HOST-PARASITE FACTORS IN GROUP A STREPTOCOCCAL INFECTIONS*

Pyrogenic and Other Effects of Immunologic Distinct Exotoxins Related to Scarlet Fever Toxins

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Pathogenic organisms growing in the dynamic environment of the host can be modified by the mechanism of selection or adaptation. As a result of the host-parasite interaction, they produce substances necessary for their survival in the host and therefore important in the pathogenesis of the infection. When removed from the host to ordinary culture media, the organisms may or may not continue to synthesize these substances. This concept, first proposed by Bail and Weil (1), later revived and applied to the anthrax bacillus (2), and more recently effectively pursued and reviewed by Smith (3), has been the basis of a technique designed to isolate factors responsible for the diverse sequelae of Group A streptococcal infections (4, 5). The method also permits the study of toxic host factors which arise as a result of tissue damage (6, 7).

By the application of this method most types of Group A streptococci, grown in the *in vivo* environment of rabbit skin, produced a soluble factor not readily obtained *in vitro* (4, 5). When tested in American Dutch rabbits, the factor enhanced the lethal and cardiotoxic properties of typhoid endotoxin and streptolysin O. In some respects, the lesions observed in the tissues of these animals resembled those characteristic of the generalized Shwartzman reaction. The factor was relatively heat-labile, being partially destroyed when heated at 56°C. for 30 minutes. Even though antibodies were not found, it was antigenic as manifested by specific immunization against its lethal enhancing activity. The activity was not related to any known factor derived from Group A streptococci.

This communication describes additional toxic activities of these factors or toxins and distinguishes three distinct immunologic types. Comparison of

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their toxic and immunologic properties with purified erythrogenic toxins of Type A relate them to the important scarlet fever group of toxins. That these toxins play a role, previously unrecognized, in Group A streptococcal infections and their non-suppurative sequelae will be suggested and discussed.

Materials and Methods

Rabbits.—Young American Dutch rabbits used throughout this investigation weighed on the average 1 kg. They were supplied from a single source and fed a diet of Nutrena rabbit pellets.

History and Source of Streptococcal Cultures.—Organisms were maintained in the lyophilized state after one or more passages through the skin of rabbits (8). All types, with the exception of T4-366, became highly mucoid after animal passage. T-28, NY-5, and T-17 came from NMRU-4, Great Lakes, Illinois; these came originally from Dr. Rebecca Lancefield, of The Rockefeller Institute. T-18, designated P-18, was isolated at NMRU-4, Great Lakes, Illinois. T-19, made available by Captain John Seal, was isolated 1/29/53 at the USNTC, Bainbridge, Maryland, designated 089704 (Fu-7). T4-366 came from Dr. Ann G. Kuttner, Bellevue Hospital, New York. Group C-C74, originally obtained from NMRI, Bethesda, Maryland, came from NMRU-4. Type 12 is a nephritogenic strain supplied by Dr. Floyd Denny, Heart Hospital, University of Minnesota, Minneapolis. The Group B was isolated from a patient in the University Hospital, University of Minnesota.

Preparation of Streptococcal Lesion Extracts (SLE).—The details for the preparation of these extracts were given previously (4, 5). Briefly, 20 to 30 ml. of an 18-hour Todd-Hewitt broth culture containing 9.0×10^7 organisms per ml. were injected intracutaneously at 40 to 60 sites over the entire shaved abdomen and thorax of American Dutch rabbits. To obtain satisfactory preparations it is essential to distribute large numbers of streptococci throughout the skin. Rabbits are highly resistant to streptococci, and one or two injections is not sufficient to overcome the innate resistance of the host. These lesions, when properly prepared, are not purulent but rather edematous with a high ratio of streptococci to inflammatory cells.

After 18 hours, moribund animals were killed and lesions excised and immediately frozen at −70°C. The partially thawed lesions were ground in a meat grinder at 5°C. and the meat and fluids suspended in cold saline at a ratio of 1 gm. of tissue to 1 ml. of 0.85 per cent NaCl solution. The mixture, after being stirred continuously for 12 to 18 hours at 2–4°C., was filtered through 4 layers of gauze to remove the tissue. The extract was then centrifuged at 30,000 R.P.M. (105,000 *G*) in the Spinco 30 head for 30 minutes at 2°C. The supernatant fluids were pooled, filtered through an 02 Selas filter, tested for sterility, and finally dispersed in glass ampoules for storage at −70°C. After the lesions were removed from the rabbit, the material was not permitted to reach a temperature above 5°C. With these precautions, contamination with other microorganisms and their products which might interfere with the activity of these preparations did not occur.

The numeral following the SLE designation represents the type of Group A streptococci used in its preparation.

Endotoxins.—Endotoxins from Gram-negative bacteria used in the enhancement experiments and for cross-tolerance were prepared as previously described (4) from N-28-1 strain of *Salmonella typhosa.* This material was highly active for the production of the local Shwartzman reaction. A purified endotoxin prepared by the method of Webster *et al.* (9) was also used in similar experiments.

Erythrogenic Toxin.—For comparative and enhancement experiments, a purified erythrogenic toxin 1014C was kindly supplied by Dr. Aaron H. Stock. Details of its preparation were previously published (10). It contained 1000 Lf per mg. total protein, and 1 Lf equalled about

60,000 STD. The material was dissolved in buffered saline and preserved with merthiolate at a concentration of 1 to 10,000.

Scarlet Fever Antitoxin.—This lot, LP316F was obtained by Dr. Aaron Stock from the Wellcome Physiological Laboratories, Beckenham, England. It contained 1760 units of antitoxin by flocculation when tested with the above toxin.

Recording of Temperatures.—Rectal temperatures were recorded on a tri-R electronic thermometer. Normal temperatures were recorded prior to injection, and animals with abnormally high temperatures, above 104°F., were not used. Food and water were not removed during the experiments.

Modified Stock's Dialyzable Medium for Production of Streptococcal Exotoxin.[1]—Twenty-five pounds of whole fresh beef heart was defatted, cubed, and ground, giving a final weight of 15 pounds. For each pound of meat, 400 ml. of pyrogen-free distilled water was added; the mixture was then stirred to give a uniform consistency. The tissue suspension, maintained in a water bath at a temperature of 40°C., was solubilized by adding 1.6 gm. of Difco trypsin for each pound of meat. The pH was maintained at 8.0 by the frequent addition of 2.5 N NaOH; this required 50 ml. at intervals of 10 to 15 minutes with constant stirring. Digestion was considered complete when the pH remained at pH 8.0. At this stage, the tissue was highly solubilized, and the resulting digest was poured into Visking cellulose casing, size 36/32; sections 4 to 5 feet in length were readily filled through a funnel. These were coiled into 12 liter pyrex bottles and immersed in pyrogen-free distilled water at a ratio of 2 liters for each pound of ground meat. The dialysis was continued at 1°C. for 48 hours. The dialysate was used for the medium; the insoluble residue and high molecular weight constituents remaining inside the casing were discarded. The dialysate was sterilized by filtration through 2 liter capacity Seitz filters and collected aseptically in 3 liter Erlenmeyer flasks in volumes of 1.5 liters in each flask.

Immediately before use, the medium was completed by the addition of 50 ml. of buffer to each liter of dialysate. The buffer contained glucose 3 gm., $NaHCO_3$ 2 gm., NaCl 2 gm., $Na_2HPO_4 \cdot 12H_2O$ 2 gm., and *l*-glutamine 200 mg., dissolved in 50 ml. pyrogen-free distilled water. Sterilization was accomplished by filtration through an O2 Selas filter.

RESULTS

Enhancement of Lethal and Tissue-Damaging Properties of Stock's Erythrogenic Toxin by Streptococcal Lesion Extract

As previously reported, streptococcal lesion extract (SLE) injected into American Dutch rabbits markedly alters the host-response as manifested by increased susceptibility to subsequent injections of Gram-negative bacterial toxins and streptolysin O (4, 5). The present experiment was designed to test in a similar manner the effect of SLE on the lethal and tissue-damaging properties of another streptococcal toxin, erythrogenic or scarlet fever toxin. It is interesting to note that at the time these experiments were done, the active component of SLE had not been identified with erythrogenic toxin. A possible relationship was not considered because SLE was heat-labile at 56°C. and gave a negative skin test in American Dutch rabbits.

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[1] I am indebted to Dr. Aaron H. Stock, Department of Microbiology, University of Pittsburgh, for the basic method of preparing this medium. In addition, his generosity in making the toxins and antitoxins available for this study is gratefully acknowledged.

To establish the MLD of Stock's erythrogenic toxin for the enhancement experiments, it was titrated as outlined in Table I. These results show that the MLD, 25 Lf for a 1 kg. American Dutch rabbit, is about the same on a weight basis as previously given by Hottle and Pappenheimer (11); they reported an MLD of 50 Lf for a 2 to 3 kg. chinchilla rabbit. For the lethal enhancement experiment and all others using sublethal doses of erythrogenic toxin doses of 15 Lf were used.

Table II gives the results of four experiments on the lethal enhancement of sublethal doses of erythrogenic toxin. In all experiments, the injection of

TABLE I

Titration of Stock's Erythrogenic Toxin in American Dutch Rabbits

Dose injected Lf	Protein	Results Death/total	Time of death
	mg.		*hrs.*
75	0.075	2/2	8
			14
50	0.050	2/2	20
			12
25	0.025	2/2	Less than 8
20	0.020	1/2	—
15	0.015	0/2	—
10	0.010	0/2	—
5	0.005	0/2	—

Rabbits weighing 1 kg. were injected intravenously with toxin diluted in 0.85 per cent NaCl solution.

SLE-18, 3 hours prior to the injection of the sublethal dose of toxin, modified the host-response toward the direction of greater susceptibility. As shown in subsequent experiments, this is a true enhancement and not an additive effect. It appears, therefore, that the lethal enhancement of toxins by SLE, as manifested in these experiments is a general phenomenon and applies to a variety of toxins including Gram-negative bacterial toxins, streptolysin O and now erythrogenic toxin.

Histopathologic Studies[2].—To compare the type and extent of tissue damage produced by erythrogenic toxin in normal and SLE-treated animals, tissues from the various groups of animals recorded in Table II were examined.

In the gross, the same degree of focal necrosis in the hearts and liver was observed after treatment with erythrogenic toxin as formerly observed with

[2] I am indebted to Dr. Joel Brunson of the Pathology Department, University of Minnesota, for the microscopic observations and his cooperation and assistance with the pathologic aspects of this problem.

typhoid toxin and streptolysin O (4, 5). As confirmed by microscopic examination, there was less kidney pathology than observed with streptolysin O and perhaps more liver damage and mitral valve involvement with erythrogenic toxin. Bilateral renal cortical necrosis, characteristic of the generalized Shwartzman reaction, was not a feature of this reaction.

Structural changes were limited almost entirely to the heart and liver. No qualitative differences were observed in the animals given 15 Lf of erythrogenic toxin in a single injection as compared with those given SLE 3 hours before

TABLE II

Lethal Enhancement of Stock's Erythrogenic Toxin by SLE-18 in Rabbits

Experiment	First injection	Second injection	Results Deaths/total
I, II, and III	SLE-18 0.5 ml.	Stock's erythrogenic toxin 15 Lf	13/15
	Saline control 0.5 ml.	" " " " "	0/15
IV	SLE-18 0.75 ml.	Stock's erythrogenic toxin 15 Lf	4/5
	Saline control 0.75 ml.	" " " " "	0/5

All injections were given in the marginal ear vein. The second injection was given 3 hours after the first. Time of death varied from 3 hours to 24 hours. Tissues, including heart, liver, kidney, and lungs were fixed in formalin-alcohol solution for histopathologic studies. All survivors were killed on the 4th day. Saline control was an 0.85 per cent NaCl in pyrogen-free water. Each experiment consisted of 10 animals, 5 test and 5 controls.

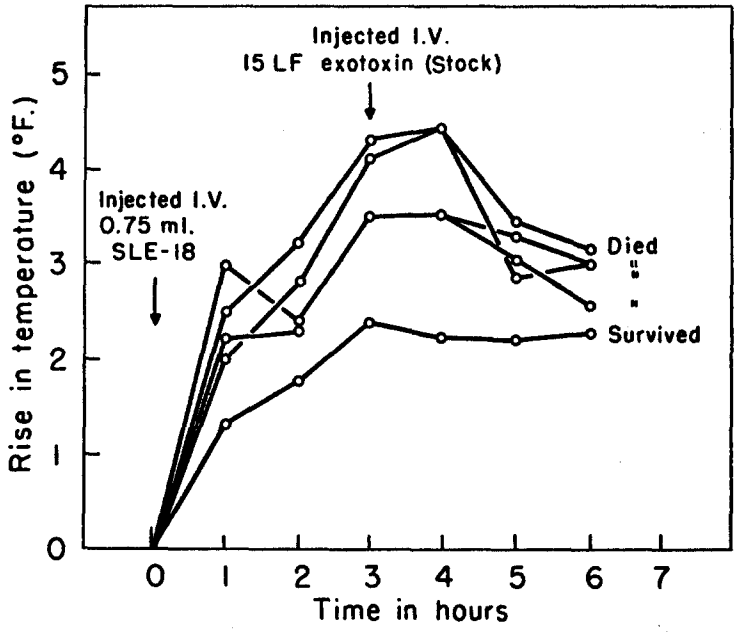
the toxin, although in the latter group the lesions appeared somewhat more extensive.

Both valvular and myocardial lesions were present in the heart. The valvular changes consisted of thickening and some increased cellularity. The thickening was associated with the presence of a fibrillar, slightly basophilic material which was slightly metachromatic when stained with toluidine blue. It did not, however, give a positive reaction when stained by the periodic acid–Schiff method. The cellular components, limited usually to aggregates just beneath the valvular endocardium, consisted of mononuclear cells with occasional heterophiles (Fig. 1).

Extensive areas of muscle necrosis were observed also. In some areas this was accompanied by calcification of the necrotic myofibers (Fig. 2) with little evidence of inflammatory reaction. In other areas, the necrosis was associated with a pronounced cellular reaction (Fig. 3), which consisted of large mononuclear cells and occasional heterophiles. These areas bore no constant rela-

tion to intramural arteries but were observed more commonly in sections from the interventricular septum and the papillary muscles of the left ventricle.

Focal to diffuse areas of hepatic necrosis were noted commonly. The focal areas had no predilection for any particular part of the lobule and consisted of small necrotic areas with an accompanying mononuclear cellular reaction. The diffuse areas commonly involved several lobules of the liver and consisted of frank infarcts accompanied by a classic reaction zone of hemorrhage, mono-



TEXT-FIG. 1. Febrile response of rabbits to SLE-18. Five rabbits were each given 0.75 ml. SLE-18 intravenously. Rectal temperatures taken at hourly intervals when plotted against time show biphasic curves. Lethality of erythrogenic toxin was markedly enhanced as shown by death of 4 of the animals in 3 hours after the injection of a sublethal dose.

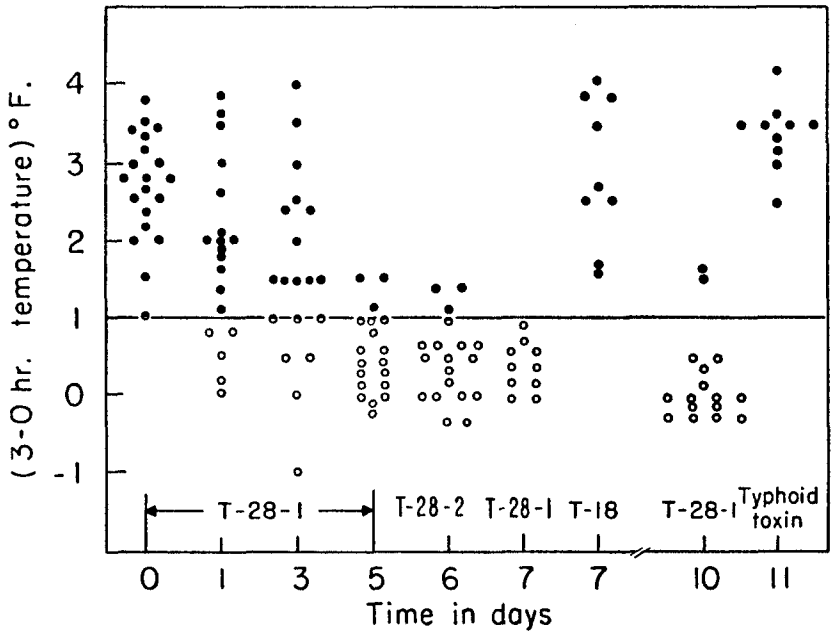
nuclear, and heterophilic cells (Fig. 4). No thrombi, however, were observed in any sections from the liver.

From these studies, it is evident that erythrogenic toxin given in a single injection can bring about marked tissue destruction. In animals pretreated with SLE, however, erythrogenic toxin is more destructive, as manifest by greater lethality and quantitative tissue damage.

Pyrogenicity as a Measure of SLE Toxicity

Increase in serum transaminase after intravenous injection of SLE, given in a preliminary report (12), indicated a primary toxicity of SLE preparations;

no doubt the release of this enzyme into the serum was a manifestation of the tissue damage observed in the preceding experiment. This observation, along with Stetson's report (13) of the pyrogenic nature of streptococcal endotoxin, made it imperative to test the SLE preparations for their pyrogenic effects.



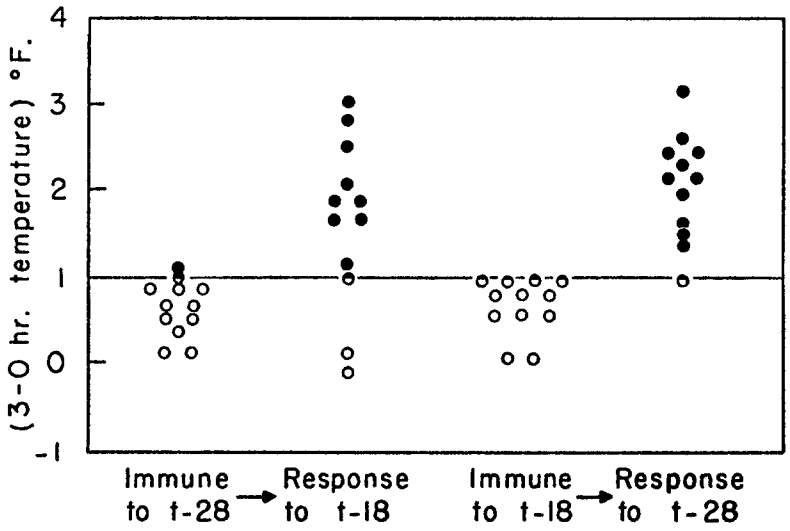
TEXT-FIG. 2. Development of specific immunity to SLE-28 toxin determined by febrile response. Twenty rabbits each received daily intravenous injections of 0.5 ml. of SLE T-28-1. Rectal temperatures were recorded before and 3 hours after injection to give the 3 — 0 hr. values recorded. Solid circles, above the line drawn at 1°F., indicate fever. Clear circles, below the line, are within the normal variation which can occur with this breed of rabbit and under the conditions of the experiment. The rabbits developed immunity on the 5th day as indicated by the absence of fever. On the 6th day as a control, the rabbits received a different preparation, SLE-28-2. On the 7th day, they were divided into 2 groups; one group was given the homologous SLE-28-1 and the other a heterologous SLE-18. Specific immunity to SLE-28 is evident by the absence of fever in the group compared with the fever observed in those receiving the SLE-18. On the 10th day all received the homologous SLE-28-1 and again the majority were immune. On the 11th day, 10 of the original 20 received a pyrogenic dose of typhoid endotoxin (10 μg.) and responded comparably to an equal number of normal animals.

Injection of 0.75 ml. quantities of SLE-18 intravenously into American Dutch rabbits produced biphasic temperature curves; these were typical of those described by Beeson (14) for Gram-negative bacterial toxins and by Stetson (13) for the endotoxins of Group A streptococci. Text-fig. 1. shows that a maximum temperature response occurs in 3 hours and, if the animals receive

a sublethal dose of erythrogenic toxin at this time, death occurs within 6 hours. It is interesting to note that in previous experiments we had empirically selected 3 hours as the optimum time to show lethal enhancement of toxins when SLE was given intravenously; at this time both serum transaminase production (12) and fever response are at a maximum.

Development of Specific Immunity to SLE as Demonstrated by Febrile Response to SLE-18 and Typhoid Endotoxin

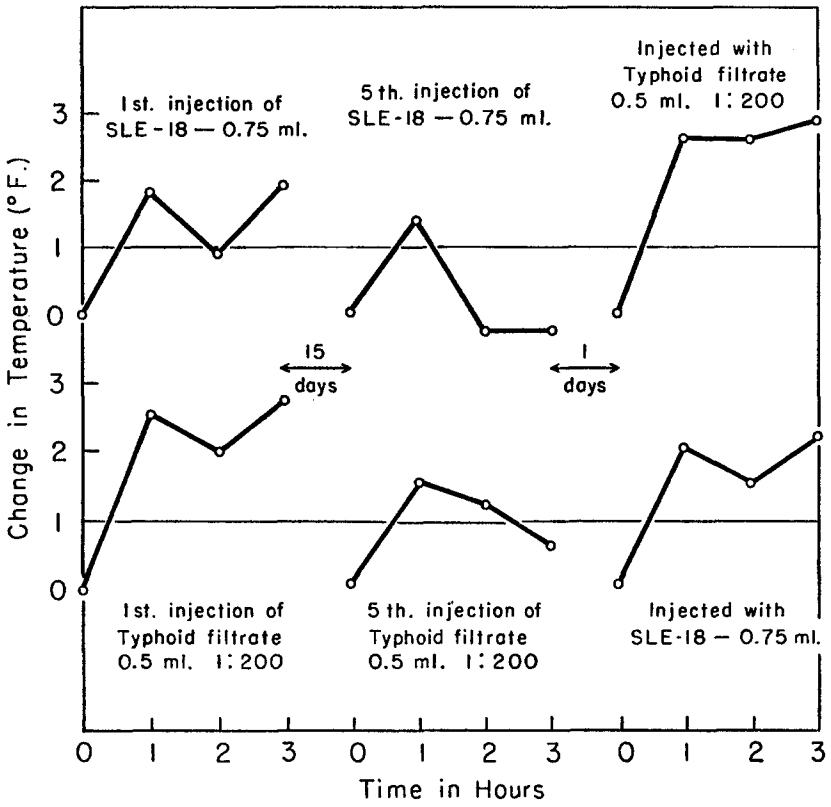
The febrile response noted in the previous experiment could have been activated by a number of contaminating pyrogens such as Stetson's strepto-



TEXT-FIG. 3. Immunologic specificity of SLE-toxins determined by cross-immunization with SLE-28 and SLE-18. Twelve rabbits were immunized by repeated daily intravenous injections of 0.5 ml. of SLE-28. When immune, as indicated by their fever response, they were given 0.75 ml. of heterologous SLE-18. Another 12 rabbits were immunized with 0.75 ml. of SLE-18 and when refractory tested for their response to the heterologous SLE-28. The same preparations and concentrations were used for both groups of animals.

coccal endotoxin (13) or the endogenous pyrogen of Landy and Shear reported to be present in high concentration in rabbit skin (15). All of these have one property in common—they produce a non-specific tolerance first described by Beeson (14). Animals which receive daily injections develop a non-specific "immunity" within 6 to 7 days. In the present studies, therefore, reliance has been placed on the development of specific immunity to differentiate between SLE and other pyrogenic toxins. Text-fig. 2. gives the results of such an experiment.

Even though the immunity developed within the same period of time as observed for the Beeson tolerance, the resistance developed was by no means

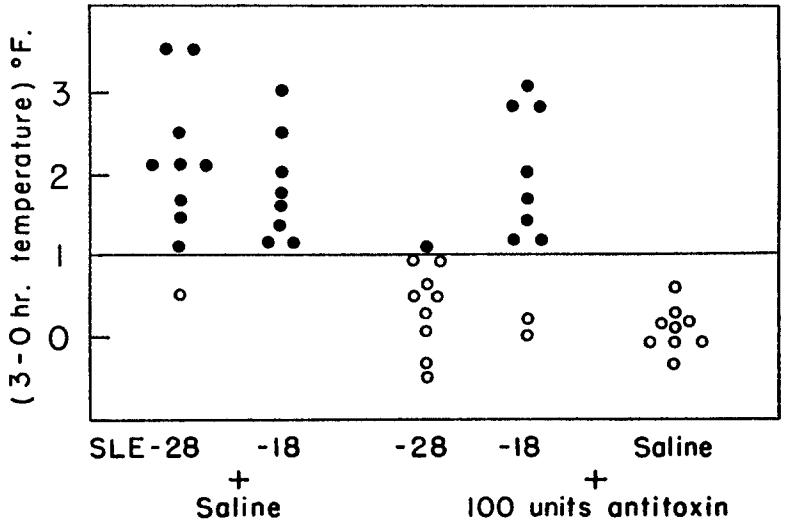


TEXT-FIG. 4. Immunologic specificity of immunity to SLE-18 shown by cross-immunization with typhoid endotoxin. Each point, in the above biphasic fever curves taken over a 3 hour period, represents the average temperature of 5 animals. Animals received all injections intravenously, and the same lots of SLE-18 and typhoid filtrate were used throughout the experiment. The 1st curve in the top series represents the response of normal animals to SLE-18. The 2nd fever curve in the top series is that obtained after the 5th injection; temperatures returned to normal in 3 hours, indicative of the immune state. One day after the last injection of SLE-18, the animals received typhoid endotoxin; the 3rd curve in the top series is typical of that given by normal animals as seen in the 1st curve, bottom series—indicating the specificity of the immunity to SLE-18. Another group of animals were given 5 injections of typhoid endotoxin over a 15 day period. When tolerant, as shown in the 2nd curve in the bottom series, and 1 day after the last injection of typhoid endotoxin, each animal received SLE-18; the 3rd curve in the bottom series is typical of those obtained with normal animals as shown in the 1st curve of the top series.

non-specific when tested with the heterologous SLE-18 or a pyrogenic dose of typhoid endotoxin. One might criticize this experiment on the basis of concentration differences between immunizing dose of toxin and the dose contained in the heterologous challenges. To overcome such criticism, complete cross-immunization experiments were done between SLE preparations of different

immunologic specificity and between SLE preparations and typhoid endotoxin.

Results of a typical cross-immunization experiment are given in Text-fig. 3. The results clearly indicate that SLE-28 and SLE-18 are immunologically distinct toxins.



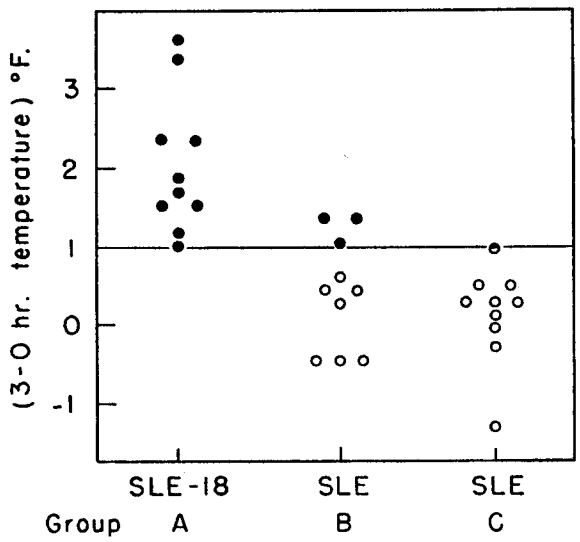
TEXT-FIG. 5. Specific neutralization of the pyrogenic activity of SLE-toxins by commercial scarlet fever antitoxin. The febrile responses given in the 1st and 2nd groups represent the activity of 0.8 ml. of SLE-28 when injected intravenously into each of 10 rabbits and 0.5 ml. of SLE-18 when injected into each of 8 rabbits. Each dose of SLE was mixed with 0.2 ml. of 0.85 per cent NaCl solution (saline). The 3rd group is the individual temperature responses of 10 rabbits which received 0.8 ml. SLE-28 mixed with 100 units of commercial antitoxin in 0.2 ml. of 0.85 per cent NaCl solution, before intravenous injection. The 4th group represents the temperature response of 10 rabbits injected intravenously with 0.5 ml. of SLE-18 plus 100 units of antitoxin; the 5th group is the antitoxin control, and each animal received 0.5 ml. of 0.85 per cent NaCl solution containing 100 units of antitoxin. Antitoxin and SLE were mixed and incubated 30 minutes at room temperature before injection.

A similar experiment was repeated using SLE-18 and typhoid endotoxin. Text-fig. 4 gives the results. From these, one can conclude that unlike the Beeson tolerance, characteristic for Gram-negative bacterial endotoxins and other pyrogens, immunity developed against SLE-toxins is specific and confers no protection against Gram-negative bacterial toxins.

Specific Neutralization of Pyrogenic Effects of SLE-28 with a Commercial Scarlet Fever Antitoxin

Another criterion for identifying pyrogens, characteristic of endotoxins, is the failure of antisera to neutralize specifically their pyrogenic effects.

Pyrogenic activity of SLE-toxins, as shown in Text-fig. 5, are specifically neutralized by a commercial scarlet fever antitoxin. It will be noted that this antiserum neutralizes only the SLE-28 and not SLE-18. As already shown in Text-fig. 3, SLE-28 and SLE-18 are immunologic distinct toxins, and it will be shown later that SLE-28 has the same immunologic specificity as Stock's erythrogenic A toxin and that the SLE-18 is a new toxin designated C. Commercial scarlet fever antitoxin is prepared by immunization of horses against the A toxin, and it would not be expected to contain the C antitoxin; this latter toxin, so far, is produced only by a Type 18 streptococcus.

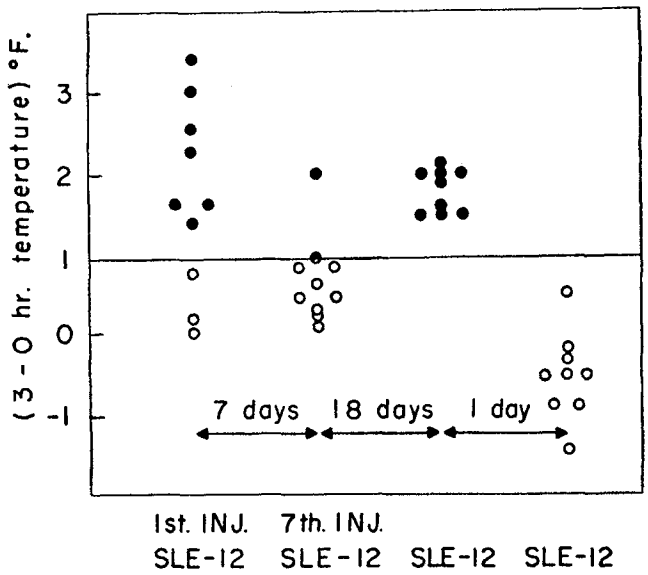


TEXT-FIG. 6. Relative SLE-toxin production by hemolytic streptococci of Groups A, B, and C. The 1st group gives the individual temperature responses of 10 rabbits after each received 0.5 ml. of SLE-18 from Group A streptococci; the 2nd and 3rd groups represent the temperature responses after each animal received 1.0 ml. of SLE prepared with Groups B and C streptococci respectively.

Distribution of SLE-Toxins within the Lancefield Groups A, B, and C

In previous experiments 2 distinct immunologic specific toxins were demonstrated; one was produced by Type 18, the other by Type 28 and both were within the Group A streptococci. It was of interest, therefore, to determine if similar pyrogenic toxins were produced by other Lancefield groups of hemolytic streptococci. SLE preparations using Groups B and C organisms were made in a manner comparable to that described for Group A. The lesions were highly edematous and in physical appearance were quite similar to those produced by the Group A streptococci. Results given in Text-fig. 6 show that SLE preparations made with Groups B and C streptococci failed to induce significant temperature responses in rabbits comparable to those observed for Group A.

Since only 2 strains were tested for SLE toxins within Lancefield groups other than A, it cannot be concluded that these toxins are restricted to Group A. These results are suggestive, and further testing will be carried out in additional strains within these and other groups.



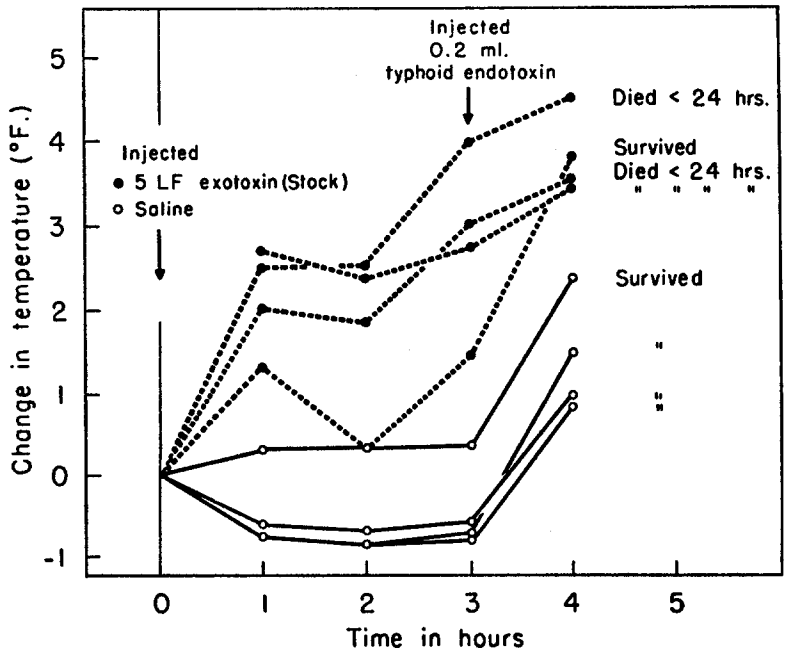
TEXT-FIG. 7. Duration of immunity and demonstration of the anamnestic response to SLE. Ten rabbits were each given daily injections of 0.5 ml. of SLE-12 for 7 days. The same dose was given on the 18th and 19th day.

Duration of Immunity to SLE Toxins and the Demonstration of an Anamnestic Response

Animals given daily intravenous injections develop specific immunity to the SLE-toxins within 5 to 7 days. Undoubtedly, a small amount of antibody will neutralize the toxin in SLE. Because the toxins are placed directly into the blood stream and because they act rapidly, it is necessary to have circulating antibody present to demonstrate immunity. In other words, if antibody is not present, the toxins can exert their injurious effects before the anamnestic response can bring forth sufficient antitoxin to neutralize their activity. The results given in Text-fig. 7 confirm these predictions and show that after 18 days the animals become less immune to intravenous injection of SLE as manifest by a significant temperature response. Challenge on the following day reveals complete immunity and demonstrates a rapid anamnestic response to these toxins.

Pyrogenicity of Stock's Erythrogenic Toxin and Its Lethal Enhancement of Typhoid Endotoxin

Specific neutralization of SLE-28 pyrogenicity by commercial scarlet fever antitoxin suggested a relationship between the SLE-toxin and erythrogenic



TEXT-FIG. 8. Pyrogenicity of Stock's erythrogenic toxin and its lethal enhancement of typhoid endotoxin. Four rabbits were each given intravenously 5 Lf of Stock's exotoxin dissolved in 0.5 ml. of 0.85 per cent NaCl solution. A similar number of rabbits served as a control group, and each received 0.5 ml. of 0.85 per cent NaCl solution (saline). After 3 hours all of the rabbits were given a sublethal dose, 0.2 ml. of typhoid endotoxin (filtrate) intravenously. A complete titration of this toxin in groups of 5 rabbits for each dilution showed that a minimal pyrogenic dose was 0.125 Lf.

toxin. Such antisera, however, are prepared by the injection of heterogeneous materials and might contain antibodies to several streptococcal products.

Results given in Text-fig. 8 show that a known purified erythrogenic toxin and SLE have similar activities as manifest by the fact that small quantities of erythrogenic toxin can induce the biphasic fever curves comparable to those observed for SLE-toxins (Text-fig. 1). In addition, the results show that erythrogenic toxin, like SLE, enhances the lethal and tissue-damaging properties of other toxins including typhoid endotoxin. Those animals which received

both erythrogenic toxin and typhoid toxin and survived longer than 24 hours showed the typical myocardial and liver necrosis observed in animals given SLE. The control animals which received saline and typhoid endotoxin showed no gross pathology when examined 3 days after injection.

Differentiation of Erythrogenic Exotoxin (Stock) and Typhoid Endotoxin by the Local Shwartzman Reaction

The pyrogenic activity of any product might be attributed to contamination with Gram-negative bacteria and their active endotoxins. In the last experiment, the biphasic temperature curves and the lethal enhancement of toxins are properties of Gram-negative bacterial toxins which could be present in

TABLE III

Differentiation of Gram-Negative Toxins (Typhoid) and Streptococcal Exotoxin (Stock) by Means of the Local Shwartzman Reactions

First injection intradermally	Second injection typhoid endotoxin intravenously 18 hrs. later	No. of rabbits	No. showing local Shwartzman reactions	No. of deaths
	ml.			
Erythrogenic toxin (Stock), 5 Lf	0.2	10	1(?)	5
Typhoid endotoxin, 0.2 ml	0.2	10	7	0
None	0.2	2	—	0
None	0.5	2	—	0
None	1.0	2	—	0

Typhoid endotoxin was undiluted sterile filtrate. In additional titrations, concentrations of erythrogenic toxin up to 15 Lf did not prepare for the local Shwartzman reaction.

the erythrogenic toxin. The same applies to the SLE toxins. To eliminate the possibility of contamination with Gram-negative bacterial endotoxins, advantage is taken of two properties of the erythrogenic toxins not shared with endotoxins. One of these, specific immunization, both active and passive, has been demonstrated in several previous experiments with SLE-toxins. The second property, originally observed by Schwab *et al.*, (4, 5) is the ability of SLE when injected intradermally, to enhance the lethal and tissue-damaging properties of toxins. In contrast, endotoxins from Gram-negative bacteria or streptococci (13) given intradermally induce the local Shwartzman reaction.

It was of importance, therefore, to compare Stock's erythrogenic toxin with a known Gram-negative bacterial endotoxin with respect to its ability to enhance the lethality or prepare for the local Shwartzman reaction. Results given in Table III show that erythrogenic toxin, given intradermally, enhanced the lethal and tissue-damaging properties of typhoid endotoxin as manifested by 5 deaths and a significant amount of carditis and liver necrosis in the sur-

vivors when examined 3 days after the injection of typhoid toxin; local Shwartzman reactions were not observed. On the other hand, typhoid endotoxin given intradermally produced local Shwartzman reactions in 7 of the 10 animals and none died or appeared ill.

These results confirm our original observations and relate SLE activity to that of erythrogenic toxin; therefore, this ability of erythrogenic toxin when given intradermally, to enhance the lethal and tissue-damaging property of typhoid endotoxin rather than to prepare the skin for the local Shwartzman reaction, is the basis of another method for differentiating these scarlet fever toxins from other exogenous and endogenous pyrogens.

Preparation and Purification of Streptococcal Exotoxins in Vitro

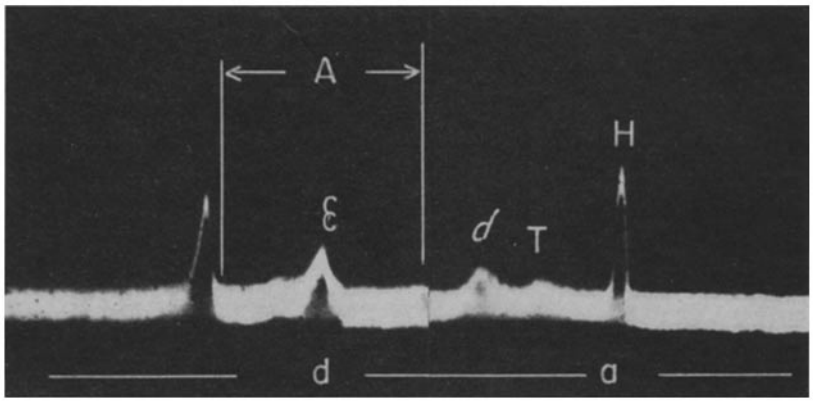
Lesion extracts such as SLE are ideal sources in which to demonstrate important parasite factors; they are not, however, suitable for isolation and purification studies because their relatively high content of serum proteins make purification or concentration difficult. Therefore, the experiments described here are preliminary to a subsequent study designed to produce and isolate these toxins from *in vitro* sources. As already pointed out, Stock and his associate (10) have made valuable contributions in this area, specifically toward the isolation and characterization of the A toxin and more recently toward the isolation of the B toxin (16).

Methods used in this phase of the problem have been those which will not dissociate natural complexes. So far we have found a close association between the toxin and hyaluronic acid. Whether or not this has any biological significance is of particular interest for the future. For these reasons, ultrafiltration and electrophoretic methods were used at hydrogen-ion concentrations and ionic strengths which at least approach the physiologic conditions of the host.

Production of Toxin.—NY-5 streptococci grown for 18 hours from the lyophilized state in 10 ml. amounts of modified Stock's medium were transferred in 1 to 2 ml. quantities to flasks containing 100 ml. of this medium. After incubation for approximately 8 hours at 37°C., the entire culture of 100 ml. was transferred to flasks containing 1500 ml. of the media; these had previously been brought to 37°C. Within 5 to 6 hours, the culture completes the logarithmic phase of growth. Short incubation periods were chosen to minimize contamination of the extracellular with intracellular products derived from lysed cells. Cell-free filtrate obtained by passing the culture through an electrically driven Sharples centrifuge and a Seitz sterilizing filter was concentrated on ultrafilters at 1°C. (17). Alundum thimbles were coated with parlodion dissolved in concentrated glacial acetic acid at a concentration of 6 per cent. The concentrated material was washed on the ultrafilters with several liters of pyrogen-free distilled water. The highly viscous material, washed off the ultrafilters with distilled water, was placed in Visking cellulose casing and dialyzed with continuous stirring against pyrogen-free water at 1°C. for 3 days to remove low molecular weight—contaminating constituents of the medium. It was assumed that they would be removed by this procedure since they had originally passed through the same membrane during the preparation of the medium. The

dialyzed produce was now finally brought to dryness by lyophilization. This crude material was highly pyrogenic and enhanced the lethality of Gram-negative endotoxins. A yield of 2.2 gm. from 20 liters of medium was obtained.

Electrophoretic Purification.—When 0.5 per cent concentrations of the crude preparation were run in an Aminco Tiselius-type electrophoretic cell, the results recorded in Text-fig. 9 were obtained. The high viscosity prevents the use of higher concentrations. In this concentration, there were two main components. As indicated by the areas under the curves, hyaluronic acid is the major component in these crude preparations; a second component of low mobility at this pH and ionic strength appeared to contain the toxin. Since there were two main components, it was possible to remove the fast moving component from the ascending



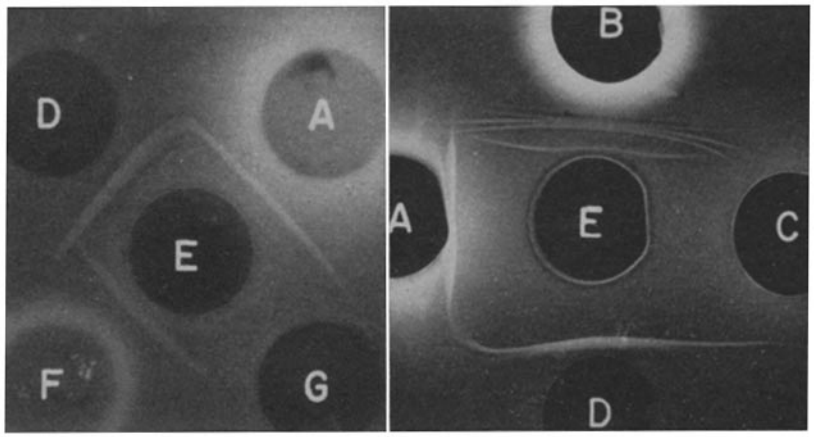
TEXT-FIG. 9. Electrophoretic pattern of streptococcal (NY-5) extracellular products. The concentration was 0.5 per cent in veronal buffer at pH 8.6 and ionic strength 0.1. Component (*T*) had an apparent isoelectric point of 7.2 determined from a pH mobility curve. Below pH 7.2 there was visible complexing with hyaluronic acid (*H*). For isolating (*A*) electrophoresis was continued until (*H*) reached the bottom of the descending arm of the cell.

limb of the cell and the slow moving from the descending. This was accomplished by the use of polyethylene (0.067″ O.D.) intravenous tubing attached to a 10 ml. syringe. By visualization through the optical system, the end of the polyethylene tubing could be observed and the components easily removed. The absence of observable components does not of course preclude the possible existence of other extracellular products at concentrations below the resolving power of the optical system. Fraction A, shown in Text-fig. 9 contained all of the extracellular products after the removal of hyaluronic acid (*H*); this fraction was dialyzed against distilled water and concentrated by lyophilization. Fractions from several runs were pooled to give sufficient material for testing.

Agar Diffusion Precipitin Analysis.—The Ouchterlony technique was used to compare, serologically, fraction A with Stock's erythrogenic toxin and sonic extracts of NY-5 and Type 18 cells. The results given in Text-fig. 10 show reaction of identity between fraction A and Stock's toxin. This commercial antisera obviously contains a number of antibodies to intracellular constituents of Group A streptococci. Neither of the toxins tested showed observable lines

of identity with any of the intracellular components. More critical use of the Ouchterlony technique may resolve more than one system in Stock's toxins and fraction A. Regardless of this possibility, the major component in fraction A is definitely related to Stock's toxin.

Pyrogenicity of Toxin in Fraction A, Its Lethal Enhancement and Erythrogenic



TEXT-FIG. 10. Agar diffusion precipitin analysis of streptococcal extracellular and intracellular components. The lettered wells in the Ouchterlony plate contained: *E*, commercial scarlet fever antitoxin (Wellcome Laboratories) 0.2 ml.; *A*, fraction A (Text-fig. 9), 0.2 ml. of a solution containing 2 mg. in 0.5 ml. of 0.85 per cent NaCl solution; *D*, Stock's exotoxin 0.2 ml. of a solution containing 560 Lf/ml.; *F*,* sonic extract of washed NY-5 streptococcal cells 0.2 ml.; *B*,* sonic extract of washed Type 18 streptococcal cells, 0.2 ml.; *G* and *C* were not significant to the experiment. Differences in the position of lines in the two experiments and the greater resolution with sonic extract of Type 18 cells can be explained by the longer time of diffusion in the *A*, *B*, *C*, *D* plate.

Activity.—To relate the toxin in fraction A with SLE toxins and Stock's erythrogenic toxin, it was tested for pyrogenicity and lethal enhancement of typhoid endotoxins. Results of these tests given in Table IV indicate that the electrophoretically isolated fraction A is highly active as a pyrogen and enhances the lethality of Gram-negative bacterial toxins.

Like Stock's erythrogenic toxin, it gives no reaction even at 15 μg. in the skin of the American Dutch rabbit. When tested in the skin of 5 humans, 0.01 μg. of Stock's toxin gave reactions showing edema and erythema; these

* Cells obtained from the Sharples centrifuge during the preparation of the toxin were washed and resuspended in distilled water. A 22 per cent suspension was placed in the Raytheon for 3 hours at 9000 kc./sec. at a temperature of 6°C. Cell debris was removed by centrifugation at 12,800 *G.* for 90 minutes. The supernatant, after passing through an 02 Selas filter was stored in sealed ampoules at −70°C. These extracts were kindly prepared by Miss Natalie Cremer of this laboratory.

appeared in 24 hours and varied in size from 15 × 20 mm. to 50 × 65 mm. On a comparative basis in a single individual, fraction A at a concentration of 0.01 μg. gave a reaction in the left arm measuring 35 × 40 mm., while the reaction to Stock's toxin in the right arm at the same concentration, measured 50 × 65 mm.

Consideration of Other Streptococcal Factors

The most probable toxic component of SLE preparation would be streptolysin O or S. SLE preparations, however, do not contain either active streptolysin

TABLE IV

Pyrogenicity of Toxin in Fraction A and Its Lethal Enhancement of Typhoid Endotoxin

First injection fraction A	Temperature > 1°F. after 3 hrs.	Second injection typhoid filtrate	Dead/total
μg.		*ml.*	
15	3/3	0.25	3/3
10	2/3	"	3/3
5	2/3	"	2/3
2.5	1/3	"	3/3
1	0/3	"	1/3
1 ml. 0.85 per cent NaCl solution	0/4	0.25 + 5 μg. fraction A	0/4
" " " " " " "	0/4	0.25 + 15 μg. fraction A	1/4

Fraction A dissolved in 0.85 per cent NaCl solution was injected intravenously into rabbits. Temperatures were recorded before and 3 hours after injection. Three animals were used for each concentration of toxin and those given in the nominator had temperature increases in excess of 1°F. 3 hours after injection. The controls received saline first injection and typhoid toxin plus fraction A 3 hours later. The failure of the combined toxins to produce significant lethality when compared with the activity observed when given singly at a 3-hour interval indicates that this phenomenon is a true enhancement and not a simple additive effect.

O or S or inactive streptolysin O, determined by the addition of a reducing agent such as cysteine. When the Group A streptococci grow in the skin of rabbits, there is an extravasation of red cells into the lesions with subsequent lysis of these cells. In other words, one might postulate that there is sufficient substrate available to absorb these lytic toxins. Stock's toxin and fraction A contained no detectable streptolysins when tested with conventional hemolytic methods controlled by the use of known streptolysins.

Another interesting streptococcal product described by Carlson *et al.* (18) is diphosphopyridine nucleotidase or DPNase; this enzyme is closely associated with streptolysin O. It was of interest to determine its presence and possible implication in the toxic manifestations of SLE. Several SLE preparations made with different types of Group A streptococci were assayed for DPN-

ase activity by the method of Kaplan *et al.* (19). Table V shows that the nephritogenic Type 12 strain produces significant quantities of DPNase *in vivo*. Types 18, 19, and NY-5-SLE preparations contained no detectable DPNase; these results are in accord with those reported for the *in vitro* production of enzyme by these types (18). The absence of this enzyme in highly toxic SLE-18,

TABLE V

Diphosphopyridine Nucleotidase (DPNase) Activity of SLE-Preparations*

SLE	DPNase
	units/ml.
SLE 12-1	375
	380
SLE 12-2	520
	545
SLE 12-3	215
	220
SLE 12-4	150
	155
SLE-18	0
	0
SLE-19	0
	0
SLE-NY-5	0
	0

SLE-12 was made with a single type 12 streptococcus. Nos. 1 to 4 represent different preparations of SLE-12. The values given for DPNase activity are duplicate determinations on samples from the same lot.

* These determinations were kindly made by Mr. Richard Hyde of this laboratory.

-19, and -NY-5 excludes this factor as the active toxin in SLE. It is not impossible, however, that the pyrogenic exotoxins of SLE may enhance any toxicity attributed to this enzyme in the same way that it enhances the lethality of streptolysin O and Gram-negative bacterial toxins.

The only component observed in fraction A other than erythrogenic toxin was desoxyribonuclease or DNase. Determinations[3] revealed a significant quantity of the enzyme in contrast to the absence of active enzyme in Stock's

[3] These determinations were kindly done by Mr. Howard Pierce in Dr. L. W. Wannamaker's laboratory, Department of Pediatrics, University of Minnesota.

erythrogenic toxin. Since fraction A and Stock's erythrogenic toxin had the same pyrogenic activity, it can be concluded that the DNase was not contributing to the toxicity of fraction A.

TABLE VI

Relationship of Stock's Erythrogenic Toxin to SLE-Toxins Determined by Cross-Immunization

Rabbits immunized with	Immune/total when tested with						
	Stock's toxin	SLE 18	SLE 12	SLE NY5	SLE 19	SLE 17	SLE 28
Stock's erythrogenic toxin	11/12 (0/5)	1/12 (1/9)	8/12 (2/10)	1/8 (1/6)	1/11 (0/9)		
SLE-18		12/12 (0/6)		1/8 (2/10)	2/8) (1/8)	1/8 (0/8)	1/12 (2/10)
SLE-12	10/10 (0/5)	0/4 (2/6)	9/10 (3/10)	2/7 (1/6)	2/10 (0/8)		8/10 (0/10)
SLE-NY5	6/8 (2/10)	0/8 (1/9)	7/8 (0/6)	6/6 (1/6)	7/7 (0/9)	7/8 (1/8)	
SLE-19		1/9 (0/9)			7/9 (0/9)		
SLE-17		2/8 (0/10)	7/8 (0/8)	0/8 (1/9)		7/8 (0/8)	7/8 (0/10)
SLE-28		0/9 (0/9)	7/10 (2/10)				10/10 (2/10)

On the average, 7 daily intravenous injections of the toxins immunized the rabbits. A single immunizing or test dose of Stock's toxin, diluted in 0.85 per cent NaCl, contained 0.125 Lf in 0.5 ml. Immunizing and test doses of the SLE preparations were undiluted; a single dose between 0.5 and 0.8 ml. depended on the pyrogenicity of the SLE. Animals were considered immune when their temperatures did not increase over 1°F. 3 hours after receiving the test dose of toxin intravenously. The bracketed results represent the febrile response of normal animals given the test dose of toxin at the same time as the immunized group; as shown, 2 of 10 normal animals may appear refractory to the toxin.

Identification of Exotoxins by Specific Immunization against Their Pyrogenic Effects

Evidence presented in this paper related the fever-inducing ability and other effects of SLE preparations to the group of toxins represented by Stock's erythrogenic toxin. A serologic or immunochemical test would be desirable for the identification of these toxins in SLE. Their relatively low concentration and close association with other antigenic extracellular products, such as one

or more of three immunologic distinct DNases (20) as shown by Halbert (21) makes a serologic test difficult until methods of production *in vitro* and purification are achieved. In the meantime, a measurement of their activity and specific neutralization by active immunization in rabbits can be utilized to identify immunologically specific toxins within this group. The technique has been fully outlined and the results for SLE-18 and -28 are given in Text-fig. 3. In addition, it was of interest to determine whether the pyrogenic assay for specificity of these toxins could be correlated with the skin-neutralization test.

By the use of this latter test, Coffey (22) recognized the multiplicity of these toxins, and Hooker and Follensby (23) found 2 toxins in NY-5 filtrates which they designated A and B.

TABLE VII

Immunologic Distinct Exotoxins Produced in vivo by Various Types of Group A Streptococci

Type of streptococcus	Type of toxin*		
	A	B	C
NY-5-10	+	+	−
18	−	−	+
28	+	−	−
12	+	−	−
17	+	−	−
19	−	+	−

* Nomenclature of Hooker and Follensby (23).

The data given in Table VI show that Stock's toxin immunized against the effects of the homologous challenge and SLE-12 but not against SLE-18, SLE-NY-5 or SLE-19. Since Stock's toxin prepared from NY-5 is the A toxin, failure to immunize against SLE-18, -NY-5, and -19 could be accounted for by either the presence of 2 toxins in these SLE preparations or an immunologically distinct toxin. Immunization with SLE-NY-5 indicated the presence of two toxins as previously shown (23). Indeed, it immunized against all of the SLE-toxins with the exception of SLE-18. The failure of SLE-toxins, except NY-5, to immunize against SLE-19, indicated that SLE-19 contained only the B toxin. It can be seen that SLE-12, -17, and -28 are related to Stock's toxin and, therefore, contain only the A toxin. SLE-18 produced an immunologically distinct toxin not related to either A or B. To continue the nomenclature of Hooker and Follensby (23), this new toxin was designated C. Small amounts of the C toxin were produced *in vitro*; further characterization of this toxin is in progress.

Table VII gives the types of toxins produced by the various Group A streptococci tested. From the results with SLE-NY-5, it is evident that the pyrogenic

assay is measuring the same activity as originally obtained with the skin-neutralization test.

Among the Group A streptococci tested, the A toxin is the most common. It is interesting that the more recently isolated Types 18 and 19 produce the C and B toxins, respectively; the nephritogenic Type 12 strain, however, makes the A toxin. Indeed, it will be interesting to correlate the types of toxin with the serologic types and to determine whether or not there are additional distinct toxins especially in the more recently isolated epidemic types.

DISCUSSION

This investigation, designed to discover new factors important in the pathogenesis of Group A streptococcal infections and their sequelae, has demonstrated a new activity associated with a familiar group of substances commonly referred to as Dick, scarlet fever, or erythrogenic toxins. The following discussion relates these findings to the classical reactions of these toxins and develops the concept that they damage tissue and modify the host-response to injury in a manner not previously recognized.

Soon after Dicks (24) and Dochez and Sherman (25) discovered scarlet fever toxin its toxicity for rabbits was described (26, 27). The time of death and the gross pathology resembled in some ways the results given here where Stock's toxin was titrated in American Dutch rabbits. In the past, few studies have been concerned with the lethal and tissue-damaging properties of the scarlet fever toxins. Perhaps the importance of these toxins was minimized because immunization with a single toxin as antigen did not confer protection against infection; it was reasoned, therefore, that the toxin must not play a role in the pathogenicity of the Group A streptococci. The reasoning, based on the diphtheria toxin analogy, neither took into consideration the multiplicity of immunologically distinct toxins nor did it recognize that an organism such as the streptococcus may not use one or more of its toxins in a single attribute of virulence such as infectivity. Even now, there is not complete agreement on the mechanism by which the toxins induce a rash in the Dick test and scarlet fever. The Dicks and others (28, 23) attributed the rash to a primary toxicity; others (29, 30) presented good evidence that the erythematous lesions are allergic reactions either to the toxins or other products of the streptococci. More recently, Rantz, Boisvert and Spink (31) favored the latter conclusion after studying the Dick test in men of military age. Positive reactions were less frequent in personnel from geographical areas with a reported *lower* incidence of streptococcal disease. They suggest that the skin sensitivity in the Dick test results from acquired hypersensitivity to a streptococcal product and not from the natural susceptibility to a true toxin.

The present results interpreted in the light of earlier studies, bring these two extreme views closer together. In the American Dutch rabbit, 15 Lf

(900,000 STD) of purified scarlet fever toxin gives no reaction in the skin. Dochez and Stevens in 1927 (29) made a similar observation; they made the logical correlation between hypersensitivity and activity when their normally refractory rabbits became skin-sensitive after streptococcal infection. It follows, therefore, that if skin activity is the only criterion of toxicity, scarlet fever toxin is not a toxin in our host. If, however, one uses the parameters of toxicity such as death, fever response, increase in serum transaminase, enhancement of the lethal and cardiotoxic properties of Gram-negative bacterial endotoxins, as set forth in this and previous papers, then one must conclude that these are true toxins, in this host, not dependent on the acquisition of hypersensitivity.

Boroff (32) described an interesting observation which, at the time, did not seem pertinent to our studies because SLE-toxins elicited no erythrogenic activity in American Dutch rabbits. He showed that erythrogenic toxin, given into the skin of sensitive rabbits at 24-hour intervals, markedly enhanced the erythrogenic and nephrotoxic properties of the toxins. Undoubtedly the enhancement activity Boroff described in the skin is manifested here in the results on lethality and tissue damage. These combined observations describe the most interesting toxic property of erythrogenic toxins; given either intravenously or intracutaneously, they modify the host-response to injury to themselves (see Table II and Text-fig. 1) and other toxins (Text-fig. 8). That hypersensitivity reactions of the anaphylactic type can be enhanced by these toxins was given in a preliminary report (33). It is not known whether Boroff's observations are an enhancement of an acquired hypersensitivity to the toxin or closely associated protein (34), an enhancement of a true toxicity within the skin or a mixture of both, depending on the previous experience of the host. This basic information must be obtained before the mechanism of the Dick test or the rash in scarlet fever can be resolved.

Another significant manifestation of a modified host-response brought about by treatment with SLE-toxins and streptolysin O was reported by Răska and Rotta (35); after confirmation of our earlier report (4), they showed that in toxin-treated animals, Group A streptococci given intranasally persist in the lungs, myocardium, and kidneys; normal animals given streptococci by the same route failed to become infected. The importance of factors which control the entrance and persistence of organisms in tissues is obvious, especially if the interesting cell-wall toxin (36) can be implicated in the evolution of the rheumatic lesion.

The pyrogenic response invoked in American Dutch rabbits by erythrogenic toxin present in SLE was the most useful parameter of toxicity. Failure to find significant quantities of other endogenous and exogenous pyrogens in the SLE was unexpected. Indeed, one would expect to find the endogenous pyrogen of Landy and Shear (37); either the Group A streptococci do not liberate this

toxin from rabbit skin or the toxin is destroyed within the lesion. The endogenous pyrogens of Atkins and Wood (38) and Bennett and Beeson (39) would not be expected in high quantities, especially if they arise from leucocytes. As pointed out earlier, the streptococcal lesions are not purulent. Although highly pyrogenic, the relatively small amount of Stetson's (13) endotoxins in the Group A streptococci combined with minimal lysis of the streptococci within the lesion perhaps explains the failure to detect them in the SLE-preparations. Stetson's failure to obtain complete cross-tolerance between streptococcal endotoxin and Gram-negative bacterial endotoxin could be explained by the assumption that his streptococcal lysate contained an active quantity of erythrogenic toxin (see Text-fig. 4).

Results obtained, using the Ouchterlony technique employing an antiserum with a multiplicity of antibodies to streptococcal cellular and extracellular products, indicate that Stock's toxins and the electrophoretically purified toxin are identical; from the significant work of Halbert (21) and Wannamaker (20) one could predict that they contain small amounts of other streptococcal products. Erythrogenic toxin is closely associated with streptococcal DNase (21). The electrophoretically purified preparation described here contained active DNase, but Stock's preparation had no detectable enzymic activity. Likewise SLE-toxins prepared with a nephritogenic Type 12 contained DPNase; others prepared with Type 18 had no activity. This type of indirect evidence indicates that neither DNase nor DPNase play a direct role in the toxicity attributed to erythrogenic toxins. Further purification and characterization of these toxins is the ultimate goal; once attained, however, one must recognize that the Group A streptococcus does not present the host with a single purified toxin. Indeed, as shown here and previously for streptolysin O (40) streptococcal endotoxin (13) and SLE-toxins (4, 5), the sequence in which the host receives the toxins may be a critical determinant in the host-response to injury.

The mechanism by which these toxins cause tissue damage is not known. In some respects the activity resembles that of Gram-negative bacterial endotoxin. The recent interesting results from Russia (41) on the physiologic response of rabbits to purified erythrogenic toxin may suggest possible mechanisms of action. In addition to the pyrogenic effects noted here, blood sugar and adrenalin increased and reached a maximum in approximately 30 minutes and 3 hours respectively.

The histopathologic studies on tissue from rabbits given one and/or two spaced injections of erythrogenic toxin are of interest; these changes indicate the ability of such toxins to produce significant damage in various tissues of the host including the mitral valve; there is neither an indication of specificity for this structure within the heart nor a suggestion that the lesions resemble those observed in any sequelae of Group A streptococcal infections. Morpho-

logically, more significant lesions have been observed by others (42–44). It is significant to note, however, that the results of these investigations were obtained in rabbits with *living* streptococci multiplying in an *in vivo* environment. Conditions would be ideal for the production of the exotoxins described in this communication.

Most of these investigators placed primary emphasis on the development of the Aschoff body as the final criterion in the identification of a rheumatogenic factor. Another approach, discussed briefly by Rantz (45) suggests that appropriate biological methods, such as immunization, might induce a resistance in human tissues to the development of the rheumatic process even though Group A streptococcal infection occurs. As an example, he cites the 8 year controlled study of Wasson and Brown (46); they injected Valdee's (47) tannic acid–precipitated erythrogenic toxin into rheumatic subjects. The results appear significant as indicated by the fall in the incidence of rheumatic recrudescence in the immunized groups. The production of additional immunologic distinct toxins by the Group A streptococci has special significance in the evaluation of these results. While the A and B toxin used by Brown and Wasson are produced most frequently by Group A streptococci (Table VII), an epidemic type such as18, isolated from naval personnel at Great Lakes, can produce another toxin as represented by Type C. As predicted by Hooker and Follensby (23), undoubtedly others exist. It is obvious, therefore, that antigenic preparations used to confirm the efficacy of such immunization procedures should include the known toxins and those not yet identified within the remaining types of Group A streptococci.

Finally as a working hypothesis, it is proposed that most Group A streptococci growing in the *in vivo* environment of the host produce one or more immunologically distinct toxins associated with the scarlet fever toxins. Transferred from the host to culture media, the organisms may or may not continue to produce the toxins. In the past, only those types capable of producing toxin *in vitro* were considered toxigenic. The primary activity of the toxins concerns their ability to modify the host-response to injury to themselves, to other toxins, and to toxic reactions such as hypersensitivity. The absence of a rash in an infected individual does not preclude damage to other tissues by these toxins. Rash is manifested only in those individuals sensitized as a result of antecedent Group A streptococcal infections. Immunization against all of the possible immunologic types of toxins produced by the Lancefield Group A streptococci may protect the tissues against critical injury even in the presence of infection.

It must be recognized that the results and concepts presented here were derived from experience with a single experimental host, the American Dutch rabbit; they may however serve as useful guides to future interpretations and

contribute by analogy to the understanding of data directly concerned with Group A streptococcal infection in man.

SUMMARY

The factors present in streptococcal lesion extracts (SLE) which enhanced the lethal and tissue-damaging properties of Gram-negative bacterial endotoxins and streptolysin O were identified with the scarlet fever group of toxins.

Toxic manifestations attributed to this group of toxins included lethality, cardiotoxic and other tissue damage, enhancement of toxicity, and pyrogenicity. Of these, the measurement of febrile response in American Dutch rabbits was the most useful parameter of toxicity.

In rabbits, repeated daily intravenous injections of 0.125 Lf of a purified erythrogenic toxin immunizes specifically against the pyrogenic activity; this technique was used to type the toxins and to distinguish them from exogenous and endogenous pyrogens; non-specific pyrogens, such as streptococcal endotoxin, were not found in SLE.

All types of the Lancefield Group A streptococci tested produced one or or more immunologically distinct toxins *in vivo* in contrast to Groups B and C which did not produce them; toxins A and B, previously distinguished by neutralization of rash-inducing activity in the skin, were produced *in vivo*. The A toxin was the most common, as indicated by its presence in extracts prepared with Types 28, 12, 17, and 10 (NY-5); B toxin was found in 10 (NY-5) and 19. A new toxin, designated C, was obtained from a Type 18.

In American Dutch rabbits, purified toxin at a concentration of 15 Lf (900,000 STD) neither gave a Dick test nor prepared the skin for the local Shwartzman reaction; by this route, however, in contrast to classical endotoxins, they enhance the lethal and tissue-damaging properties of sublethal doses of these and other toxins.

These properties of the immunologic distinct exotoxins as demonstrated in American Dutch rabbits suggest by analogy their importance in the pathogenesis of streptococcal disease in man. Evidence that might implicate them in sequelae, in addition to scarlet fever, is discussed.

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EXPLANATION OF PLATE 20

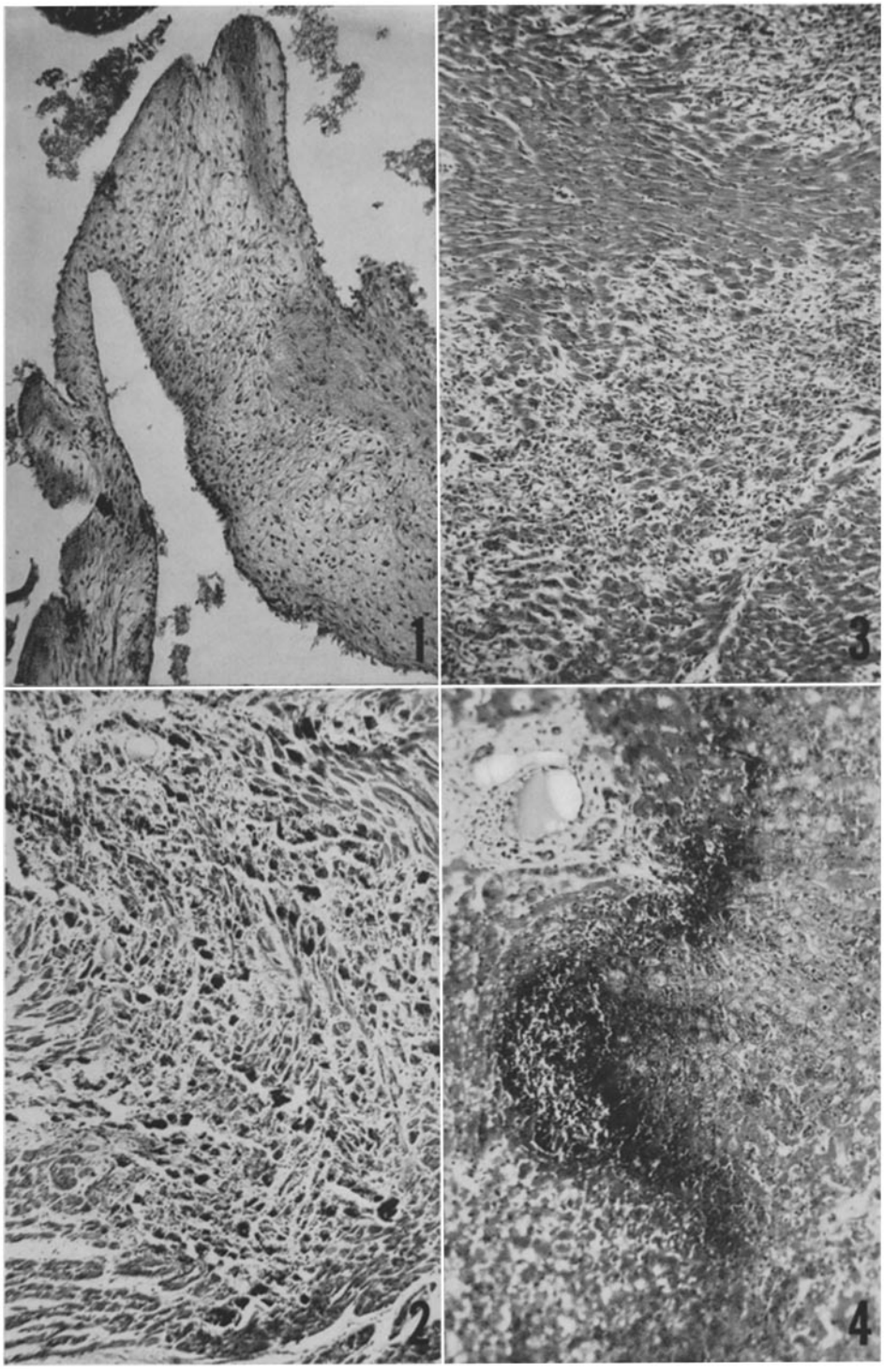
All sections were stained with hematoxylin and eosin.

FIG. 1. Section from the mitral valve of a rabbit given 0.75 ml. of SLE-18 intravenously followed in 3 hours with 15 Lf of Stock's erythrogenic toxin intravenously. The animal died in 24 hours. The valve appears edematous, and there is a moderate increased cellularity. × 70.

FIG. 2. Section from the myocardium of a rabbit killed 4 days after administration of 0.5 ml. of SLE-18 intravenously followed in 3 hours with 15 Lf of Stock's erythrogenic toxin. The section shows an extensive area of muscle necrosis with calcification of necrotic fibers, but there is no inflammatory reaction. × 70.

FIG. 3. Section from the myocardium of a rabbit given 0.5 ml. of saline intravenously followed in 3 hours with 15 Lf of Stock's erythrogenic toxin. The animal was killed 4 days after injection. There is extensive muscle necrosis accompanied by a pronounced cellular reaction which consists predominantly of mononuclear cells. × 70.

FIG. 4. Section from the liver of a rabbit which was killed 2 days after administration of 0.5 ml. saline followed in 3 hours with 15 Lf of Stock's erythrogenic toxin. The section shows a large infarct, surrounded by a heavy zone of hemorrhage and inflammatory cells. × 320.



(Watson: Host-parasite factor in Group A streptococcal infections)