to sonic vibration for 3 hours. The vibrated material was centrifuged in a Spinco No. 40 rotor at 10,000 R.P.M. for 30 minutes to remove large fragments of the wall. Rhamnose determinations have shown that approximately 75 per cent of cell wall material remains in suspension (5).

Preparations still less complex were obtained by treatment of the cell walls with filtrates of *Streptomyces albus* culture, which as reported by McCarty (14) contain a group of enzymes, one of which is capable of reducing the polysaccharide to much simpler units. Equal volumes of the filtrate and a cell wall suspension sufficiently concentrated to produce an O.D. of 0.6 at the final dilution, were mixed and incubated at 37°C. until no further reduction in optical density could be detected. A pH of 7.8 was maintained.

Finally, the C polysaccharide hapten, containing approximately 5 per cent N, was prepared by Fuller's formamide extraction technique (15).

Fig. 1. graphically compares the lesion-producing activities of this spectrum of cell wall fractions relative to their nitrogen or rhamnose content. Increased levels of activity were produced by removal of protein material from the cell wall by treatment with papain. Fragmentation by sonic vibration further in-

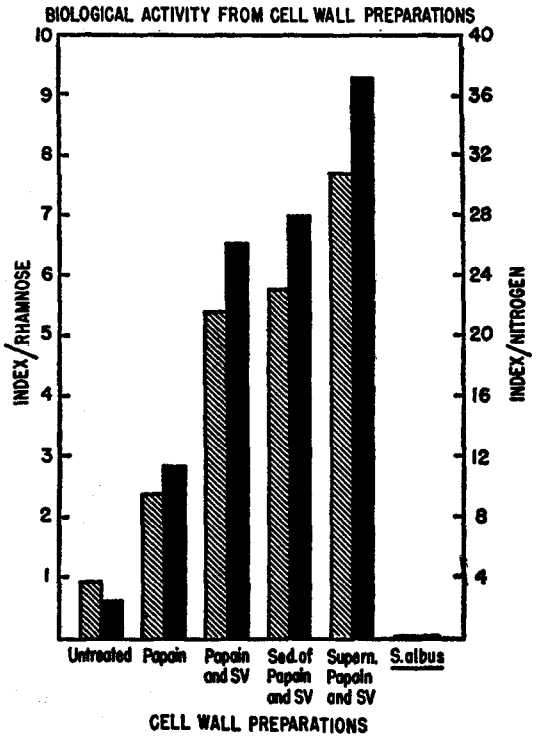

FIG. 1. Lesion-producing capacities of cell wall fractions in varying states of complexity. The shaded bar represents the index relative to the rhamnose content; the solid bar, relative to the nitrogen content. From left to right the fractions shown are: (*a*) untreated cell walls with associated protein; (*b*) papain-treated cell walls; (*c*) cell walls, papain-treated, sonic vibrated 2½ hours; (*d*) cell walls, papain-treated, sonic vibrated 3 hours, sediment of centrifugation at 11,500 *G* for 15 minutes; (*e*) the supernate from the preceding centrifugation; and (*f*) papain-treated cell walls, sonic-vibrated, exposed to filtrates of *S. albus*.

creased activity. Results obtained on injection of fractions treated with filtrates of *S. albus* demonstrated that simplification beyond limits will lead to reduction and finally to loss of activity. Cell walls incubated with filtrates of this culture until no further reduction in optical density could be detected, either failed to produce lesions or caused minimal lesions which appeared very late. Following formamide extraction, preparations consistently failed to produce lesions.

Studies on the "Granule" Fraction.—The appearance of "granules" in prepa-

rations of disrupted cells has been noted by others (16). These may represent artifacts of processing. The separation of these morphological entities provides a means for objective analysis. Although their morphology in the electron microscope and their density are obviously distinctive, the chemical data in Table I showing a slightly lower rhamnose to nitrogen ratio suggest they are cell wall fragments. The lesion-producing activity of the "granule" suspension was compared to that of cell walls following enzyme treatments. As shown in Table II, cell walls and "granules" show parallel changes in activity.

Particles of Varying Complexity Derived from a Fraction of a Whole Cell Extract.—The studies thus far presented on toxic activity derived from cell walls, reveal an interesting relationship between particle size or complexity

TABLE II

Effect of Papain and S. albus Filtrate on Activity of Cell Walls and "Granules"

Preparation	$\frac{\text{Index}}{\text{Nitrogen}}$	$\frac{\text{Index}}{\text{Rhamnose}}$
Cell walls, untreated	2.4	0.94
Cell walls, papain	11.4	2.37
Cell walls, papain and *S. albus*	0.0	0.0
"Granules," untreated	2.8	0.94
"Granules," papain	7.0	2.45
"Granules," papain and *S. albus*	0.0	0.0

and ability to produce the nodular lesion of dermal connective tissue. As an alternative approach to this study, a fraction of the extract of whole cells disrupted by sonic vibration without glass beads was used as a starting material, instead of the isolated cell walls. Previous studies on differential centrifugation of a sonic extract of Group A streptococcal cells demonstrated that, in extracts prepared in this manner, most toxic activity could be sedimented at 75,000 *G* in 1 hour in the Spinco No. 40 rotor. The sediment obtained was referred to as 75p60 (3). This fraction, repeatedly washed, contains mainly cell wall fragments. The 75p60 fraction so obtained was subjected to a procedure based on rather subtle differences in density, designed to separate the particles of varying configuration or complexity and to relate these differences to toxicity.

The proteolytic enzymes pepsin, trypsin, chymotrypsin, and papain have previously been shown not to reduce toxic activity (3). A 75p60 fraction, washed three times with neutral phosphate buffer, was treated with a combination of trypsin and chymotrypsin at pH 8.5, followed by treatment with papain at pH 7.0. The treated extract was centrifuged through a discontinuous sucrose gradient. For this separation sucrose solutions of densities 1.15 (1.25 ml.), 1.10 (1.25 ml.), and 1.05 (1.5 ml.) were layered in cellulose centrifuge tubes,

overlaid with 1 ml. of the enzyme-treated fraction (3 to 5 mg. dry weight), and centrifuged at 30,500 R.P.M. for 45 minutes in a Spinco SW 39 rotor. Fractions were collected by puncturing the tubes and aspirating the clearly visible bands into a syringe. Following dialysis against six changes of water, of five liters each, sucrose sedimentation showed that the fractions again sedimented to the same level from which they were collected, demonstrating that actual separation of components with different sedimentation characteristics had been achieved. Table III, showing the toxicity of these fractions following intradermal injection into rabbits, demonstrates that the heterogeneity in density reflects pronounced differences in activity between fractions of different density. The notation 1.10/1.15 represents the fraction collected

TABLE III

Relative Lesion-Producing Activity of Sucrose Gradient Fractions from Enzyme-Treated 75p60-4 Preparation

Fraction	Index / Nitrogen	Index / Rhamnose
75p60-4*	5.0	2.2
Sediment	5.4	1.5
1.10/1.15	20.1	6.0
1.05/1.10	6.65	4.0
Supernate	0.4	5.4

* Initial preparation was the 75p60-4 fraction. Other fractions are designated according to the approximate location in the sucrose gradient.

between sucrose densities of 1.10 and 1.15. Most significant is the observation that a fraction of intermediate density is the most active.

Studies on the Nature of the Minimal Polysaccharide Complex Possessing Complete Toxicity: Chemical Deproteinization.—Further deproteinization of the 1.10/1.15 sucrose fraction from the previous experiment was attempted by shaking with chloroform according to the procedure of Sevag (8). The material recovered from this procedure was further treated by shaking with a freshly prepared suspension of $Zn(OH)_2$ (9). A concentration of approximately 5 per cent wet volume of $Zn(OH)_2$ at near neutral pH was used. Traces of zinc ions were removed from this treated fraction by repeated centrifugal washings following precipitation of the polysaccharide with acidified ethanol.

In addition, a fraction of cell walls dispersed by sonic vibration was treated with phenol according to the procedure outlined by Westphal *et al.* (17) for the isolation of lipopolysaccharides from Gram-negative bacteria. The cell wall fraction was heated at 65°C. for 10 minutes in a mixture of phenol-water (1:1, weight/volume). On cooling, the aqueous layer containing the polysaccharide

was separated from the phenol, and residual phenol was removed with ether. Traces of the ether were removed under vacuum.

Both deproteinized fractions, the 1.10/1.15 fraction from whole cells and the cell wall suspension, were found to contain approximately 6.3 times as much rhamnose as nitrogen. Table IV shows the activities of these fractions before and after treatment. The results show that these attempts to remove the protein remaining after enzyme treatment do not significantly alter lesion-producing activity.

Effect of Periodic Acid on Toxic Activity.—To provide more direct chemical evidence to support the importance of the carbohydrate to the activity of the complex, the sucrose 1.10/1.15 fraction was treated with periodate. The selectiveness of this material for the oxidation of polyhydroxy compounds, has

TABLE IV

Effect of Chemical Deproteinization on Toxicity of Enzyme-Treated Fractions

Fraction	Index / Nitrogen	Index / Rhamnose
1.10/1.15 fraction	26.0	5.9
1.10/1.15 fraction, deproteinized*	20.1	3.9
Vibrated cell walls	25.2	5.2
Vibrated cell walls, deproteinized‡	30.7	6.2

* Deproteinized by shaking with chloroform.

‡ Deproteinized by heating with phenol.

made this an important reagent for the study of polysaccharides (18). The fraction was incubated for 30 minutes at 37°C. with sufficient periodic acid to give a final concentration of 0.002 M. The reaction was halted by the addition of 1/9 volume of a 5 per cent solution of sucrose to the reaction mixture. After dialysis for 48 hours against distilled water, this fraction, which gave a positive Schiff's test, and an untreated control similarly diluted, were injected into rabbits. The results are given in Table V. The positive Schiff's test following treatment and dialysis is strong evidence that oxidation of the polyhydroxy units within the polysaccharide had occurred. The results indicate that this reaction markedly reduced lesion production and serve to illustrate further that the polysaccharide is an essential component of the toxic macromolecule.

Analysis of Degradation of the Active Complex by S. albus Filtrates.—Assuming that the basic unit of toxicity consists of a macromolecular complex composed of subunits of the group-specific polysaccharide, the enzymatic degradation of a toxic preparation by filtrates of the *S. albus* culture was examined more critically. Table VI shows that the relative lesion-producing activity of a fraction so treated can be markedly reduced. The rate of change

in optical density observed during treatment of this fraction was initially rapid, after which turbidity reduction continued, but the rate was markedly reduced.

While these results have been frequently observed, they are not always predictable. Occasionally, fractions obtained from whole cell extracts and treated with *S. albus* filtrates show an initial reduction in turbidity followed by a rapid increase. Examination by phase microscopy of a sample removed during the period in which turbidity was increasing, showed particulation. Related to these changes in optical density is the alteration in lesion-producing activity. Aliquots of a 75p60-4 fraction from sonic vibrated whole cells undergoing *S.*

TABLE V

Effect of Limited Periodic Acid Treatment on Toxic Activity

Fraction	Index	$\frac{\text{Index}}{\text{Nitrogen}}$
Control	291	26
Periodate-treated	34	0.8

* A partially purified extract of whole cells, the 75p60-4 fraction, was the toxin preparation used.

TABLE VI

Effect of S. albus Filtrate on Activity of the 75p60-1 Fraction

Sample	$\frac{\text{Index}}{\text{Nitrogen}}$	$\frac{\text{Index}}{\text{Rhamnose}}$
75p60-1	3.6	11.8
75p60-1 + *S. albus* filtrate	0.1	0.37

albus treatment were removed after the initial reduction in optical density. After turbidity had increased, an additional aliquot was removed. By way of comparison, a preparation of cell walls and a preparation of "granules," both containing rhamnose concentrations comparable to that of the 75p60-4 fraction, were treated with equal volumes of the enzyme filtrate. Aliquots were removed at varying intervals. Fig. 2 shows a comparison of the lesion-producing capacities of these fractions as a percentage of the untreated controls. The aliquot of the 75p60-4 fraction removed after the initial reduction in turbidity, showed a drop in lesion-producing activity. However, aliquots removed after the sharp increase in turbidity, showed levels of activity only slightly reduced from the original index. These observations suggest that a portion of the 75p60-4 fraction is reduced initially by the enzymes in the filtrate beyond critical limits of complexity required for optimal activity, but by an undefined reassociation of these units, a portion of the lesion-producing capacity can be restored. It was

consistently observed that the toxic effect of both the cell wall and granule preparations was reduced as treatment was continued.

Chemical Analysis of the Most Active Fraction Obtained by Sucrose Zone Centrifugation.—The chemical composition of the 1.10/1.15 fraction from discontinuous sucrose gradient fractionation is given in Table VII. This frac-

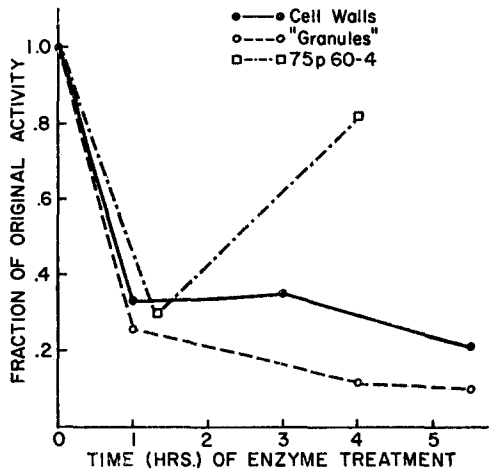

FIG. 2. Comparison of the effect of *S. albus* enzymes on lesion-producing activity of vibrated cell walls, cellular "granules" and a 75p60-4 fraction from a whole cell extract. The 75p60-4 fraction exhibited an increase in optical density after an initial reduction.

TABLE VII

Relative Composition of the 1.10/1.15 Fraction Derived from Extract of Sonic Disrupted Cells

Component	Per cent of dry weight	Mole ratio
Nitrogen	7.1	15.74
Rhamnose	39.0	6.63
Hexosamine	24.4	3.83
Phosphorus	1.0	1.00

tion derived from the whole cell extract, represents the best definition thus far of the fundamental unit of lesion-producing activity. Although the nitrogen and phosphorus content of these preparations is only slightly reduced from that observed by Schwab *et al.* (3) in the most active component separated by countercurrent electrophoresis, it may be noted that the rhamnose and hexosamine values are increased by approximately 50 per cent. This relative increase appears to be directly related to the enzymatic removal of protein. The value obtained for hexosamine by the Elson-Morgan reaction includes the

probable contribution of muramic acid (19) to the reaction which has been identified by others as being a component of the C polysaccharide (20). Although the absence of lipid cannot be concluded from this analysis, no evidence has been found to suggest the presence of these elements on repeated extraction with acidified ethanol-ether. The ultraviolet absorption spectrum shows essentially only terminal absorption in the far ultraviolet with no peak absorption around either 280 or 260 mμ.

DISCUSSION

The evidence presented here and in other reports clearly establishes the essential role of the group-specific C polysaccharide in the production of the

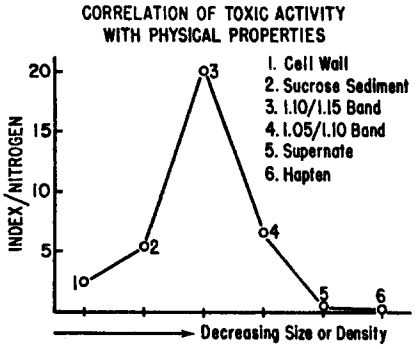

FIG. 3. Diagrammatic representation of the relationship of particle size to toxicity. Points on abscissa arbitrarily spaced. Fractions: (1) untreated cell walls; (2) to (5) fractions obtained from an enzyme-treated 75p60 preparation by discontinuous sucrose gradient fractionation, arranged according to decreasing size or density; and (6) C polysaccharide extracted with hot formamide.

chronic, remittent multinodular lesion of connective tissue. This evidence may be summarized as follows: (*a*) correlation of activity with rhamnose in particulate material (reference 3, this report); (*b*) derivation of activity from purified cell walls (this report); (*c*) failure of various proteolytic enzymes, ribonuclease, and chemical deproteinization to destroy activity (reference 3, this report); (*d*) destruction of activity by periodic acid oxidation (this report); (*e*) destruction of activity by *S. albus* filtrates (this report); (*f*) destruction of activity with a bacterial glucosaminidase (21); (*g*) neutralization by antibodies specific for C polysaccharide (4).

It is apparent, however, that not all forms of the C polysaccharide are capable of injuring connective tissue, since a fragment sufficiently complex to be considered a complete hapten has no observable effect when injected intradermally. The data accumulated thus far have not allowed a final defini-

tion of the minimal unit possessing complete toxic activity. This report demonstrates that the capacity to produce the chronic lesion of connective tissue is limited to a relatively narrow range of particle size by the C polysaccharide complex. This is very clearly illustrated by the studies on a spectrum of particles derived either from purified cell walls or from a fraction of a sonic extract of whole cells, as summarized in Fig. 3. Maximum activity, on a weight basis, is associated with particles of intermediate size, in a spectrum extending from large cell wall fragments to the soluble hapten with a molecular weight of about 8,000 (22).

Any explanation of this association between size of the polysaccharide-containing fragment of the cell wall and the ability to injure connective tissue is highly speculative on the basis of our present information. The following possibilities warrant further investigation.

The exposure of certain essential configurations or combining groups as dispersion proceeds up to a critical point is one explanation which is being considered. The reduction in activity accompanying further dispersion would reflect the removal or alteration of such groups.

An alternative concept considers the colloidal properties which are associated with particles of certain size (23). In this concept the large cell wall fragments, and the soluble polysaccharide, lack essential properties associated with the intermediate C polysaccharide complexes having colloidal dimensions. Two observations are consistent with this possibility. Chemical analyses have demonstrated no qualitative, and only moderate quantitative, differences between the composition of fractions differing in relative toxicity. Secondly, the increase in toxicity following the development of particulation or increased optical density during the course of exposure to *S. albus* filtrates, suggests a requirement in addition to chemical composition or even configuration.

It is also well known that numerous substances assume vastly different physical and biological properties as their state of polymerization changes (23). Studies on the toxic properties of silica are very interesting in this regard, as indicated by Dale and King (24). These authors also suggest that the silicic acid which dissolves from the surface of quartz particles in the tissues becomes in part colloidal and this colloidal silicic acid may be responsible for silicotic lesions. The ability of silicic acid to bring about agglutination, etc., of red blood cells is also related to the polymers of silicic acid which are of a size possessing colloidal properties (25).

It is conceivable that the surface or colloidal properties associated with certain C polysaccharide complexes of appropriate size, could have great influence on the reaction with biocolloids of connective tissue. It is also possible that the size or physical state of the polysaccharide material influences the rate of its removal from tissue and in part accounts for the long duration of the inflammatory process induced by its presence. Thus, the small C polysaccharide molecule studied by Schmidt (22) is very rapidly excreted following injection into the mouse; whereas, it is probable that large cell wall fragments are readily phagocytized. The C polysaccharide complex of inter-

mediate size, which in addition is a poor antigen under natural conditions (4), may thus be well suited to remain in tissue for a relatively long period of time.

SUMMARY

The component of Group A streptococci which is responsible for the chronic, remittent, multinodular lesion of connective tissue is derived from the cell wall. Further evidence is given to support the essential role of the group-specific C polysaccharide in the production of this lesion.

A series of particles containing the group-specific C polysaccharide was prepared, ranging in size from large cell wall fragments to the relatively small hapten. A comparison of the lesion producing capacity of the particles in this spectrum revealed that maximum toxic activity is associated with C polysaccharide complexes of intermediate size. The discussion considers colloidal properties associated with C polysaccharide complexes of a certain size, and the influence particle size has on persistence in tissue, as possible explanations of the relationship between the size of the C polysaccharide complex and its ability to produce the chronic lesion of connective tissue.

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