Rash Induction by Intracellular Products of Group A Streptococci.* (25911)

NATALIE CREMER AND DENNIS W. WATSON
Dept. of Bacteriology, University of Minnesota, Minneapolis

Extensive work(3,4,5,12) has been done on electrophoretic separation of intracellular components of group A streptococci. However, there are fewer reports on biologic activity *in vivo* of such fractions(11). This study was undertaken to investigate chemical separation, antigenic constitution and intradermal biologic activity of fractions obtained by starch electrophoresis.

Materials and methods. Preparation of streptococcal extracts. Five to 6-hour cultures of group A streptococci, types 18 and 4 and strain NY5 were grown in a completely dialyzable medium(13). After harvesting the bacteria by centrifugation in a Sharples, they were washed 3 times with distilled water and resuspended in distilled water. 30 g wet weight plus 100 ml water. Thirty-ml aliquots were disrupted in a 9 kc Raytheon, 2½ to 3 hours, 130 plate voltage, temperature 4°C. Sonic extracts were cleared of cellular debris by centrifugation 1 hour, 12,800 to 15,000 g in a refrigerated Serval. Supernates were dialyzed against saline or balanced salt solution for 24 hours at 2°C. filtered through an 02 Selas filter, placed in ampules and frozen at −70°C. Similar sonic extracts of *Salmonella typhosa* Ty2 were prepared. Bacteria grown on veal infusion agar were exposed to sonic oscillation for ½ hour. *Fractionation of sonic extracts.* One streptococcal type, 18, was separated by starch electrophoresis(7), veronal buffer pH 8.6, ionic strength 0.1, potential gradient 500 volts, 16 hours. After completion of run, the block was cut in 1½ cm strips, from point of origin. Five to 6 blocks were done in succession, comparable strips pooled and eluted with 100 ml distilled water by washing on a sintered glass filter of medium porosity under suction. Pooled fractions were concentrated to about ⅓ the original volume of extract by ultrafiltration(2). Starch control fractions were processed in the same way. All procedures were done in a 1°C cold room. An aliquot of each fraction was saved for chemical analyses; the remainder was dialyzed against 0.85% saline, placed in ampules and frozen at −70°C. *Chemical analyses of fractions.* Each pooled fraction was analyzed for RNA by the orcinol method (1), for total phosphorus by the method of Fiske and Subbarow(6), for DNA by the diphenylamine reaction(1) and tyrosine by the Folin-Ciocalteau method. *Serologic activity of fractions.* Each fraction was checked with T18 type specific antiserum, group A specific antiserum, and with antiserum prepared against either viable T18 streptococci or against T18 streptococcal sonic extracts. The Ouchterlony technic was used. *Biologic activity of sonic extracts and fractions obtained by starch electrophoresis.* Injections of whole sonic extracts, 1.0 to 1.5 ml, and fractions 0.5 ml, were made intradermally on the shaved abdominal surface of American Dutch rabbits, average weight 1 kg. *Intradermal control injections.* Intradermal injections of 1.0 ml were made into rabbits with sterile balanced salt solution; sterile 0.85% saline; trypsin (Difco) 5 mg/ml in balanced salt solution, final pH 7.8; histamine acid phosphate 0.275 mg/ml suspended in 0.9% saline equivalent to 0.1 mg histamine/ml (Eli Lilly and Co.); concentrated extracellular products of T18 containing 4 mg lyophilized material per ml; supernate from rabbit epithelial homogenate with and without added cysteine.

Results. Chemical analyses of fractions. These data are given in Table I. *Serologic study on fractions.* Fractions 1 through 7 taken from the positive side of the origin formed multiple lines of precipitate with T18 antiserum (Fig. 1). No lines of precipitate formed with fractions beyond 8 or with fractions taken from the negative side of the origin. Fractions 0 through 7 reacted with type specific M antiserum, fractions 0, 4, 5, 6, 7 forming one line of precipitate, fractions 1 and 3 two lines, and fraction 2 three lines.

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TABLE I. Chemical Analyses on Fractions from Starch Electrophoresis.*

No. fraction†	μg/ml Tyr	P	DNA	RNA
−1 through −5	—	—	—	—
0	—	—	—	—
1	28	—	—	—
2	100	9	—	—
3	310	43	—	92
4	460	127	—	800
5	400	290	—	3160
6	240	404	—	5000
7	53	210	85	2580
8	—	91	440	660
9	—	54	610	190
10	—	28	360	12
11	—	8	120	—
12 through 16	—	—	—	—

* Tests on starch control fractions read between .0-.4 O.D. In reading test fractions, starch control fraction was set at .0 O.D.

† Fractions taken from negative side of origin (0) are designated as minus.

Only fractions 1 and 2 reacted with group A specific antiserum. One broad line of precipitate was formed with each of the 2 fractions.

Intradermal activity of sonic extracts and fractions. Slight to considerable edema developed 3 to 6 hours after injection of sonic extracts and certain fractions into rabbits. Fractions 0 through 8 were the most active in eliciting an early reaction. The edema gradually subsided during the next 24 to 48 hours. Approximately 96 hours post-injection, 2 types of lesions began to develop in some of the animals, a remittent nodular reaction, grossly resembling that previously described by Schwab and Cromartie(9,10) and a spreading erythematous eruption resembling that described years ago by Rivers(8). The latter lesion appeared the 3rd to 4th day post-injection. Its advancing border was marginated, slightly raised from the normal surrounding skin. There was very little edema or induration. The bright red lesion faded the 6th to 8th day. Heavy desquamation of the involved area began the 9th to 11th day. After the original lesion faded, a fresh, bright red eruption (secondary lesion) occurred adjacent to the original lesion (primary lesion) in about 10% of the reactants. Several days later the new extension faded and eventually desquamated. Rarely a tertiary extension adjacent to the secondary lesion occurred about the 10th to 11th day post-injection. Tables II, III, and IV present these data. Figs. 2 and 3 show the reaction.

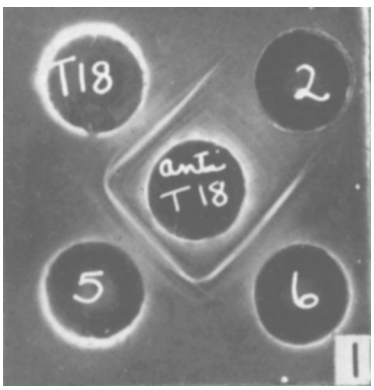

FIG. 1. Ouchterlony plate showing antigenic heterogeneity of starch fractions. Center well antiserum to T18 extract. Peripheral wells, crude sonic extract of T18 and fractions, as numbered.

TABLE II. Intradermal Activity of Strep. Extracts in Causing an Erythematous Eruption.

Streptococci	No. of rabbits	No. developing lesion Primary	Secondary	Tertiary
T4	10	5	—	—
NY5	47	25	—	—
T18	142	62	10	4

Studies on erythematous eruption. Upon centrifugation of crude sonic extracts at 105,400 g for 1 hour in a refrigerated Spinco, both sediment and supernate were active in producing the eruption; the activity causing recurrent nodular formation was concentrated primarily in the sediment. By intradermal injection of supernate only, the erythematous

TABLE III. Erythematous Eruption Caused by I.D. Injection of Certain Fractions.*

No. fraction	No. of rabbits developing lesion Primary lesion	Secondary lesion
C†	—	—
1	5	—
2	5	—
3	5	2
7	5	—
8	2	—
10	2	—
13	2	—

* Result of 2 separate experiments. Fractions −4 through 15 were each inj. into 9 rabbits. All fractions not listed did not produce this reaction.

† Starch control.

TABLE IV. Nodule Formation Caused by I.D. Injection of Certain Fractions.*

No. fraction	No. of rabbits developing nodules
C†	—
1	4
2	8
3	8
4	3

* As under Table III. † Starch control.

eruption occurred in absence of nodular formation.

To determine extent and duration of capillary damage, after development of the eruption, 5 ml of a 1% saline solution of trypan blue was injected intravenously. The first 2 days after appearance of the lesion, the dye appeared immediately over the eruption area. On the 4th-8th day, accelerated appearance of dye occurred only at the periphery of the lesion. By the 9th day there was no accelerated appearance of dye, the animal became uniformly blue as time elapsed.

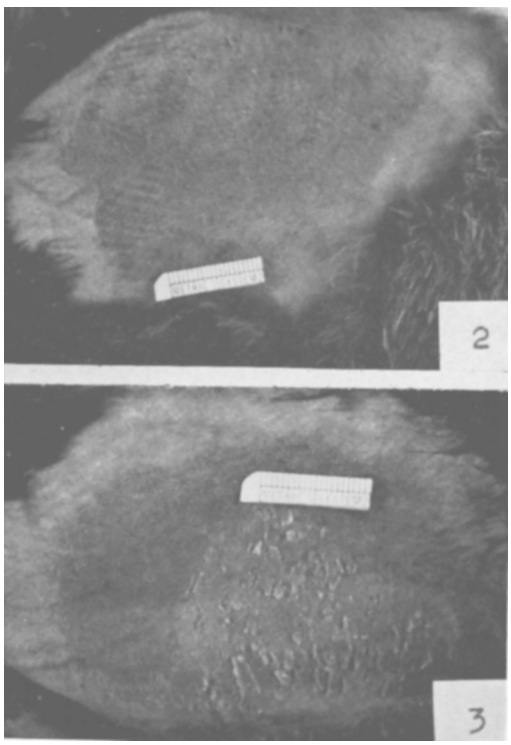

FIG. 2 (top). Primary erythematous eruption showing marginated, slightly raised advancing border.

FIG. 3 (bottom). Fresh secondary extension of the eruption adjacent to the now desquamating original lesion, 6 to 8th day post-inj.

The variability in rabbit response made it difficult to study the mechanism of the reaction. Using the same extract, rabbits showing a positive reaction varied from 8 out of 10 to 0 out of 10. The variability was not correlated with aging of the extract. The lesion could be the result of a humoral or delayed type hypersensitivity present in only certain rabbits. Therefore 2 groups of rabbits were given multiple subcutaneous and intradermal injections over the back and legs with equal parts of Freund's complete adjuvant and streptococcal extract. Three weeks later both groups reacted with strong Arthus reactions to an intradermal injection of homologous sonic extract. One group, 6 out of 11 rabbits, developed a rash with 2 of the reactants showing a secondary extension of the lesion. In the other group, 0 out of 8 rabbits developed a rash. The presence of circulating antibody did not insure either production of a positive reaction or inhibition of the reaction.

Intradermal activity of control injections. These data are given in Table V. Only *S. typhosa* extract produced a lesion similar to streptococcal extracts.

TABLE V. Intradermal Activity of Control Injections in Causing an Erythematous Eruption.

I.D. injection	No. of rabbits	No. with typical reactions	No. with minimal reactions*
Conc. extracellular products of T18	10	0	0
Histamine	"	0	2
Epithelial homogenate	"	0	1
Idem with cysteine	"	0	3
Trypsin	"†	0	1
Saline	29	0	7
Balanced salt solution	53	0	6
S. typhosa sonic extract	10	3‡	

* Streaks of erythema; similar reactions in test animals were called negative.

† Within 5 to 15 min. after inj., all rabbits developed hemorrhage at inj. site. This reaction proceeded to lysis of epithelium during next hours.

‡ Two of the 3 reactants developed a secondary extension of the reaction. All developed a necrotic lesion. None formed remittent nodules.

Discussion. By sonic disruption of bacteria, a heterogenous population of macromolecules is probably obtained. Thus, common antigenic and toxic groups might be attached to molecules of various sizes and charges which would be dispersed widely in starch electrophoresis. The antigenic heterogeneity of the fractions and wide dispersal of the active material causing the erythematous eruption substantiate this idea.

Rashes and eruptions are found in many human streptococcal infections. This reaction resembles the eruption seen in human erysipelas. The eruption may be due to a preexisting state of hypersensitivity in some of the rabbits or to induction of a hypersensitive state by the streptococcal injection. Alternately the streptococcal injection may activate release of deleterious host products which in turn produce the reaction. Intravenous injection of trypan blue revealed persisting capillary damage at periphery of the lesion at a time when the secondary eruption normally occurred. The latent period necessary for production of the rash supports either theory. The negative results obtained in this study following both lines of reasoning do not necessarily rule out either hypothesis.

Summary. Intradermal injection of crude sonic extracts and fractions obtained by starch electrophoresis of group A streptococci produced a spreading erythema in a variable percentage of rabbits. Recurrence of the lesion developed in about 10% of rabbits after abatement of primary lesion. The variability in rabbit response made it difficult to characterize the active principle and to determine the underlying mechanism of the reaction.

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Production of Normal-Lived Erythrocytes with Erythropoietin.* (25912)

D. C. VAN DYKE AND N. I. BERLIN

Donner Lab. of Medical Physics, University of California, Berkeley; and National Cancer Inst., N.I.H., Bethesda, Md.

Although erythropoietic hormone may be an important factor in normal regulation of erythropoiesis, it can be obtained in significant amount only from animals whose erythropoiesis is markedly disturbed(1). If erythropoietin is part of the normal mechanism of erythropoiesis, one would expect administration of this hormone to result in production of normal erythrocytes. If production of erythropoietin is an emergency measure designed to flood the circulation with hastily or incompletely formed red cells, one might expect to find evidence of this in abnormal morphology, incomplete or abnormal hemoglobinization, increased fragility, or in shortened average survival time in the circulation. Gordon *et al.*(2) investigated the properties of erythrocytes produced under the stimulus

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