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Production of Generalized Shwartzman Reaction with Group A Streptococcal Factors.* (20237)

JOHN H. SCHWAB, DENNIS W. WATSON, AND WILLIAM J. CROMARTIE.

From the Department of Bacteriology and Immunology, University of Minnesota, Minneapolis, and the Departments of Bacteriology and Medicine, University of N. Carolina, Chapel Hill.

Recent studies by Thomas, Denny and Floyd(1-3) suggest that the mechanisms which bring about the generalized Shwartzman reaction may play a role in the pathogenesis of rheumatic fever and other disorders. Their studies involve the preparation of rabbits for the generalized Shwartzman reaction by infection with group A streptococci and provocation of the reaction by the injection of toxins from Gram-negative organisms. They observed that heat-killed group A streptococci and culture filtrates did not possess the property of preparing rabbits for this reaction and it was necessary to utilize products of Gram-negative organisms for the provoking injection. This paper is concerned with observations which indicate that when group A streptococci multiply in an *in vivo* environment a soluble material is produced which possesses the property of preparing rabbits for the generalized Shwartzman reaction. This reaction may be provoked in rabbits, prepared with this factor, not only by toxins from Gram-negative bacteria, but, in addition, by reduced filtrates of group A streptococcal cultures containing active streptolysin O.

Materials and methods. Streptococcal skin lesion extract. The preparation and extraction of this lesion extract was described by Watson and Cromartie(4). This technic consists of intracutaneous injection of 30 ml of an 18-hour Todd-Hewitt broth culture of type 28 group A streptococci in about 30 sites over the shaved abdomen and thorax of 2 kg New Zealand white rabbits. The moribund animals were killed 24 hours after injection of the streptococci, the lesion ground in a meat grinder and extracted overnight at 2°C by stirring with 1 to 2 ml of saline per g of tissue. This was filtered through gauze and centrifuged in the Spinco 30 head at 25,000 rpm for ½ hour. This supernatant was filtered through a Selas bacterial filter and kept frozen in sealed ampules at −70°C. Four lots of this material were used in the work reported in this paper. They are referred to as streptococcal lesion extract 108, 109, 112, or 116. The total nitrogen of extracts 108 and 109 was 1.7 mg N per ml. Extract 116 contained 3.24 mg N per ml. As a preparing dose 0.5 ml of extract 108 or 109 was used. A preparing dose of extract 116 was 0.25 ml. Lesion extract 112 was prepared as above and was then concentrated 10 fold by lyophilization and dialyzed against physiological saline for 24 hours.

Bacterial toxins. Typhoid toxin was prepared from N-28-1 strain of *Salmonella typhosa*. The organism was cultured 9 days in nutrient broth under semi-anerobic conditions. It was then filtered through a Selas bacterial filter and stored in the refrigerator under aseptic conditions Six lots of the toxin were produced. The potency of each lot was

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determined by the injection of 6 rabbits with doses varying from 1.0 ml to 4.0 ml. The toxicities of all preparations were quite constant. Three to 4 ml were required to kill a normal 1 kg American Dutch rabbit. Depending upon the type of experiment, the weights of the rabbits varied from 0.6 to 2 kg. The rabbits were injected on the basis of 1 ml per kg; the maximum dose was 1.0 ml since the susceptibility of a 2 kg rabbit was about the same as that of a 1 kg animal. *Reduced culture filtrate of group A streptococci.* A type 3, D-58 strain was grown for 16 hours in Todd-Hewitt media and then reduced and stored as described by Todd(5). It was titered for streptolysin O activity using standard anti-streptolysin O. Two preparations of this filtrate were used in the work reported in this paper. They are referred to as reduced streptococcal culture filtrate I or II. The streptolysin O titer of both lots was 12.5 combining units per ml. *Normal skin extract.* Sterile broth was injected intracutaneously in place of the streptococcal culture. The skin was then treated exactly as for the preparation of streptococcal lesion extract with the exception that after grinding and the addition of saline it was then subjected to sonic disintegration for 30 minutes in a Raytheon 9 KC oscillator. *Turpentine lesion extract.* This preparation was made exactly as was the streptococcal lesion extract with the exception that 15.0 ml of sterile wood turpentine was injected intracutaneously in place of the streptococcal culture. *Potentiation of toxins and production of the generalized Shwartzman reaction.* American Dutch rabbits weighing about 1 kg were used. The preparing material was injected intradermally or intravenously. After an interval of 4 to 16 hours the provoking injection was given intravenously.

Results. I. Potentiation of lethal effect of typhoid toxin by intradermal injection of streptococcal lesion extract. To determine if products of *in vivo*-grown streptococci could prepare rabbits for the local Shwartzman reaction, the following experiment was performed: 16 rabbits were injected intradermally with 0.5 ml of lesion extract 108 or 109. Sixteen hours later "typhoid toxin" was injected intravenously. As controls, 8 rabbits were injected intradermally with 0.5 ml of "typhoid toxin" and after an interval of 16 hours were injected intravenously with 1.0 ml of the same material. Another control group of 36 rabbits was given a single intravenous injection of 1.0 ml of "typhoid toxin." The results of this experiment are illustrated in Table I. No local lesions were produced in the 16 rabbits receiving lesion extract intradermally and "typhoid toxin" intravenously. However, 13 of the 16 animals in this group died within 24 hours after receiving the injection of "typhoid toxin." Local lesions characteristic of the Shwartzman reaction developed in 50% of the control animals receiving "typhoid toxin" intradermally and the same material intravenously. None of these animals died. Two of the control animals receiving only one intravenous injection of typhoid toxin died. These observations indicated that the intradermal injection of lesion extract in the amount used did not prepare the skin site for the local Shwartzman reaction. However, such an injection did appear to bring about a marked alteration in the general susceptibility of the animals to the intravenous injection of "typhoid toxin" possibly comparable to the preparation of an animal for the generalized Shwartzman reaction.

TABLE I. Potentiation of Lethal Effect of "Typhoid Toxin" by Intradermal Injection of Streptococcal Lesion Extract.*

Procedure	No. of rabbits	No. showing local lesions	No. dead*
0.5 ml lesion extract I.D., "typhoid toxin" I.V. 16 hr later	16	0	13
0.5 ml "typhoid toxin" I.D., "typhoid toxin" I.V. 16 hr later	8	4	0
Single inj. of "typhoid toxin"	36	0	2

* Animals observed for 72 hr. Deaths occurred within 4-24 hr.

II. *Preparation of animals for generalized Shwartzman reaction by intravenous injection of streptococcal lesion extract.* Forty rabbits were prepared with an intravenous injection of lesion extract 109 or 116. After an interval of 4 hours "typhoid toxin" was injected intra-

TABLE II. Preparation of Rabbits for Generalized Shwartzman Reaction by Intravenous Injection of Streptococcal Lesion Extracts.

Preparative material*		No. of rabbits	No. dead†	Microscopic lesions
ml				
.5	lesion extract I.V.	40	35	6 animals which survived over 8 hr studied—4 showed heart lesions, 1 showed renal lesions
.5	normal skin extract	4	0	3 animals studied—0 showed lesions
.5	turpentine lesion extract	4	0	2 " " " " "
2.0	reduced filtrate of 24 hr broth culture of streptococci	6	0	5 " " " " "

* All rabbits inj. intrav. after 4 to 16 hr with "typhoid toxin" as the provocative dose.
† Rabbits were observed 72 hr. All deaths occurred within 28 hr.

venously. As controls for this experiment the following groups of animals were studied: 4 animals were injected intravenously with 0.5 ml of normal skin extract as the preparative injection; 4 animals were treated in a similar manner using 0.5 ml of turpentine lesion extract as the preparative injection; 6 rabbits were prepared with an intravenous injection of reduced filtrate of a 24-hour broth culture of group A streptococci containing 12.5 combining units of streptolysin O per ml. All of these animals were injected intravenously 4 hours after the preparative dose with 1.0 ml "typhoid toxin" as the provocative material. Table II summarizes these observations. Of the 40 animals receiving streptococcal lesion extract as the preparative material, 35 died within a period of 24 hours. In striking contrast, none of the animals receiving normal skin extract, turpentine skin lesion extract, or a filtrate of a 24-hour broth culture of group A streptococci as the preparative material died. These studies indicate that the intravenous injection of streptococcal skin lesion extract markedly alters the susceptibility of rabbits to the lethal effect of the "typhoid toxin." It seems likely that the lethal effect produced involved the mechanisms of the generalized Shwartzman reaction. This possibility was supported by microscopic studies of the hearts and kidneys of rabbits treated in this manner; these animals died or were sacrificed at various intervals up to 72 hours after the injection of the provocative material. The results of these studies are illustrated in Table II. The lesions observed were limited to the animals prepared with streptococcal skin lesion extract. The *lesions in the hearts* consisted of scattered areas in which the myofibers had undergone necrosis. Depending upon the duration of the process at the time of study various degrees of cellular reaction were seen in the areas of necrosis. Between 48 and 72 hours many mononuclear cells with basophilic cytoplasm were seen along with multinucleated giant cells. The myocardial necrosis appeared to be the same as that described by Thomas *et al.*(1-3). No lesions comparable to those described by these workers as fibrinoid necrosis of the coronary arteries were noted. Microscopic studies on control animals receiving only one intravenous injection of "typhoid toxin" revealed no lesions in any of the tissues studied. Sections from one rabbit given sufficient "typhoid toxin" to kill the animal in about 18 hours displayed none of the lesions noted above and showed only areas of hemorrhage in the lung and liver.

III. *Production of generalized Shwartzman reaction using group A streptococcal products as preparative and provocative material.* The work of Shwartzman(6) indicates that it is difficult to obtain a factor from streptococci capable of preparing for the Shwartzman reaction, but a provoking factor is produced. If such a factor could be obtained, it should be possible to bring about the generalized Shwartzman reaction using products derived entirely from group A streptococci. The following experiments were carried out to determine whether such a factor is produced. Groups of rabbits were prepared by the intravenous injection of streptococcal lesion extract 109 or 116. After an interval of 4 hours,

TABLE III. Production of Generalized Shwartzman Reaction Using Group A Streptococcal Products as the Preparative and the Provocative Material.*

Preparative material	Provocative material	No. of rabbits	No. dead†
Streptococcal lesion extr. 109 or 116	Reduced streptococcal culture filtrates	16	13
Single I.V. inj. of 2 ml reduced streptococcal culture filtrate		12	0
Lesion extr. 109	1 ml conc. lesion extr. 112	4	0
" " "	1 ml lesion extr. 109	4	0
" " "	3 ml filtrate of 24 hr culture of type 28 streptococci	6	0
Streptococcal lesion extr. 116	2 ml reduced sterile broth	9	0

* Preparative and provocative materials given I.V. with an interval of 4 hr.
† Animals observed for 72 hr.

TABLE IV. Incidence of Myocardial and Renal Lesions in Rabbits Prepared with an Intravenous Injection of Streptococcal Skin Lesion Extract and Provoked with Various Streptococcal Products.

Provocative material*	Time of death or sacrifice, hr	No. of animals studied	No. showing myocardial lesions	No. showing renal lesions
Reduced streptococcal culture filtrate	24–72	15	11	3
Single I.V. inj. of reduced streptococcal culture filtrate	72	2	0	0
Streptococcal lesion extr. 109	72	3	0	0
Reduced sterile broth	72	5	0	0

* Preparative and provocative materials given I.V. with an interval of 4 hr.

the following materials were injected intravenously to determine their provocative properties: 3.0 ml of a filtrate of a 24-hour broth culture of type 28 streptococci; 1.0 ml of concentrated streptococcal lesion extract 112; 1.0 ml streptococcal lesion extract 109 and 2.0 ml of reduced streptococcal culture filtrate with a titer of 12.5 hemolytic units per ml of streptolysin O. Twelve control rabbits were given 2.0 ml of the reduced streptococcal filtrate without the preparative injection. Another control group was prepared with the lesion extract as described above and after 4 hours received 2 to 4 ml of sterile, reduced Todd-Hewitt broth as a provoking dose. Table III illustrates these observations. Using death as an indication of the generalized Shwartzman reaction, only the material containing active streptolysin O was capable of provoking this reaction. *Microscopic studies* of the tissues also revealed that only the material containing active streptolysin O produced lesions characteristic of the generalized reaction. The results of the microscopic studies are summarized in Table IV. The heart lesions indicated in the table are the same as those described above and appear to be the same as those described as myofiber necrosis by Thomas *et al.*(1-3). These lesions are illustrated in Fig. 1 and 2. No lesion resembling fibrinoid necrosis of the coronary arteries (1-3) was seen in this group of animals. Three of the animals in which the reaction was provoked with filtrate containing active streptolysin O showed characteristic cortical necrosis of the kidney. There was widespread coagulative necrosis of the tissues of the cortex. In many of the glomeruli the capillaries were occluded by hyaline thrombi. These lesions are illustrated in Fig. 3 and 4.

IV. *Immunization of animals against preparatory factor in streptococcal lesion extract.* Rabbits were given one intravenous injection and 3 to 5 intradermal injections of 0.5 ml of streptococcal lesion extract 109 or 116 at weekly intervals. Seven to 10 days after the last injection the animals were given a preparative dose of the lesion extract intravenously

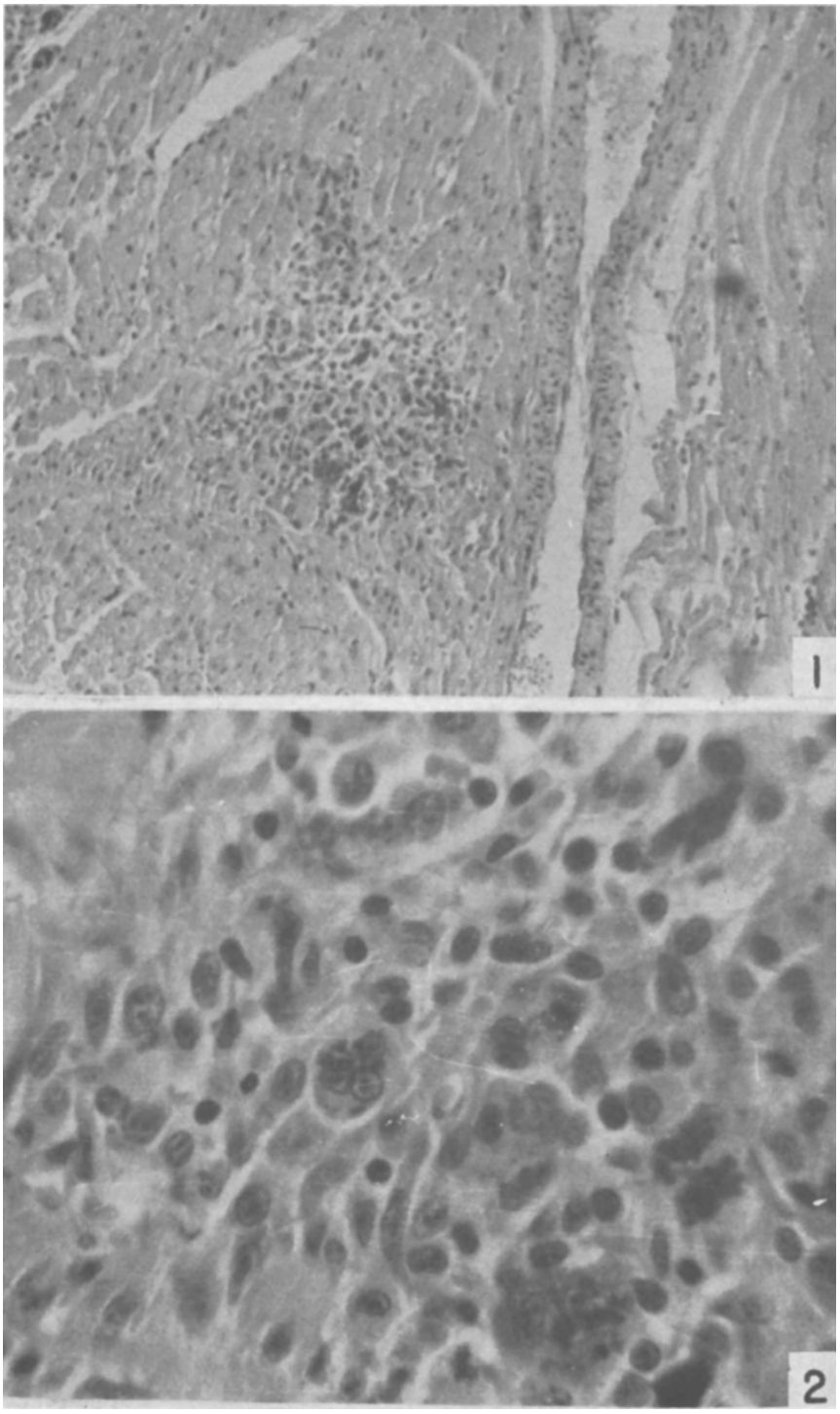
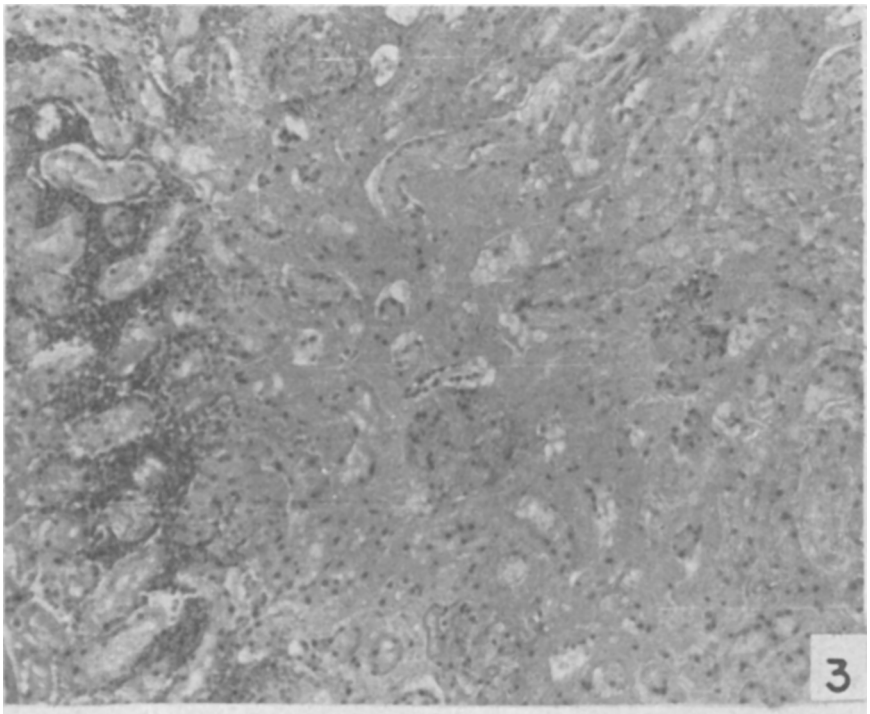


FIG. 1. Heart: Rabbit prepared with 0.5 ml skin lesion extract intravenously. Reaction provoked with 2 ml reduced filtrate broth culture of streptococci which contained 12.5 combining units of streptolysin 0. Sacrificed after 72 hr. Focal necrosis of cardiac muscle with associated inflammatory reaction. Hematoxylin and eosin stain. × 200.

FIG. 2. Higher magnification of the lesion shown in Fig. 1. Mononuclear cells and giant cells present in the area of focal necrosis. Hematoxylin and eosin stain. × 900.



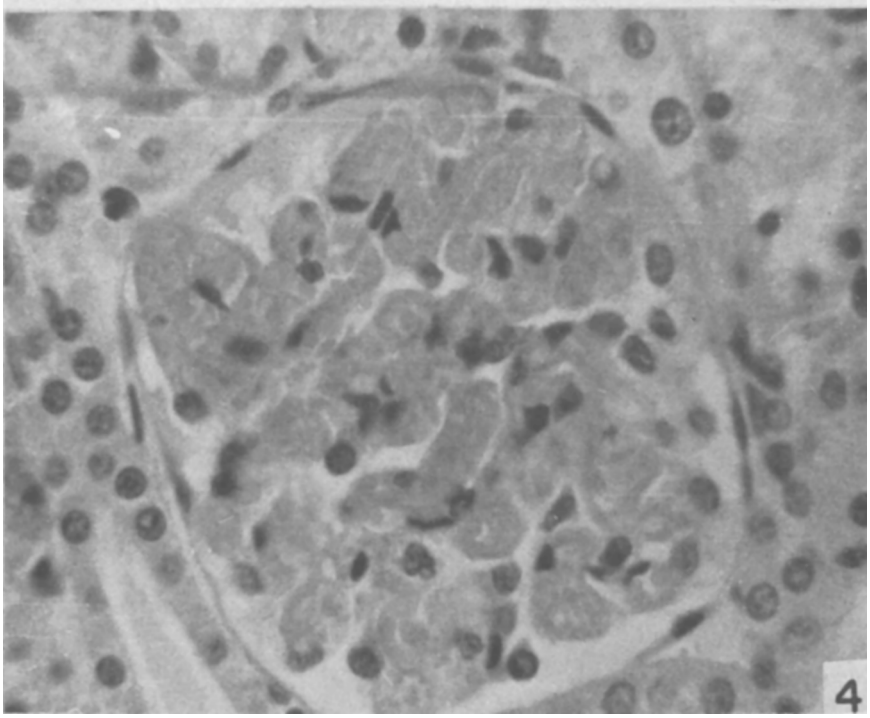


FIG. 3. Photomicrograph of section of kidney. Rabbit prepared with 0.5 ml streptococcal lesion extract intravenously. Reaction provoked by intrav. inj. of 2 ml reduced filtrate of broth culture of streptococci containing 12.5 combining units of streptolysin O. Animal died after 19 hr. Cortex shows diffuse necrosis with an associated zone of cellular infiltration beneath the capsule. Hematoxylin and eosin stain. × 200.

FIG. 4. Photomicrograph of glomerulus. Reaction produced in same manner as described above for Fig. 3. Animal died at 6 hr. Glomerular capillaries are filled with a homogenous acellular material. Hematoxylin and eosin stain. × 900.

TABLE V. Immunization of Rabbits against the Preparative Factor in Streptococcal Lesion Extracts.*

Exp. No.	Immunized	Control
1	1/6†	4/5†
2	0/9	8/8
3	1/6	6/7
Total	2/21	18/20

* Rabbits prepared with homologous lesion extr. followed after 4 hr by "typhoid toxin."
† Dead/total.

and 4 hours later received a provocative dose of "typhoid toxin." The non-immunized control rabbits received the same preparative and provocative injections. The results of 3 such experiments are shown in Table V. A total of 18 of 20 control rabbits died in contrast to 2 out of 21 in the protected group. These results indicate that the rabbits can be immunized against the preparative material present in the lesion extract.

Discussion. It has been shown that the intradermal or intravenous injection of a soluble material produced when group A streptococci multiply *in vivo* possesses the property of increasing the susceptibility of rabbits to the lethal effect of filtrates of the Gram-negative organism, *Salmonella typhosa*. It is believed that this increased lethal effect involves the mechanisms of the generalized Shwartzman reaction. However, it should be pointed out that there are several differences between the phenomenon observed in this paper and the classical Shwartzman reaction. In our experiments no local reaction has been observed upon intradermal preparation with the whole lesion extract, but the generalized reaction is produced by this preparing route. It is possible that the hyaluronidase which is present in the lesion extract may allow a more rapid diffusion of the active factor into the circulation, thus giving the effect of an intravenous preparative dose. The observation of death of the animals prepared with the lesion extract is definitely more prominent than is usually observed in the generalized Shwartzman reaction. There is also a reduced incidence of renal necrosis which is usually characteristic of the generalized reaction. However, the cardiac lesions recorded in this paper are similar to those reported by Thomas *et al.*(1-3) as typical of a generalized Shwartzman reaction. Finally, the time interval between the preparative and provocative injections can apparently be reduced to a much shorter time in our experiments than is possible in the classical reaction described by Shwartzman(6). Of primary significance is the observation that reactions in rabbits can be provoked with reduced filtrates of streptococcal cultures. Filtrates in which the streptolysin O activity was maintained appeared to be the most effective provocative material under the conditions of these experiments. The fact that streptolysin O is the active factor in the provocative injection is difficult to prove without having a pure product with which to work. Further studies to clarify this point are being carried out at the present time.

The fact that rabbits can be prepared and provoked for the generalized Shwartzman reaction with factors derived entirely from group A streptococci adds significance to the possible role of this reaction in the pathogenesis of the nonsuppurative sequellae associated with group A streptococcal infections.

The most common lesions observed were found in the heart; however, renal cortical necroses were noted in a number of animals prepared with skin lesion extract and provoked with culture filtrate in which the streptolysin O activity was maintained. The cardiac lesions were similar to those referred to by Thomas *et al.*(1-3) as myofiber necrosis. The fibrinoid necrosis observed in the arteries of the heart by Thomas *et al.* was not observed in these studies.

It is of interest to note the small quantity of the active factor in the lesion extract which is required to prepare the animals. The protein concentration of the extract, based on total nitrogen, is approximately 2%. Electrophoretic studies indicate that most of this is rabbit serum protein. Thus, the 0.25 ml volume injected must contain a minute amount of the active material. The *nature of the resistance* of the rabbits immunized to the preparing factor is not known. It is probably not the same as that developed against bacterial pyrogens as described by Morgan(7), and Beeson (8,9). Studies, not yet complete, indicate that the resistance described in this paper is type

specific. The *nature of the soluble material* extracted from group A streptococcal skin lesions which is responsible for preparing animals for the generalized Shwartzman reaction is being investigated. The precise relationship of this product and the provoking factor in the reduced culture filtrate to the generalized Shwartzman reaction is under study.

Summary. A soluble factor from group A streptococcal skin lesions prepared American Dutch rabbits for the generalized Shwartzman reaction. The reaction can be provoked in rabbits prepared with this material not only with toxins from Gram-negative organisms but also by reduced filtrates of streptococcal cultures with a high streptolysin O content. The tissue changes associated with the reaction brought about with these materials include myocardial necrosis and cortical necrosis of the kidney. Although these reactions simulate those observed in the classical generalized Shwartzman reaction, points at difference are discussed. It was possible to immunize rabbits against the preparative activity of the soluble factor by repeated injections of the streptococcal skin lesion extract.

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Renal Function During Viomycin* Administration to Dogs. (20238)

JOHN H. MOYER AND CARROLL A. HANDLEY. (With the technical assistance of Miss C. Polk Smith, Mr. A. P. Kimball, and Mrs. Elizabeth Muchmore.)

From the Departments of Pharmacology and Medicine, Baylor University College of Medicine, Houston, Texas.

An intermittent dosage schedule for administering Viomycin (2 g twice a week) to patients with pulmonary tuberculosis, proved to be free of nephrotoxicity(1). It is not apparent whether this absence of toxicity is due to administering the antibiotic intermittently or due to smaller doses than those employed in the original studies when nephrotoxicity(2,3) was observed. Therefore, before giving larger doses of Viomycin to patients, a preliminary study of nephrotoxicity and electrolyte disturbances using larger daily doses (15 to 60 mg/kg/day) in dogs seemed indicated. The purpose of the present communication is to report these observations.

Methods. Six female dogs were studied. The dogs were observed for a control period of two weeks during which time renal function (clearance) and electrolyte (plasma) studies were completed. Then they were placed on a Viomycin regimen receiving one-half the daily dose intramuscularly in the morning and one-half in the evening. Three of the dogs received a dose of 15 mg/kg for 3 months which was then increased to 30 mg/kg for an additional 3 months. Three of the dogs were started on 30 mg/kg. After 3 months this was increased to 60 mg/kg which they received for an additional 3 months. After the drug was started, renal function studies were repeated once a month as long as Viomycin was being administered. The dogs were weighed daily throughout the study. Creatinine was used to measure glomerular filtration rate, p-aminohippurate (PAH) for renal

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