THE ENDOTOXIC PROPERTIES OF LYSATES OF GROUP A HEMOLYTIC STREPTOCOCCI*

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Plates 68 and 69

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The bacterial endotoxins are generally considered to be products of Gram-negative species and to be closely associated, if not identical, with the somatic antigens of these bacteria (1, 2). This concept may require revision, however, in view of the evidence that streptococci may elaborate materials which possess certain of the properties of endotoxins.

While there has been no clear demonstration of the production of endotoxins by these or other Gram-positive bacteria, the capacity of suspensions and culture filtrates of streptococci to produce fever (3–5) and the Shwartzman phenomenon (6, 7) has been recognized for some time. Duval and Hibbard (8) reported that hemolytic streptococci contained an endotoxin which produced fever and toxemia on injection into normal rabbits. However, this material was prepared by the intraperitoneal injection of large numbers of living cocci into immunized animals, and it is not certain that the toxic properties of the resulting peritoneal fluid derived from the injected bacteria. The endotoxin prepared by Green (9) by extraction of disrupted streptococci produced, in normal and rheumatic human subjects, local and systemic reactions which may, however, have been due to the existence of delayed type hypersensitivity to streptococcal antigens in these subjects.

It has recently been found in this laboratory that mechanical disruption of group A beta hemolytic streptococci yields crude lysates which produce the characteristic effects of endotoxin when injected into normal rabbits. Attempts to fractionate these lysates and to identify the active material are now under way. The present communication is concerned with a description of some of the biological effects of these lysates and a comparison between these effects and those produced by Gram-negative bacterial endotoxins.

Materials and Methods

Rabbits.—Hybrid albino rabbits, each weighing approximately 1.5 kilos, were maintained on a standard pellet ration which did not contain an antibiotic supplement.

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Streptococci.—Strains of group A streptococci belonging to the serological types 1, 3, and 12 were used. The type 1 strain was obtained from Dr. Alan Bernheimer and the types 3 and 12 strains from the Streptococcal Diseases Laboratory, Cheyenne.

Streptococcal Lysates.—The streptococci were grown in 2 liter flasks containing 1 liter of neopeptone infusion broth. After 24 hours, the streptococcal cells were harvested by centrifugation and washed three times with sterile pyrogen-free distilled water. The washed cells were then suspended in approximately 10 times their packed volume of distilled water, killed by exposure to 65°C. for an hour, and subjected to disruption in the "Mickle" disintegrator (10) for 30 minutes. The glass beads used in the disintegrator were superbrite No. 112, obtained from the Minnesota Mining and Manufacturing Co., St. Paul; before use, they were cleaned in dichromate–sulfuric acid solution, thoroughly washed in pyrogen-free distilled water, and sterilized by autoclaving.

The suspension of disrupted streptococcal cells was centrifuged at high speed to remove the cell walls and any intact cocci, and the opalescent supernatant solution was stored at 4°C. until used, with merthiolate 1:100,000 as a preservative. The lysates were used without further dilution in all the experiments to be described. The sterility of each preparation was checked periodically, and in no case was contamination with Gram-negative or other bacteria detected. Some of the lysates from type 1 streptococci were kindly prepared by Dr. Angelo Taranta by a slightly different procedure than that outlined above. No appreciable differences were noted between the lysates prepared from the streptococci of types 1, 3, and 12, and the term streptococcal lysates will be used throughout this communication without specific designation of the serologic type involved.

Endotoxins.—Several preparations of "lipopolysaccharide" endotoxin derived from *E. coli* were kindly furnished by Mr. Aaron Lane, Difco Laboratories, Detroit. Two of these, designated 0111 and 0127, were used throughout these experiments; 100 μg. doses were used either for preparation of the skin for the Shwartzman phenomenon or for the intravenous doses.

EXPERIMENTAL

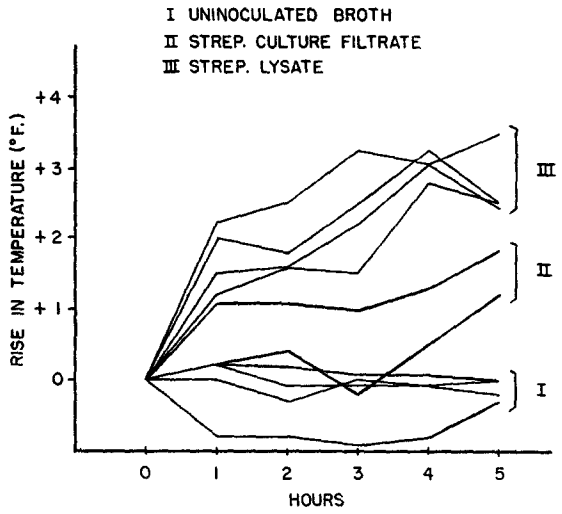
Reactions to Intradermal Injections of Streptococcal Lysates

Following the intradermal injection of streptococcal lysates, delayed local inflammatory reactions appeared in all the normal rabbits tested. These reactions were characterized by erythema, induration, and edema, and reached maximum size and intensity about 24 hours after the injections. In general, the reactions resembled those produced by Gram-negative bacterial endotoxins, except that erythema and edema were somewhat more prominent and the lesions tended to be rather more diffuse. Primary hemorrhagic necrosis (11) was never observed in these lesions, and in animals receiving no further treatment the inflammatory reactions subsided over a period of 2 or 3 days. Histologically, the skin reactions exhibited intense cellular infiltration with polymorphonuclear leucocytes and macrophages, without appreciable evidence of necrosis or irreversible tissue damage.

Intravenous injections of *E. coli* endotoxin given at the height of these skin reactions caused the development, within the next few hours, of the characteristic hemorrhagic necrosis of the local Shwartzman phenomenon (6). This phenomenon could not be produced regularly, but was observed in 11 of the 23 rabbits so treated. Hemorrhage and necrosis could also be produced in the skin

lesions resulting from the injection of streptococcal lysate by local infiltration of the inflamed tissue with epinephrine 1:1000. This effect of epinephrine had been observed earlier (11) in the case of skin lesions induced by endotoxins or by tuberculin in specifically hypersensitive rabbits. Fig. 1 illustrates the severe tissue damage resulting from this procedure. In contrast to the rather low incidence of Shwartzman reactions occurring in lysate-induced skin lesions, the hemorrhagic reactions to epinephrine appeared in greater or lesser degree in all the skin lesions so tested.

It was concluded from these experiments that the skin reactions produced in normal rabbits by the intradermal injection of streptococcal lysates resembled



TEXT-FIG. 1. The febrile response of normal rabbits to intravenous injections of 2.0 ml. amounts of streptococcal culture filtrate or streptococcal lysate. Each line represents a single animal.

those produced by Gram-negative bacterial endotoxins in appearance, in timing, in being prepared for the Shwartzman phenomenon and in their susceptibility to the hemorrhage-inducing effect of locally injected epinephrine.

Reactions to Intravenous Injections of Streptococcal Lysates

Pyrogenic Effect.—In preliminary experiments it had been found that rabbits receiving intravenous injections of suspensions of living or heat-killed streptococci developed pronounced elevations of body temperature. Streptococcal lysates were found to produce a similar effect, and in addition elicited other systemic reactions closely resembling those known to be produced by endotoxins (1). The temperature curves obtained in a typical experiment are presented in Text-fig. 1.

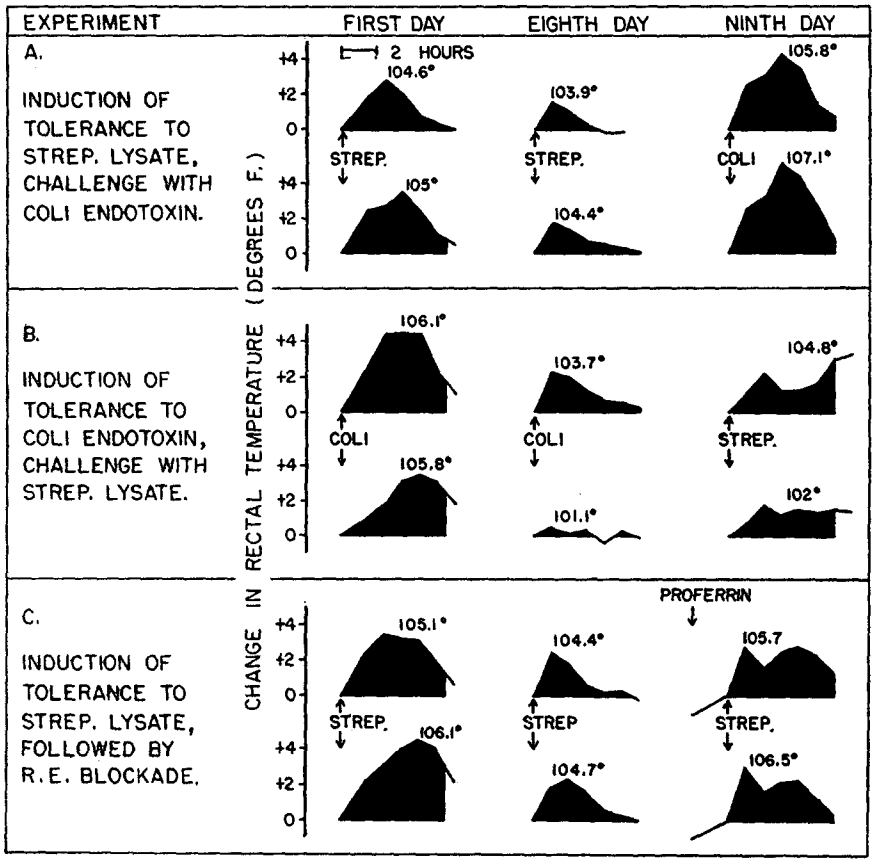
The uninoculated culture medium was non-pyrogenic. The 24 hour culture filtrate produced a mild febrile response, although in similar experiments the culture filtrates were often completely without pyrogenic effect. The rectal temperature following the injection of streptococcal lysate showed a well marked and sustained elevation, comparable to that produced by endotoxins. Both the inoculum and the 24 hour culture were checked for purity in each experiment, and no evidence of contamination could be found. In some experiments in which glass beads of a larger size were used, effective disruption of the streptococcal cells was not achieved, and the supernatant fluid in these cases was without pyrogenic activity. In other experiments, samples of the uninoculated culture medium were subjected to all of the manipulations performed in the preparation of lysates, including shaking in the disintegrator with glass beads, and these remained essentially non-pyrogenic. These experiments indicate that the pyrogenic material in the streptococcal lysates was actually derived from the streptococcal cells, rather than from the culture medium or glassware.

Induced Tolerance to the Pyrogenic Effect.—Beeson (12) found that repeated daily injections of Gram-negative bacterial pyrogens in rabbits produced, after a week or 10 days, a state of non-specific tolerance to these materials. When the streptococcal lysates were tested in this fashion, it was found that there was a steady decrease in the degree and duration of the febrile response to successive injections. In Text-fig. 2 are presented the temperature curves obtained in a representative experiment after the initial and eighth daily injections of streptococcal lysate, together with similar data obtained with *E. coli* endotoxin; it will be seen that a similar degree of tolerance was obtained with both materials.

Animals rendered tolerant to one Gram-negative bacterial endotoxin or pyrogen exhibit diminished febrile responses to pyrogens derived from other Gram-negative bacterial species (12, 13). It was therefore of interest to determine whether the rabbits made tolerant to streptococcal lysate would exhibit cross-tolerance to *E. coli* endotoxin and *vice versa*. To this end, the animals of experiments A and B (Text-fig. 2) were appropriately tested on the 9th experimental day, and it will be seen that the animals made tolerant to streptococcal lysate exhibited no tolerance to *E. coli* endotoxin, while those tolerant to the Gram-negative bacterial endotoxin showed some, but not complete, tolerance to the pyrogenic effect of the streptococcal lysate. Similar results were obtained when these experiments were repeated with two other streptococcal lysate preparations; in each case, the lysate-tolerant animals showed no cross-tolerance to *E. coli* endotoxin, while the endotoxin-tolerant animals exhibited marked to moderate tolerance to the streptococcal lysate.

It seemed possible that this apparently anomalous finding might be explained simply on the basis of dosage relationships. For example, if the amount of pyrogenic material in the streptococcal lysate dose had been equivalent to only 1 μg. of the *E. coli* endotoxin, the rabbits made tolerant to this dose would have been expected to develop the observed febrile response when challenged with 10 μg. of endotoxin (12). On the other hand, animals made tolerant to this latter dose would have been expected not to develop fever after challenge with lysate. In order to clarify this matter, another experiment was performed in which

rabbits received increasing daily doses of *E. coli* endotoxin for 10 days and then five daily doses of 200 μg. each. These animals, which showed almost complete tolerance to these large doses of endotoxin, were then tested with streptococcal

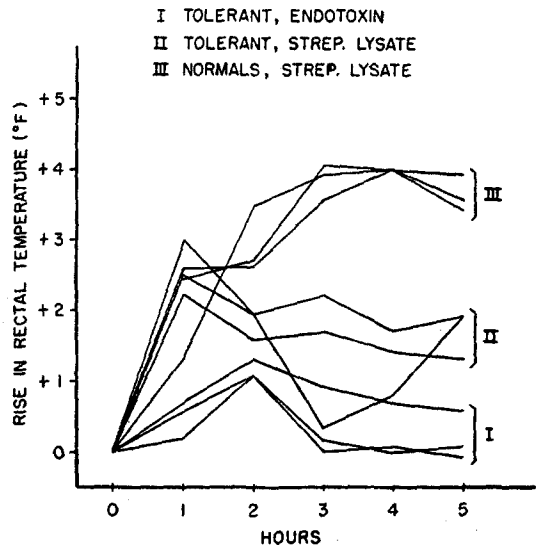


TEXT-FIG. 2. Tolerance to the pyrogenic effect of streptococcal lysate. The two rabbits of Experiment A received eight daily injections of 2.0 ml. of a streptococcal lysate preparation, and the febrile responses to the first and last of these injections are charted; on the 9th day, these animals were given 10 μg. of *E. coli* endotoxin and exhibited normal febrile responses. In Experiment B, the order of these injections was reversed; the two rabbits were given 10 μg. of *E. coli* endotoxin daily for 8 days and were then challenged with 2.0 ml. of streptococcal lysate. The rabbits of Experiment C were treated like those of Experiment A, except that on the 9th day they received intravenous injections of 3.0 ml. of colloidal saccharate of iron oxide 2 hours before a final injection of streptococcal lysate.

lysate and were found to develop distinct temperature elevations (Text-fig. 3). These experiments indicated that only partial cross-tolerance exists between these two pyrogenic substances.

The intravenous injection of certain materials capable of producing reticuloendothelial blockade serves to abolish the state of induced tolerance to Gram-negative bacterial pyrogens (13, 14). In Text-fig. 2 are included data which indicate that colloidal iron saccharate, an effective blockading agent (15), produces some reversal of the tolerance to streptococcal lysate.

Elicitation of the Local Shwartzman Phenomenon.—Intravenous injections of streptococcal lysates in doses similar to those used in the preceding experiments



TEXT-FIG. 3. Cross-tolerance between streptococcal lysate and *E. coli* endotoxin. The curves of group I represent the febrile responses of three rabbits to the last of 15 daily intravenous injections of *E. coli* endotoxin, given in increasing dosage, and the absence of a typical pyrogenic effect in these animals indicates the high degree of tolerance attained. The curves of group II were obtained when these same animals were subsequently given intravenous injections of 2.0 ml. of streptococcal lysate. Although the degree of response of these animals was strikingly less than that of normal control rabbits receiving the same doasge of streptococcal lysate (curves of group III), cross-tolerance was evidently not complete.

were sometimes effective in eliciting the appearance of homorrhagic necrosis in rabbit skin sites which had been injected 24 hours earlier with either streptococcal lysate or *E. coli* endotoxin. When larger doses were used, typical Shwartzman reactions were produced in the majority of animals tested (Table I).

The capacity to elicit the local Shwartzman phenomenon is not limited to endotoxins; various non-specific materials including starch and agar (6) are capable of eliciting hemorrhage and necrosis in suitably prepared skin sites. With these latter materials, the hemorrhage develops much more rapidly than with endotoxins (1) and is usually apparent within 20 to 30 minutes. The reactions produced by streptococcal lysates, however, appeared approx-

imately 3 hours after the intravenous injections, as is ordinarily the case with Gram-negative bacterial endotoxins.

Elicitation of the Generalized Shwartzman Phenomenon.—The capacity to produce bilateral renal cortical necrosis and the other lesions of the generalized Shwartzman phenomenon in the rabbit is perhaps a more specific property of bacterial endotoxins than any of the effects described above (1). The generalized Shwartzman phenomenon is usually produced by two appropriately spaced intravenous injections of endotoxin, the characteristic lesions appearing several hours after the second injection (1, 6). However, the phenomenon also occurs in a high percentage of rabbits after a single injection of endotoxin, provided that

TABLE I

Elicitation of the Local Shwartzman Phenomenon by Streptococcal Lysates

Intradermal injection	Intravenous injection of streptococcal lysate	No. of rabbits	No. showing Shwartzman phenomenon
	ml.		
E. coli endotoxin	1.0	6	0
	2.0	4	1
	5.0	6	4
Streptococcal lysate	1.0	8	1
	2.0	11	2
	4.0	4	2
	5.0	6	4

The intradermal injections consisted of either 100 μg. of *E. coli* endotoxin or 0.2 ml. of undiluted streptococcal lysate, and were given 24 hours prior to the intravenous injection of streptococcal lysate. One of the rabbits receiving the 4.0 ml. intravenous dose of streptococcal lysate was found dead after 24 hours, and showed bilateral renal cortical necrosis; this observation is unexplained.

the animals have been pretreated with cortisone (16) or with reticuloendothelial blockading agents (15). In the latter case, the experimental animals also show a heightened susceptibility to the lethal effect of endotoxin.

In Table II are shown the results of attempts to produce the generalized Shwartzman phenomenon with streptococcal lysates. The double injection method consistently failed, with the doses of lysate used. However, in cortisone-treated rabbits and in those receiving previously an injection of saccharated iron oxide, single intravenous injections of streptococcal lysate produced typical gross and microscopic lesions (Figs. 2 and 3). It will also be noted that some of the animals which had received saccharated iron oxide died after the injection of streptococcal lysate, while normal animals receiving the same dosage of lysate in this and other experiments exhibited no noticeable signs of toxemia.

Enhancement of Antibody Production.—An interesting effect of Gram-negative bacterial endotoxins on the immune response to protein antigens has recently been described (17–19). This consists of a marked stimulation of antibody production in animals receiving endotoxin with the immunizing dose of antigen, as compared with animals receiving the antigen alone in like dosage. A similar enhancing effect was found to occur in experiments in which streptococcal lysates were tested in animals undergoing immunization with ovalbumin. Although the enhancing effect was only moderate, it was demonstrable in each of the three experiments performed, which are summarized in Table III. It has been pointed out (19) that small quantities of endotoxin, which produce a vigorous febrile response but are smaller than those ordinarily used to elicit the

TABLE II

Elicitation of the Generalized Shwartzman Phenomenon by Streptococcal Lysates

Preliminary treatment	Intravenous injections of streptococcal lysate		No. of rabbits	No. showing bilateral renal cortical necrosis
	First	Second		
	ml.	*ml.*		
None	2.0	2.0	14	0
	5.0	5.0	7	0
Cortisone, 25 mg. daily for 3 days	2.0	None	4	2
Proferrin, 3 ml.	2.0	None	2	1
	4.0	"	4	3
	7.0	"	2	1

One animal from each of the groups receiving proferrin was subsequently found dead; none of these showed bilateral renal cortical necrosis in the gross at autopsy. The remainder of the animals were sacrificed at 24 hours and the diagnosis of renal cortical necrosis, based on inspection of the kidneys, was confirmed in each case by histological examination.

Shwartzman phenomenon, produce only a moderate enhancing effect on antibody formation. The results obtained with streptococcal lysates in these experiments are consistent with those which would have been expected with small doses of endotoxin.

Other Effects.—The intravenous injection of streptococcal lysate was observed to produce peripheral vasomotor effects similar in nature and in timing to those previously observed with endotoxin (11, 20). Severe and prolonged constriction of the arteries and arterioles of the rabbits' ears occurred during the first half-hour after the injection and hyperemia of the iris was noted during the 2nd and 3rd hours. These effects were not observed in all of the test animals, but occurred in a majority of those receiving the larger doses of lysates. Polymorphonuclear leucopenia, another characteristic effect of endotoxin administration (11), was also observed irregularly in animals receiving streptococcal lysates.

Thomas (21) has found that an alteration of fibrinogen occurs in rabbits given intravenous injections of endotoxin. Plasma from such animals yields an abundant precipitate following admixture of heparin in the cold. This precipitate, which appears to consist of a fibrinogen-

TABLE III

Effect of Streptococcal Lysate on Antibody Production

Immunizing injection	Streptococcal lysate	Rabbit No.	Capillary precipitin reaction					
			Ovalbumin concentration					NaCl
			10^{-2}	10^{-3}	10^{-4}	10^{-5}	10^{-6}	
	ml.							
Ovalbumin, 5.0 mg. i.v.	None	1	−	−	−	−	−	−
		2	−	−	±	+	±	−
		3	−	−	±	−	−	−
		4	−	−	−	±	−	−
		5	−	−	−	−	−	−
		6	−	−	−	−	−	−
		7	−	−	−	−	−	−
		8	−	−	±	−	−	−
		9	−	−	−	−	−	−
Ovalbumin, 5.0 mg. i.v.	2.0	1-0	−	−	±	−	−	−
		1-1	−	−	+	+	−	−
		1-2	−	−	−	±	−	−
		1-3	−	−	−	−	−	−
		1-4	−	−	+	+	−	−
		1-5	−	±	++	+	−	−
		1-6	−	−	−	−	−	−
Ovalbumin, 5.0 mg. i.v.	5.0	1-7	−	−	−	±	−	−
		1-8	−	−	±	+	±	−
		1-9	−	+	++	++	±	−
		2-0	−	−	+	±	−	−
		2-1	−	−	±	+	−	−

Injections of ovalbumin solution were made into the marginal ear vein. Streptococcal lysate was injected 15 minutes earlier into the marginal vein of the opposite ear. Blood was obtained by cardiac puncture between the 17th and 22nd day after the immunizing injections. The sera were tested against falling dilutions of ovalbumin in capillary precipitin tubes; the concentrations of ovalbumin are expressed as grams per milliliter (*i.e.*, 10^{-2} equals 10 mg./ml). The precipitation reactions were read after incubation at 37°C. for 2 hours followed by overnight refrigeration, and the results recorded as follows: − indicates no reaction; ± indicates a faint or questionable reaction; + indicates a column of precipitate approximately 1 mm. in height; ++ indicates a column 2 or more mm. in height.

heparin complex, redissolves on warming and forms again when the solution is chilled. Normal rabbit plasma contains little or none of the heparin-precipitable fibrinogen.

In rabbits injected with streptococcal lysates there appeared in the circulation moderate amounts of heparin-precipitable fibrinogen. Thomas (22) has

presented evidence which indicates that the intracapillary fibrinoid deposits responsible for the occlusive lesion of the generalized Shwartzman phenomenon are derived, in part at least, from altered fibrinogen, and the capacity of the streptococcal lysates to induce this lesion may well be related to their capacity to cause this alteration of fibrinogen.

It has been observed that rabbits receiving intravenous injections of Gram-negative bacterial endotoxins exhibit severe hemorrhagic lesions at the sites of local injections of epinephrine (23). In the present study, it was found that streptococcal lysates may be substituted for endotoxins in this phenomenon as well. In Fig. 4 is shown a typical skin lesion produced by the intradermal injection of epinephrine in a rabbit which had received, 1 hour earlier, an intravenous injection of streptococcal lysate. The hemorrhage was barely visible 6 hours after the injection of epinephrine, but was well marked at the end of 24 hours, as illustrated.

Reactions of Hypersensitive Rabbits to Streptococcal Lysates

While the reactions to streptococcal lysates appear to be similar to those produced by endotoxins, it has been pointed out that these latter reactions, in turn, closely resemble the reactions of delayed hypersensitivity (11). The possibility that the normal rabbits employed in the present study possessed specific acquired delayed hypersensitivity to group A streptococcal antigens does not seem likely, since these microorganisms are not natural pathogens for rabbits and the occurrence of previous infection in all of the experimental animals is improbable in the extreme. On the other hand, it seems well established that nucleoprotein-containing extracts of beta hemolytic streptococci cross-react, both serologically and in skin tests, with similar extracts of alpha hemolytic or non-hemolytic streptococci (9, 24). It is thus possible that the observed reactions to group A streptococcal lysates were cross reactions, based on the existence in the normal rabbits of delayed hypersensitivity to enterococci or streptococci of other groups.

If the reactivity of normal rabbits to streptococcal lysates were dependent on hypersensitivity to streptococcal antigens, this reactivity would be expected to increase following intradermal streptococcal infections (24). Consequently, twenty rabbits were inoculated intradermally with 0.25 ml. of a 24 hour broth culture of group A type 1 streptococci. After 10 days to 2 weeks, animals of this group received either intradermal or intravenous injections of streptococcal lysates, and their reactions were compared with those of normal rabbits of the same age receiving similar injections.

The local delayed inflammatory reactions produced by intradermal injections of streptococcal lysates in the hypersensitive rabbits were significantly larger and more intense than those in normal control animals. Shwartzman reactions produced in these skin areas were also notably larger and more violent and occurred in a higher proportion of rabbits tested (Table IV, Fig. 5). In addition

to the increased susceptibility of the hypersensitive rabbits to the local effect of streptococcal lysates, these animals also showed unequivocally heightened susceptibility to the systemic effects described in an earlier section. Text-fig. 4

TABLE IV

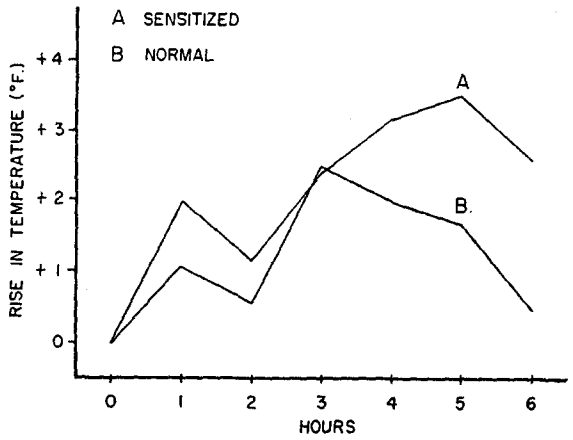
Elicitation of Shwartzman Phenomena in Hypersensitive Rabbits by Streptococcal Lysates

Shwartzman phenomenon	Rabbits	No.	Hemorrhagic necrosis in skin site	Bilateral renal cortical necrosis	Remarks
Local	Normal	1	None		
		2	5 × 5 mm.		
		3	None		
		4	Petechiae		
		5	None		
		6	"		
	Hypersensitive	7	60 × 50 mm.		Dead in 24 hrs.
		8	25 × 25 mm.		" " " "
		9	None		
		10	35 × 25 mm.		
		11	60 × 60 mm.		
Generalized	Normal	12		Negative	
		13		"	
		14		"	
		15		"	
		16		"	
	Hypersensitive	17		Negative	
		18		Positive	Dead in 24 hrs.
		19		Negative	
		20		"	
		21		Positive	

Rabbits were prepared for the local Shwartzman phenomenon by the intradermal injection of 0.2 ml. undiluted streptococcal lysate, and the subsequent intravenous challenging injection consisted of 2.0 ml. of the same preparation given 24 hours later. The generalized Shwartzman phenomenon was produced by two injections of 2.0 ml. streptococcal lysate, given intravenously 24 hours apart. The hypersensitive rabbits had been infected intradermally with group A streptococci 2 weeks previously; at the time of the experiments recorded above, the sites of the streptococcal infections usually showed a nodule with surrounding erythema and some induration, and hemorrhagic necrosis was often elicited in such areas by the intravenous injections of streptococcal lysate.

shows the higher and more prolonged febrile response seen after intravenous injection of a lysate preparation. Diarrhea and other signs of toxemia were frequently encountered in these animals, and it was further found that the generalized Shwartzman phenomenon could be produced by two injections of

streptococcal lysate spaced 24 hours apart, while normal control rabbits failed to react in this way (Table IV).



TEXT-FIG. 4. Febrile response of hypersensitive rabbits to streptococcal lysate. Each curve represents the average response of five rabbits. The test dose of streptococcal lysate was 1.0 ml., given intravenously. The hypersensitive rabbits had been infected intradermally with homologous group A streptococci, 2 weeks previously.

DISCUSSION

While the chemical nature of the active material in the streptococcal lysates remains to be determined, it would seem permissible to refer to it as an endotoxin, within the usual meaning of that term. Virtually all the characteristic effects of Gram-negative bacterial endotoxins have been reproduced with the lysates, and no essential points of difference were observed. It does appear that the endotoxic activity of the streptococcal lysates is low, and this may account in part for earlier failures to detect the activity.

The active material appears to have been derived from the streptococcal cells themselves, although other interpretations of the data are possible: for example, the streptococci may have concentrated or activated some potentially endotoxic material from the culture medium, or the streptococcal cultures may have been contaminated by some undetected endotoxin-producing microorganism. While these and other possibilities cannot be categorically ruled out, it seems more likely that the endotoxin was streptococcal in origin. It is hoped that indentification of the active material may permit more definite conclusions in this regard.

The higher degree of reactivity of hypersensitive rabbits to the streptococcal lysates raises the possibility of an allergic basis for the biological activity of this material, an hypothesis advanced earlier in the case of Gram-negative bacterial endotoxins (11). Alternatively, it may be argued that the exaggerated reactions of the hypersensitive rabbits represent merely an additive effect, with an

allergic component superimposed on the endotoxic effect. The difficulty in distinguishing between delayed hypersensitivity reactions and those produced by endotoxins makes any final analysis of this problem impossible at present.

The data presented may be helpful in interpreting several earlier reports concerning certain effects of streptococci and their products. The toxicity of streptococcal lysates produced *in vivo* (8) and of streptococcal skin lesion extracts (25) may have been due to streptococcal endotoxin. Likewise, the capacity of living streptococci to prepare rabbits for the local and generalized Shwartzman phenomena (7) may be more readily explained. The apparent ability of streptococcal suspensions to confer antigenicity on dextran (26) and connective tissue antigens (27) may be an example of the adjuvant effect of endotoxin (18, 19).

SUMMARY

Suspensions of group A streptococci were subjected to mechanical disruption by shaking with glass beads. The supernatant solutions produced severe delayed inflammatory reactions on intradermal injection into normal rabbits. Intravenous injections caused systemic reactions characterized by fever, leucopenia, and peripheral vasomotor phenomena. These effects were more pronounced in rabbits which had previously been infected with group A streptococci.

The local and generalized Shwartzman phenomena were reproduced with these crude streptococcal lysates, and the data suggest that streptococci possess an endotoxin similar to those of Gram-negative bacterial species.

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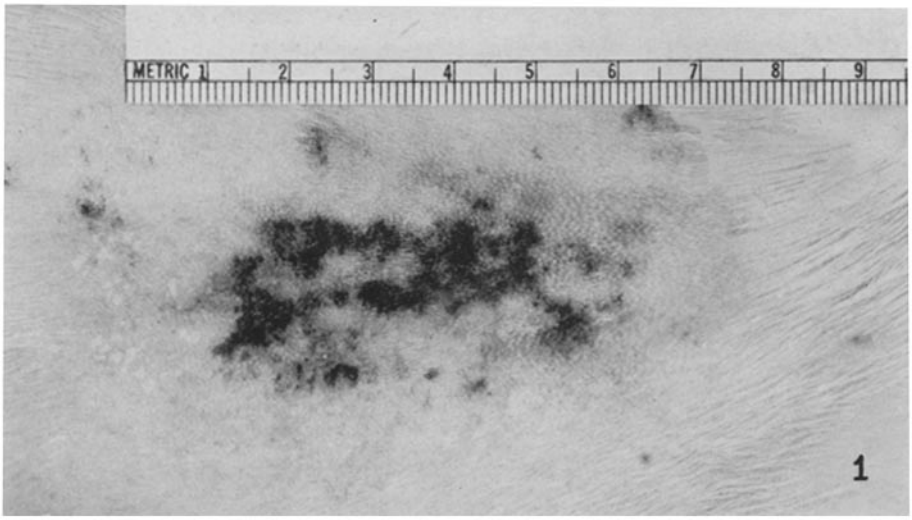
EXPLANATION OF PLATES

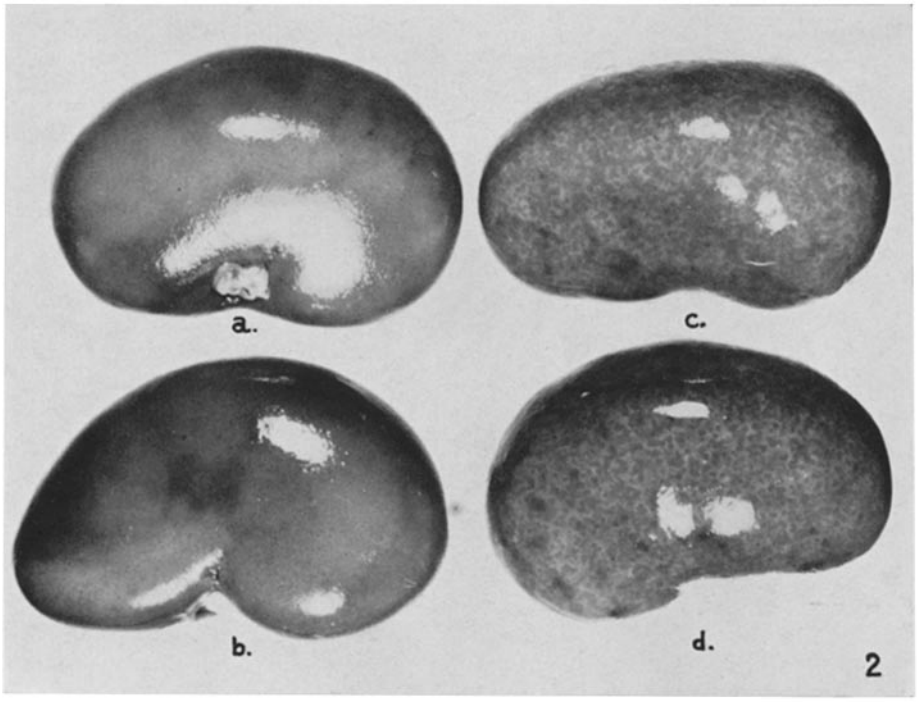
PLATE 68

FIG. 1. Hemorrhage produced by local injection of epinephrine into an area of skin injected 24 hours previously with streptococcal lysate. × 1.1.

FIG. 2 *a* and 2 *b*. Normal rabbit kidneys. × 1.6.

FIG. 2 *c* and 2 *d*. Rabbit kidneys, showing bilateral cortical necrosis, after the intravenous injection of streptococcal lysate in animals which had been treated with cortisone (Table II). 1.60.





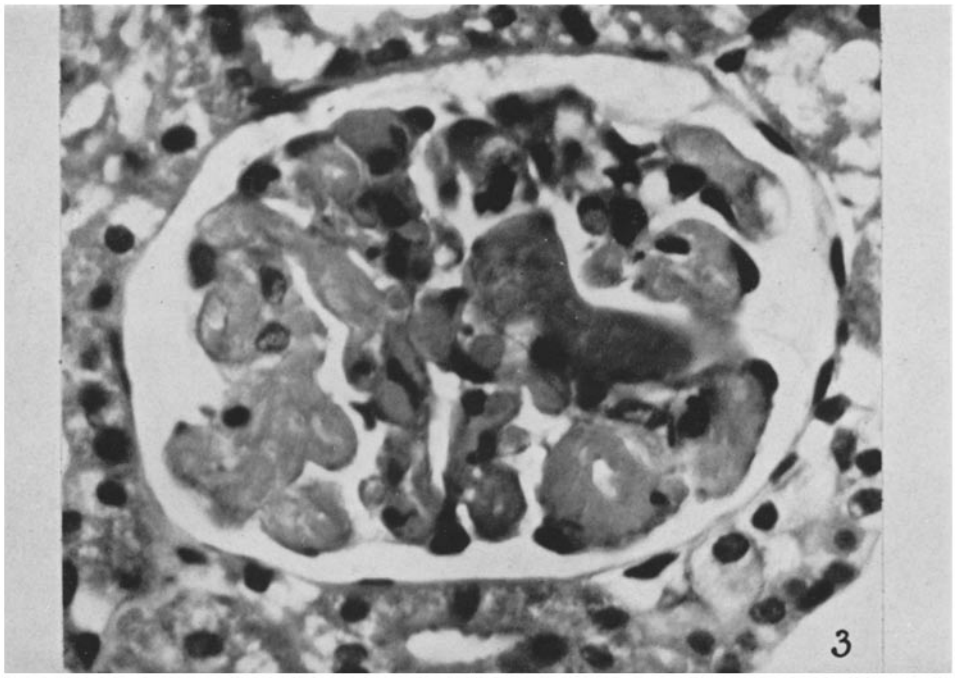
(Stetson: Streptococcal endotoxin)

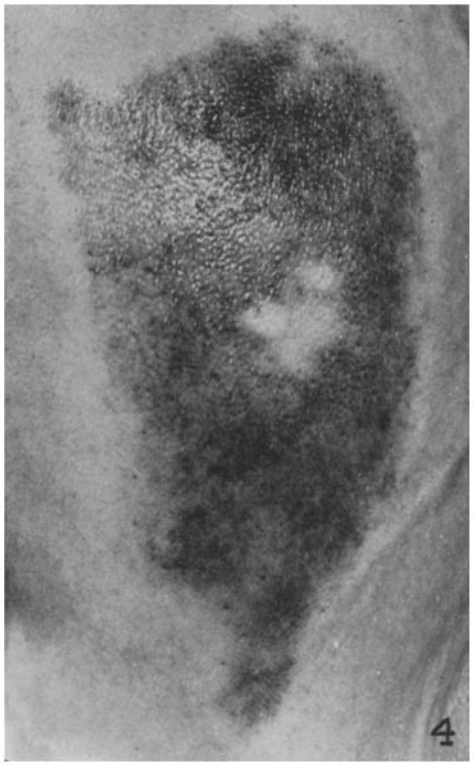
PLATE 69

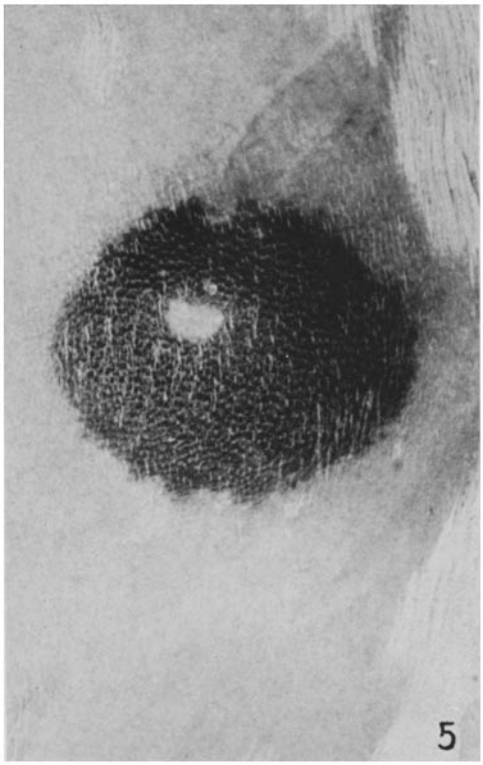
FIG. 3. A glomerulus from the kidney illustrated in Fig. 2 *d*, showing occlusion of the capillaries by homogeneous eosinophilic material. Hematoxylin-eosin. × 370.

FIG. 4. Hemorrhagic lesion produced by the intradermal injection of 0.2 ml. of epinephrine 1:1000 in a rabbit which had received, 1 hour previously, an intravenous injection of streptococcal lysate. × 1.2.

FIG. 5. Typical local Shwartzman reaction produced by streptococcal lysate in a hypersensitive rabbit. The photograph was taken 6 hours after the intravenous challenging injection. × 1.2.







(Stetson: Streptococcal endotoxin)