STUDIES ON THE GENERALIZED SHWARTZMAN REACTION*

III. Lesions of the Myocardium and Coronary Arteries Accompanying the Reaction in Rabbits Prepared by Infection with Group A Streptococci

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(Received for publication, January 10, 1953)

Following the original description of the cutaneous Shwartzman reaction (1), various workers reported that skin tissue could be prepared for the reaction by local infection with a variety of microorganisms, including hemolytic streptococci (2), anthrax bacilli (3), tubercle bacilli (4), pneumococci (5), *Oidium albicans* (6), *Haemophilus influenzae* (7), and vaccinia virus (3, 8). When a culture filtrate from Gram-negative microorganisms capable of provoking the Shwartzman reaction was injected intravenously in animals with such skin infections, hemorrhagic necrosis occurred at the infected skin site within a few hours.

Several years before the recognition of the Shwartzman phenomenon, Sanarelli (9) reported a series of experiments which appear to have been the first demonstration of what is now termed the generalized Shwartzman reaction. This investigator produced a systemic infection with *Vibrio cholerae* in rabbits, by intravenous injection of live microorganisms, and on the following day gave an intravenous injection of filtrate from *Escherichia coli* or *Bacillus proteus* cultures. The majority of the animals died within 24 hours, and were found at autopsy to have bilateral cortical necrosis of the kidneys. Other workers have since demonstrated that this renal lesion can be consistently produced by the intravenous administration of two doses of endotoxin from a variety of Gram-negative bacteria, and it is regarded as the identifying pathological feature of the generalized Shwartzman reaction (2) (10–12). It is initiated by the appearance within the glomerular capillaries of masses of homogeneous, eosinophilic material resembling fibrinoid (12, 13); necrosis involving all structures in the cortex occurs several hours after occlusion of the glomeruli.

* This work was done under the sponsorship of the Commission on Acute Respiratory Diseases, Armed Forces Epidemiological Board, and was supported in part by the Office of The Surgeon General, Department of the Army.

The studies were also aided by grants from the National Institutes of Health, Public Health Service (HG 923), Bethesda, and the Minnesota Heart Association, Minneapolis.

In addition to the renal lesion, Sanarelli also observed extensive hemorrhagic necrosis in the walls of the small intestines, and found that large numbers of cholera vibrios were present in the involved tissues. The intestinal lesion did not occur when the order of injection was reversed, with toxin preceding the injection of vibrios, nor when heat-killed or autolysed vibrios were injected. In view of these findings, he suggested that the intestinal component of the generalized reaction was a manifestation of the selective affinity of these microorganisms for the intestine.

Observations which may be analogous were subsequently made by other workers, with other varieties of infecting agents. Bordet (6) injected *Bacillus proteus* and *Escherichia coli* culture filtrates intravenously in guinea pigs with systemic infections by *Corynebacteria* and tubercle bacilli, and observed disseminated reactions of hemorrhagic necrosis at the foci of infection in many organs. Gratia and Linz (14) produced extensive hemorrhagic lesions in the mesenteric lymph nodes and peritoneal membranes of rabbits with testicular vaccinia infections, by an intravenous injection of *E. coli* filtrate.

These findings suggest that infection by living microorganisms may not only bring about a state of preparation for the typical generalized Shwartzman reaction, but may also prepare particular organs or tissues for selective damage, depending upon the characteristics of the infecting agent. The present study represents an attempt to explore this possibility, employing infection by Group A hemolytic streptococci to prepare rabbits for the Shwartzman reaction.

It was found that Group A streptococci are capable of causing preparation for both the cutaneous and generalized Shwartzman reactions in rabbits, depending upon the route and extent of infection. In their gross appearance, the skin hemorrhages of the local reaction and the lesions in internal organs of the generalized reaction did not differ from corresponding lesions produced by two injections of meningococcal toxin. Microscopically, however, a new lesion was encountered in the hearts of some of the animals with streptococcal infection; this lesion was confined to the coronary arteries and consisted of necrosis and deposition of fibrinoid material within the walls of these vessels. Preliminary accounts of these observations have been presented elsewhere (15–17).

The present paper is concerned with a description of the local and generalized Shwartzman reactions in streptococcus-infected rabbits, the conditions which are necessary for the production of fibrinoid lesions of the heart, and a presentation of photographic material to illustrate histopathological details of the lesions.

Materials and Methods

Rabbits.—Albino hybrid rabbits of either sex, weighing 1.5 to 2.5 kilos, were employed in all experiments to be described. They were maintained on a diet of Purina rabbit pellets and water.

Streptococci.—A strain of type 1 Group A streptococcus, originally cultured from an infected human pharynx, was obtained from Dr. George E. Murphy of the Rockefeller Institute. This strain, designated MA-1 in this laboratory, was used in all the experiments to be described. The microorganisms were grown in bacto-brain heart infusion (Difco Laboratories) for 18 hours, and various dilutions of these cultures in this broth were used for experimental infections. Between experiments the streptococci were maintained by passage on blood agar, with occasional returns to a parent stock of lyophilized culture.

Bacterial Toxin.—Meningococcal toxin was prepared from a strain of meningococcus (44-B) which was obtained from Dr. Gregory Shwartzman, of the Mount Sinai Hospital, New York. The methods for growing the bacteria and preparing toxin have been described in a previous paper (12). Dilutions of the toxin were made in physiological saline, and the dosage will be designated by the dilution employed in each experiment. The toxin was injected intravenously, in the marginal ear vein, in a volume of 2 cc. A highly purified polysaccharide toxin derived from *Serratia marcescens* was supplied by Dr. Murray Shear, of the National Institutes of Health; the preparation and properties of this material have been described elsewhere (18).

I. THE LOCAL AND GENERALIZED SHWARTZMAN REACTIONS IN STREPTOCOCCUS-INFECTED RABBITS

The Local Shwartzman Reaction.—Cutaneous infections were produced in the abdominal skin of rabbits by the intradermal injection of broth suspensions containing varying numbers of the MA-1 strain of streptococcus, in a volume of 0.2 cc. After various periods of time, an intravenous injection of meningococcal or *S. marcescens* toxin was given. The dose of toxin was an amount sufficient to provoke the Shwartzman reaction in rabbits prepared by intradermal injection of toxin; with most batches of meningococcal toxin, this amount was represented by 2 cc. of a dilution of 1–40 or 1–80, and with *S. marcescens* toxin by an amount of 0.2 mg.

The extent of the local skin reaction to streptococcal infection, before the injection of toxin, varied with different doses of streptococci. With undiluted broth cultures, or with 10^{-1} dilutions, there was invariably a vigorous inflammatory reaction within a few hours, and on the following day the skin presented an area of redness and swelling 3 to 4 cm. in diameter. With 10^{-2} or higher dilutions of streptococci, the skin reactions were milder and occurred in a smaller percentage of animals.

When toxin was injected intravenously 24 hours after the streptococci, extensive reactions of hemorrhage and necrosis occurred in the involved skin within 2 or 3 hours, in the majority of rabbits prepared with large infecting doses. In their gross appearance, the lesions were indistinguishable from the usual Shwartzman reaction produced with intradermal toxin. When fewer streptococci were used for preparation, or when longer time intervals between infection and the injection of toxin were employed, the incidence of the reaction diminished sharply. With dilutions higher than 10^{-2}, or with time intervals longer than 5 days, no reactions occurred. These observations are illustrated in Table I. When the order of injection was reversed, and streptococci injected 24 hours after toxin, the results were negative.

Living streptococci were necessary for preparation of the skin. No reactions occurred with heat-killed suspensions of streptococci, or with sterile filtrates of streptococcal cultures. The administration of large doses of penicillin 12

TABLE I

Production by S. marcescens Toxin of the Local Shwartzman Reaction in Skin Sites Infected by Group A Streptococci

Dosage of streptococci*	Time interval‡	Local Shwartzman reaction	
		No. of rabbits	No. positive
	days		
Undiluted	1	6	3
	3	6	4
	5	5	2
	10	6	0
10^{-2}	1	6	2
	3	6	1
	5	3	0
	10	3	0

* Each animal received an intradermal injection of 0.2 cc. of the indicated dilution of an 18 hour broth culture of streptococci.

‡ Interval between the intradermal injection of streptococci and the intravenous injection of 0.2 mg. of *S. marcescens* toxin.

TABLE II

Prevention by Penicillin of the Local Shwartzman Reaction in Cutaneous Streptococcal Infections

Group	Local Shwartzman reaction	
	No. of rabbits	No. positive
Penicillin-treated*	5	1
Untreated	5	4

All rabbits prepared by intradermal injection of undiluted broth culture of streptococci; 2 days later all received 0.2 mg. *S. marcescens* toxin by vein.

* Each rabbit received two injections of 200,000 units of crystalline penicillin G intraperitoneally, 12 and 16 hours before toxin.

hours before the injection of toxin prevented the occurrence of the Shwartzman reaction, as is shown in Table II.

The Generalized Shwartzman Reaction in Systemic Streptococcal Infections.— In the course of the foregoing experiments, it was noted that some of the rabbits with severe cutaneous streptococcal infections became extremely ill within a few hours after the intravenous injections of meningococcal or *S. marcescens* toxin, at about the time when hemorrhage appeared in the skin.

Some of these animals showed increasing evidences of prostration during the next 24 hours and then died, and at autopsy they were found to have bilateral cortical necrosis of the kidneys. The observations indicated that a severe skin infection was accompanied by sufficient systemic involvement to prepare the animals for the generalized as well as the local Shwartzman reaction.

The production of systemic infection by an intravenous injection of streptococci resulted in a markedly enhanced state of preparation for the generalized Shwartzman reaction. A set of experiments designed to determine the optimal dosage of streptotococci is illustrated in Table III.

TABLE III

The Production by Meningococcal Toxin of Bilateral Cortical Necrosis of the Kidneys in Rabbits with Systemic Streptococcal Infection

Dose of streptococci*	No. of rabbits	No. dead‡	No. with renal cortical necrosis
Undiluted	9	9	7
10^{-1}	8	8	7
10^{-2}	9	6	3
10^{-3}	10	2	0
10^{-4}	4	0	0
10^{-6}	4	0	0

* All rabbits injected intravenously with 1 cc. of indicated dilution of 18 hour culture; 48 hours later all received 2 cc. of a 1–40 dilution of meningococcal toxin by vein.

‡ Numbers refer to rabbits which died within 48 hours after the injection of toxin. All surviving were sacrificed at 48 hours.

Rabbits weighing approximately 1.5 kilos each were given an intravenous injection of 1 cc. of various dilutions of cultures of the MA-1 strain of streptococci. 2 days later they received an intravenous injection of 2 cc. of a 1–40 dilution of meningococcal toxin.

Bilateral cortical necrosis of the kidneys and death occurred in the majority of animals given streptococci in concentrations ranging from undiluted cultures to 10^{-2} dilutions. The duration of the state of preparation for the reaction was found to depend upon the dose of streptococci administered. The animals given undiluted cultures remained prepared for 5 or 6 days, while those receiving 10^{-2} dilutions failed to react when toxin was injected later than the 2nd day after infection. When the order of injections was reversed, and streptococci given 24 hours after toxin, the generalized Shwartzman reaction did not occur.

As in the local Shwartzman reaction, it was essential that living streptococci be used. Intravenous injections of heavy concentrations of heat-killed microorganisms, or of 10 to 15 cc. amounts of sterile filtrates of whole cultures, failed to prepare animals for the generalized Shwartzman reaction. Pre-

vention of the reaction by penicillin was demonstrated in infected animals by the following experiment:—

8 rabbits received an intravenous injection of an undiluted culture of the MA-1 strain. On the 3rd and 4th days thereafter, 4 of the animals were given penicillin intraperitoneally, in a dose of 200,000 units twice daily. On the 4th day meningococcal toxin was injected intravenously in all 8 in a dilution of 1–10.

Renal necrosis occurred in 3 of the 4 untreated animals but in none of the penicillin-treated group.

The Kidney Lesions of the Generalized Reaction.—In their gross and microscopic appearance, the renal lesions in animals injected with streptococci followed by meningococcal toxin were similar to those previously observed when the generalized Shwartzman reaction was produced by two injections of toxin (12). In both instances, the earliest change in the kidney was the appearance of homogeneous, eosinophilic material within the lumen of the glomerular capillaries, 3 to 4 hours after the provoking injection of toxin. Several hours later, necrosis of the tubules and other cellular elements in the cortex became evident, presumably as a sequel to interruption of the glomerular circulation. The nature of the material occluding glomerular capillaries is not known. It was stained deep blue by azocarmine, and was strongly positive in the Hotchkiss-McManus modification of the Schiff reaction. In some specimens it bore a histological resemblance to fibrin, and appeared to be deposited in layers around the inner surface of the capillary; an illustrative glomerulus is shown in Fig. 1. In others it presented a more homogeneous appearance, occurring in masses which filled the glomerular capillary lumen; such a glomerulus is shown in Fig. 2. The histological characteristics of the material were similar to those suggested by Altschuler and Angevine (19) as criteria for the designation of fibrinoid (13).

Histological examination of other organs, including the lungs, liver, spleen, gastrointestinal tract, thymus, and lymph nodes, revealed varying degrees of hemorrhage and necrosis similar to the lesions caused by two injections of meningococcal toxin. Involvement of these tissues was most marked in the animals with severe renal necrosis.

II. LESIONS OF THE MYOCARDIUM AND CORONARY ARTERIES

Two different types of heart lesion occurred after the injection of meningococcal or *S. marcescens* toxin in rabbits with systemic streptococcal infection, and the incidence of either type was related to the dosages of streptococci and toxin employed. In severe generalized reactions, produced by large doses of toxin in animals infected with undiluted or 10^{-1} dilutions of streptococcal cultures, extensive areas of acute myofiber necrosis were observed in both ventricles. With smaller doses of streptococci and toxin, and with optimal

timing of the injections, the incidence of myofiber necrosis was sharply reduced and a new lesion involving the coronary arteries was encountered. The section to follow is concerned with a description of these lesions and the experimental conditions under which each was found to occur.

Myofiber Necrosis.—The histological appearance of the lesion of myofiber necrosis is illustrated by Figs. 3 to 5. Fig. 3 shows an early, acute lesion in the right ventricle of a rabbit which received an undiluted culture of streptococci by vein followed 48 hours later by 0.2 mg. of *S. marcescens* toxin; this animal died 3 days after toxin. Many of the myofibers in such lesions were deeply basophilic, with greatly enlarged nuclei which were sometimes packed in masses resembling syncytia. In similarly treated rabbits which survived for a week

TABLE IV

Occurrence of Cardiac Myofiber Necrosis in Streptococcus-Infected Rabbits after an Injection of Meningococcal Toxin

Dose of streptococci*	No. of rabbits	No. with myofiber necrosis
Undiluted	12	7
10^{-1}	10	6
10^{-2}	10	2
10^{-4}	4	0
Two injections of 1–40 toxin intravenously, no streptococci	24	9

* All animals received 1 cc. of the indicated dilution of streptococci intravenously, followed 48 hours later by 2 cc. of a 1–40 dilution of meningococcal toxin. The animals which survived were sacrificed and autopsied 48 hours after toxin.

or longer after toxin, illustrated by Fig. 4, the areas of myofiber necrosis contained numerous multinucleated giant cells, and deposits of calcium in and around the damaged myofibers were frequently encountered. Occasionally, collections of large mononuclear inflammatory cells with abundant basophilic cytoplasm were seen in the vicinity of areas of myofiber necrosis; one such collection, located close to a blood vessel, is shown in Fig. 5.

The incidence of myofiber necrosis, and its relation to the dose of streptococci employed for infection, are shown in Table IV. It will be seen that the lesion occurred in more than 50 per cent of rabbits receiving undiluted or 10^{-1} dilutions of streptococci, in a smaller percentage given the 10^{-2} dilution, and in none of those receiving higher dilutions.

The lesion did not appear to be the result of any special property of the streptococcus in preparing rabbits for the generalized Shwartzman reaction, since similar areas of myofiber necrosis were produced in uninfected animals by two intravenous injections of meningococcal or *S. marcescens* toxin. As is

shown in Table IV, the incidence of the lesion was approximately the same as in rabbits prepared by streptococcal infection.

Fibrinoid Necrosis of the Coronary Arteries.—In rabbits which were infected with streptococci in doses smaller than those required for the production of myofiber necrosis, a lesion of the coronary arteries occurred within 2 days after the injection of meningococcal or *S. marcescens* toxin. This lesion, which was not encountered in organs other than the heart, consisted of the accumulation of masses of homogeneous, eosinophilic material with the staining properties of fibrinoid, within the substance of the arterial wall. When

TABLE V

Occurrence of Fibrinoid Necrosis of the Coronary Arteries in Streptococcus-Infected Rabbits after an Injection of Meningococcal Toxin

Procedure	No. of rabbits	No. dead	Fibrinoid necrosis of coronary arteries*	
			No.	Per cent
10^{-2} streptococci intravenously, meningococcal toxin 48 hours later	54	37	28	51
10^{-2} streptococci intravenously, no toxin	24	0	0	0
Meningococcal toxin intravenously, no streptococci	24	0	0	0
Two injections of meningococcal toxin intravenously	30	14	0	0

The dose of meningococcal toxin was 2 cc. of a 1–40 or 1–80 dilution intravenously.
* See text for description of lesion.

present in the media or adventitia, it was usually associated with varying degrees of necrosis of the wall; when confined to the intima, necrosis was less evident. For convenience, the lesion will be referred to in the text to follow as "fibrinoid necrosis."

The incidence of fibrinoid necrosis in the coronary arteries is shown in Table V. In a series of rabbits which received an intravenous injection of a 10^{-2} dilution of streptococci, followed 48 hours later by a 1–40 or 1–80 dilution of meningococcal toxin, fibrinoid necrosis occurred in 28 of 54 animals. In control rabbits given similar doses of streptococci without toxin, or toxin without streptococci, or toxin preceding instead of following streptococci, no arterial lesions occurred. Moreover, as is shown in Table V, fibrinoid necrosis was not encountered in any of a group of 30 rabbits in which typical generalized Shwartz-

man reactions were produced by two intravenous injections of meningococcal toxin.

The Histopathology of Fibrinoid Necrosis.—A series of photomicrographs is shown in Figs. 6 to 22, to illustrate the histological appearance of the arterial lesion in a representative series of rabbits. Subendothelial deposits of homogeneous eosinophilic material within the intima of the coronary arteries in 5 animals are shown in Figs. 6 to 10. The histological appearance of the material was strikingly similar to the substance occluding the glomerular capillaries in animals with renal cortical necrosis, as is indicated by a comparison of the artery in Fig. 6 with the glomerulus in Fig. 2; both of these sections are from the same rabbit. The deposits in coronary arteries were also found to be strongly Schiff-positive, as was previously shown for the glomerular material.

In Fig. 10, the subintimal fibrinoid material appears to be extending into the media of the artery. More advanced deposits of fibrinoid involving the media and adventitia, with necrosis of the vessel wall, are shown in Figs. 11 to 16. The focal nature of the lesion is indicated by Fig. 12, which shows an area of necrosis and fibrinoid infiltration in one segment of the artery, with an immediately adjacent section of the wall which seems relatively intact. Depositions of fibrinoid which involved not only the adventitia but also areas outside the arterial wall are illustrated in Figs. 15 and 16.

Hemorrhage into the arterial wall at the site of fibrinoid deposition was observed in some hearts, and varying degrees of myofiber necrosis and inflammation occurred in the myocardium adjacent to the involved vessel. Illustrative lesions are shown in Figs. 11 and 14.

In some of the hearts with fibrinoid necrosis, collections of mononuclear cells with basophilic cytoplasm were encountered close to the involved arteries. In the animals which died 24 to 36 hours after toxin, as is shown in Figs. 6 and 8, the inflammatory cells were loosely scattered in the area just outside the adventitia. More extensive periarterial cellular infiltrations occurred in rabbits surviving for longer periods. For example, Figs. 15 and 16 show vessels with adventitial accumulations of fibrinoid associated with collections of inflammatory cells immediately adjacent to the wall; these sections are from a rabbit which was sacrificed 3 days after the injection of toxin.

Deposits of fibrinoid also occurred within the substance of the mitral and aortic valves or on the valve surfaces. Illustrative valve lesions are shown in Figs. 17 to 19. The affected valves also showed swelling, fragmentation of the ground substance, and large numbers of mononuclear or multinuclear cells with basophilic cytoplasm. Many of the mononuclear cells in such areas had the appearance of Anitschkow cells, as is illustrated in Fig. 20.

A search for bacteria in the heart tissue was made in all specimens showing fibrinoid necrosis. In one longitudinal section through the wall of an artery with subintimal fibrinoid, shown in Figs. 21 and 22, a collection of bodies re-

sembling chains of cocci was seen, apparently within the intima of the vessel. No microorganisms were demonstrable within the lumen of the involved artery, or in other parts of the heart. This observation could not be duplicated in any of the other hearts examined, but is considered to be of sufficient interest to warrant photographic mention.

The lesion of fibrinoid necrosis was apparently confined to the arteries of the heart, as far as could be determined. Examination of histological preparations of the lungs, liver, spleen, and kidneys from animals with the coronary lesion failed to reveal any accumulations of fibrinoid in the vessels of these tissues.

Factors Affecting the Incidence of Fibrinoid Necrosis

The optimal conditions for the production of fibrinoid necrosis were studied in groups of rabbits given various doses of streptococci and meningococcal

TABLE VI

The Influence of the Dosage of Streptococci on the Incidence of Fibrinoid Necrosis of the Coronary Arteries

Dose of streptococci	No. of rabbits	No. with renal cortical necrosis	No. with fibrinoid necrosis of coronary arteries
10^{-1}	8	7	0
10^{-2}	9	5	5
10^{-4}	6	0	0

All rabbits received 2 cc. of a 1–80 dilution of meningococcal toxin intravenously 48 hours after the streptococci.

toxin, with various time intervals between the injections. It was found that certain dosage and timing factors were of crucial importance in determining the incidence of the coronary artery lesion.

The Effect of the Dosage of Streptococci.—The difference in the incidence of fibrinoid necrosis in rabbits prepared with different doses of streptococci is illustrated by the results of the following experiment.

Three groups of rabbits were given intravenous injections of streptococci in 10^{-1}, 10^{-2}, and 10^{-4} dilutions, respectively. 2 days later, they received 2 cc. of a 1–80 dilution of meningococcal toxin. All the animals died or were sacrificed between 24 and 36 hours after administration of toxin.

The incidence of fibrinoid necrosis of the coronary arteries is shown in Table VI.

Fibrinoid necrosis occurred in 5 of the 9 rabbits which received the 10^{-2} dilution of streptococci, but in none of those given the 10^{-1} or 10^{-4} dilutions.

The results indicate that for this strain of streptococcus there exists a relatively narrow range of optimal dosage for the production of the lesion.

This experiment also illustrates the lack of correlation between the occurrence of arterial fibrinoid necrosis and bilateral cortical necrosis of the kidneys. The incidence of the renal lesion was approximately the same in the groups receiving the 10^{-1} and 10^{-2} dilutions of streptococci, while the arterial lesion occurred only in the latter group. The occurrence of coronary fibrinoid necrosis in the absence of demonstrable kidney damage was demonstrated in other experiments in which a total of 15 rabbits were found to have coronary lesions without any gross or microscopic evidence of renal necrosis.

TABLE VII

The Influence of the Dosage of Meningococcal Toxin on the Incidence of Fibrinoid Necrosis of the Coronary Arteries in Streptococcus-Injected Rabbits

Experiment No.	Dose of toxin	No. of rabbits	No. with fibrinoid necrosis of coronary arteries
1	1–40	12	3
	1–80	12	5
	1–160	12	0
2	1–80	10	0
	1–160	10	3

All rabbits received 1 cc. of a 10^{-2} dilution of streptococci. The toxin was given in the indicated dilutions in 2 cc. amounts 48 hours after the streptococci.

The Effect of the Dosage of Meningococcal Toxin.—The possible existence of an optimal range of toxin dosage is indicated by the results in Table VII. In the first experiment shown, a somewhat higher incidence of fibrinoid necrosis occurred with the 1–80 dilution of meningococcal toxin than with 1–40. The narrowness of the effective range of dosage is indicated by the absence of lesions in the animals receiving the 1–160 dilution. In the second experiment, in which a different batch of toxin was employed, the 1–160 dilution was more effective than 1–80.

Although the differences among the groups are not great, the results suggest that large doses of toxin may be less active than smaller amounts in producing fibrinoid necrosis.

The Effect of the Time Interval between the Injection of Streptococci and Toxin.—The importance of the timing of injections is indicated in Table VIII, which summarizes the results of the following experiment.

Four groups of rabbits were prepared by an intravenous injection of a 10^{-2} dilution of streptococci, and received the provoking injection of meningococcal toxin after intervals of

1, 2, 4, and 6 days respectively. Sections of the heart tissue of all were taken between 24 and 36 hours after toxin.

Fibrinoid necrosis of the coronary arteries occurred in 3 of 4 rabbits which were given toxin at the 2 day interval, but in none of the other animals.

The Effect of the Time Interval between the Injection of Toxin and Death.— The length of time which elapsed between the injection of toxin and the death or sacrifice of the animals appeared to have an important effect on the incidence of fibrinoid necrosis. No lesions were found in rabbits dying within 6 hours or less, and the majority were seen in animals which died or were sacri-

TABLE VIII

The Effect of the Time Interval between Injections of Streptococci and Toxin on the Incidence of Fibrinoid Necrosis of the Coronary Arteries

Time interval	No. of rabbits	No. with fibrinoid necrosis of the coronary arteries
days		
1	8	0
2	4	3
4	4	0
6	5	0

TABLE IX

The Incidence of Fibrinoid Deposition in the Coronary Arteries in Rabbits Sacrificed at Different Times after the Injection of Toxin

Day sacrificed	No. of rabbits	No. with fibrinoid necrosis of the coronary arteries
2	4	3
4	4	1
8	4	0

ficed between 24 and 48 hours after toxin. When the animals survived for longer periods, the incidence of lesions was sharply diminished. This finding is illustrated by the following experiment:—

12 rabbits were given an injection of a 10^{-2} dilution of streptococci, and 48 hours later all received toxin in a dilution of 1-80. All appeared ill on the 2nd day after toxin, and 4 were sacrificed. On the 4th day the survivors seemed to have recovered from their illness, and 4 were sacrificed. The remaining 4 were killed on the 8th day.

The incidence of fibrinoid deposition in the coronary arteries was markedly different in the three groups. Of the 4 examined on the 2nd day, 3 had the lesion, as contrasted with only 1 of the 4th day group, and none of the 8th day group. These observations, summarized in Table IX, indicate that the accumulation of fibrinoid in the vessel walls is a process which does not continue for longer than a day or two after toxin, and may be a reversible phenomenon.

Streptococcal Bacteriemia in Animals with Fibrinoid Necrosis.—The presence of streptococcal bacteriemia did not appear to be a necessary condition for the occurrence of fibrinoid necrosis, nor was the incidence of bacteriemia enhanced by the injection of meningococcal toxin. Blood cultures were performed at the time of sacrifice or death in a consecutive series of 24 rabbits in which the arterial lesion was produced by the method described above using 10^{-2} streptococci, a 1–80 dilution of meningococcal toxin, and an interval of 2 days between the injections. The blood cultures were positive in 8 animals and negative in 16, an incidence of bacteriemia not significantly different from that observed in control rabbits given the same dose of streptococci without toxin.

DISCUSSION

It has been shown that a streptococcal infection of the skin prepares the area for provocation of the local Shwartzman reaction by Gram-negative bacterial toxin, and a systemic infection produced by intravenous injection of streptococci brings about a state of preparation for the generalized reaction. In both cases living streptococci are required, as indicated by the finding that heat-killed organisms and culture filtrates are without effect, and vigorous penicillin treatment prevents both the local and generalized reactions.

With the exception of the lesion of the coronary arteries, the generalized reaction in streptococcus-infected rabbits resembled that which results from two intravenous injections of meningococcal toxin. The same type of hemorrhagic and necrotizing lesion occurred in the renal cortex, and occlusion of the glomerular capillaries by homogeneous material with the appearance of fibrinoid was demonstrated in both reactions. Both groups of animals exhibited various degrees of hemorrhagic necrosis in the lungs, liver, and spleen. Necrosis of the cardiac myofibers was a conspicuous lesion in the animals with severe streptococcal infection, but also occurred after two injections of meningococcal toxin in uninfected animals. In some respects, this type of myocardial damage resembled the lesions of myofiber necrosis described by Murphy and Swift (20) in rabbits subjected to repeated streptococcal infections over long periods of time.

The occurrence of fibrinoid necrosis of the coronary arteries following the injection of meningococcal or *S. marcescens* toxin was only observed in rabbits which were prepared for the reaction by systemic streptococcal infection, and only under optimal circumstances involving the dosages of streptococci and toxin, the time interval between the injections, and the duration of life after the injection of toxin. With the strain of streptococcus employed in these experiments, a broth dilution of 10^{-2} yielded the largest number of positive results. The amount of toxin was also of importance, and large amounts appeared to be less effective than smaller doses. The optimal time interval between the injections was 48 hours, and the optimal time for examination of the heart tissue was between 24 and 48 hours after toxin. Under these conditions ap-

proximately 50 per cent of the rabbits showed necrotizing lesions of the coronary arteries, with accumulations of fibrinoid material within the arterial walls and also in the mitral and aortic valves. The vascular lesion was confined to the arteries of the heart, and was not demonstrated in other organs.

The origin of the vascular fibrinoid material is a problem of much interest. In its histological appearance and staining properties it closely resembles the homogeneous eosinophilic material deposited in the lumen of the glomerular capillaries in the early stages of development of bilateral cortical necrosis of the kidneys. In this situation it seems to be derived from the blood, rather than from an alteration in the capillary wall, and it is conceivable that deposition of similar material passing from the blood into the coronary artery walls might account for the observed lesions. More definitive information concerning the nature of the material is required before this point can be clarified.

Although bilateral renal cortical necrosis occurred in many of the animals with fibrinoid necrosis of the coronary arteries, a sufficient number of rabbits developed the latter lesion without renal necrosis to indicate that the two types of damage are not interdependent. If the kidney lesion is taken as the identifying characteristic of the generalized Shwartzman reaction, the change in the coronary arteries is not, strictly speaking, a component of this reaction. It is possible that it may represent a selective effect of streptococcal infection on the heart, and the lesion of fibrinoid necrosis may be analogous to the local Shwartzman reaction produced in cutaneous streptococcal infections. Since streptococcal bacteriemia was present at the time of death in only one-third of the animals with fibrinoid necrosis, it did not seem to be an essential condition for the development of the lesion. However, the existence of streptococcal infection in the involved tissues is not excluded by negative blood cultures. In a study to be reported elsewhere (21) it has been found that Group A streptococci may be demonstrated in cultures of various organs of infected rabbits for long periods of time after blood cultures have become negative. The possibility that this microorganism may be capable of localizing in certain tissues in the course of a systemic infection is supported by the demonstration of chains of cocci within the wall of a coronary artery, shown in Figs. 21 and 22. It is conceivable that this type of localization is responsible for selective preparation of the coronary arteries for the Shwartzman reaction. To settle the point will require more definitive techniques for the identification of streptococci in tissues; studies in this direction are in progress.

These are obvious points of resemblance between the vascular lesions of fibrinoid necrosis produced in rabbits and the changes in blood vessels which occur in certain human disease states, characterized by fibrinoid deposition, such as rheumatic fever, periarteritis nodosa, thrombotic thrombocytopenia, and disseminated lupus erythematosis. The question whether there is a meaningful relationship between the experimental and natural lesions is not within the scope of the present report.

SUMMARY

Cutaneous and systemic infections of rabbits by Group A streptococci bring about a state of preparation for, respectively, the local and generalized Shwartzman reactions, produced by intravenous injection of meningococcal or *S. marcescens* toxin.

With maximal systemic streptococcal infections, the lesions of the generalized Shwartzman reaction do not differ from those caused by two successive intravenous injections of Gram-negative bacterial toxins. The characteristic lesions of the reaction are bilateral cortical necrosis of the kidneys, hemorrhagic necrosis in the lungs, liver, and spleen, and myofiber necrosis in the myocardium.

Under optimal conditions involving the dosages of streptococci and toxin, and the time interval between the injections, a new lesion consisting of necrosis and the accumulation of fibrinoid material in the walls of the coronary arteries occurred in approximately 50 per cent of animals within 48 hours after the injection of meningococcal toxin.

Fibrinoid necrosis was not observed in the arteries of tissues other than the heart. It did not occur in control rabbits injected with streptococci alone or toxin alone, nor in animals with the generalized Shwartzman reaction produced by two intravenous injections of toxin.

Streptococcal bacteriemia was present at the time of death in one-third of the animals with fibrinoid necrosis. In one animal, a group of bodies resembling cocci in chains was seen within the wall of a coronary artery with fibrinoid necrosis.

A series of photomicrographs to illustrate the pathological changes in the hearts and kidneys of streptococcus-infected rabbits subjected to the Shwartzman reaction is presented.

The authors are deeply indebted to Professor James R. Dawson, Jr., for his advice and counsel throughout these experiments.

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EXPLANATION OF PLATES

The photographs were made by Mr. Henry Morris.

All sections are from formalin-fixed tissue, stained with hemotoxylin and eosin.

Procedure.—All rabbits received 1 cc. of streptococcal suspension intravenously, followed 48 hours later by meningococcal or *S. marcescens* toxin. Figs. 3 to 5 are from 3 animals given a 10^{-1} dilution of streptococci and 0.2 mg. of *S. marcescens* toxin. The other sections are from rabbits given a 10^{-2} dilution of streptococci and 2 cc. of a 1–80 dilution of meningococcal toxin. The time of death in each instance is recorded in the figure legend.

PLATE 50

FIG. 1. Glomerulus of rabbit 18-03. The animal died 7 hours after toxin. The glomerular capillaries contain dense masses of eosinophilic material, much of which appears to be deposited in layers along inner surface of capillaries. × 350.

FIG. 2. Glomerulus of rabbit 18-42. Died 36 hours after toxin. The material in the capillaries has a more homogeneous appearance than in Fig. 1. × 350.

FIG. 3. Right ventricle of rabbit 6-81. Died 3 days after toxin. Necrosis of myofibers with early inflammatory reaction. × 200.

FIG. 4. Left ventricle of rabbit 5-16. Sacrificed 7 days after toxin. An area of myofiber necrosis with infiltration by mononuclear inflammatory cells and multinucleated giant cells. Several eosinophilic fragments of disintegrating muscle fibers are present in this lesion. × 110.

FIG. 5. Left ventricle of rabbit 5-14. Sacrificed 6 days after toxin. A focal accumulation of inflammatory cells near a blood vessel. Lesions of myofiber necrosis similar to that shown in Fig. 4 were present in other parts of this heart. × 200.

FIG. 6. Coronary artery in left ventricle of rabbit 18-42, with deposition of fibrinoid material beneath endothelium. There is also distortion of cells in the vessel wall, and early infiltration by inflammatory cells around vessel. Died 36 hours after toxin. A glomerulus from this rabbit is shown in Fig. 2. × 50.

FIG. 7. Coronary artery in left ventricle of rabbit 14-69, with subendothelial fibrinoid. Died 30 hours after toxin. × 350.

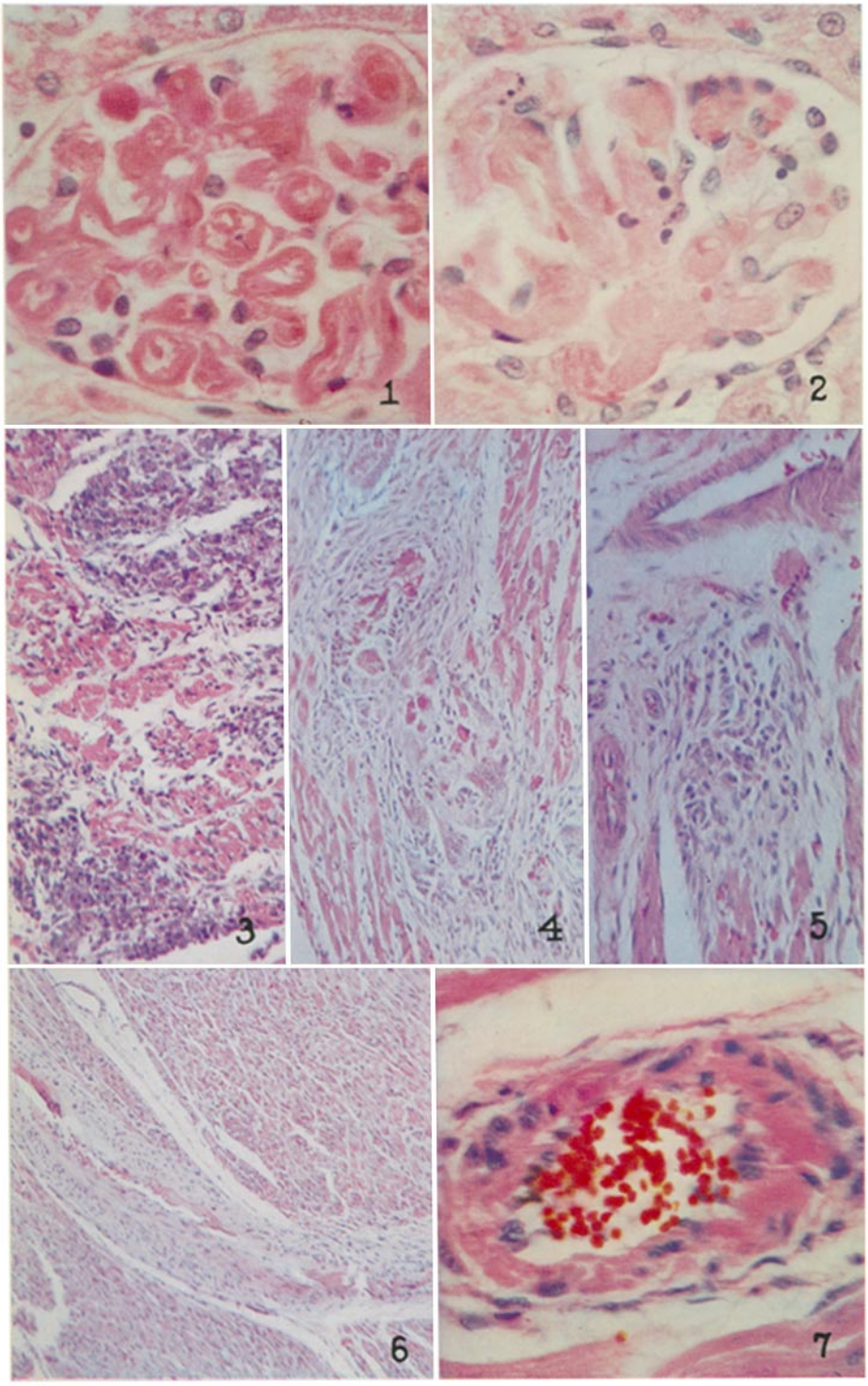

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PLATE 51

FIG. 8. Coronary artery in left ventricle of rabbit 13-49, with subendothelial fibrinoid, early necrosis of wall, and infiltration by inflammatory cells around vessel. Died 48 hours after toxin. × 225.

FIG. 9. Coronary artery in left ventricle of rabbit 18-46, with subendothelial fibrinoid. Died 36 hours after toxin. × 250.

FIG. 10. Coronary artery in ventricular septum of rabbit 17-84, with fibrinoid which appears to be extending from intima into media. Died 36 hours after toxin. × 225.

FIG. 11. Coronary artery in left ventricle of rabbit 21-34, with necrosis and hemorrhage in the wall, necrosis of adjacent myofibers, and inflammatory cell infiltration in myocardium. Died 30 hours after toxin. × 50.

FIG. 12. Coronary artery in ventricular septum of rabbit 23-71, with necrosis and fibrinoid deposition in upper portion. Note area of relatively intact vessel wall in lower third of section. Died 28 hours after toxin. × 225.

FIG. 13. Coronary artery in left ventricle of rabbit 18-11, with heavy deposit of fibrinoid occupying media and adventitia at one side of vessel. Died 48 hours after toxin. × 225.

FIG. 14. Coronary artery in left ventricle of rabbit 18-10, with fibrinoid and necrosis involving one side of vessel, and adjacent area of myofiber necrosis and inflammation. Died 24 hours after toxin. × 200.

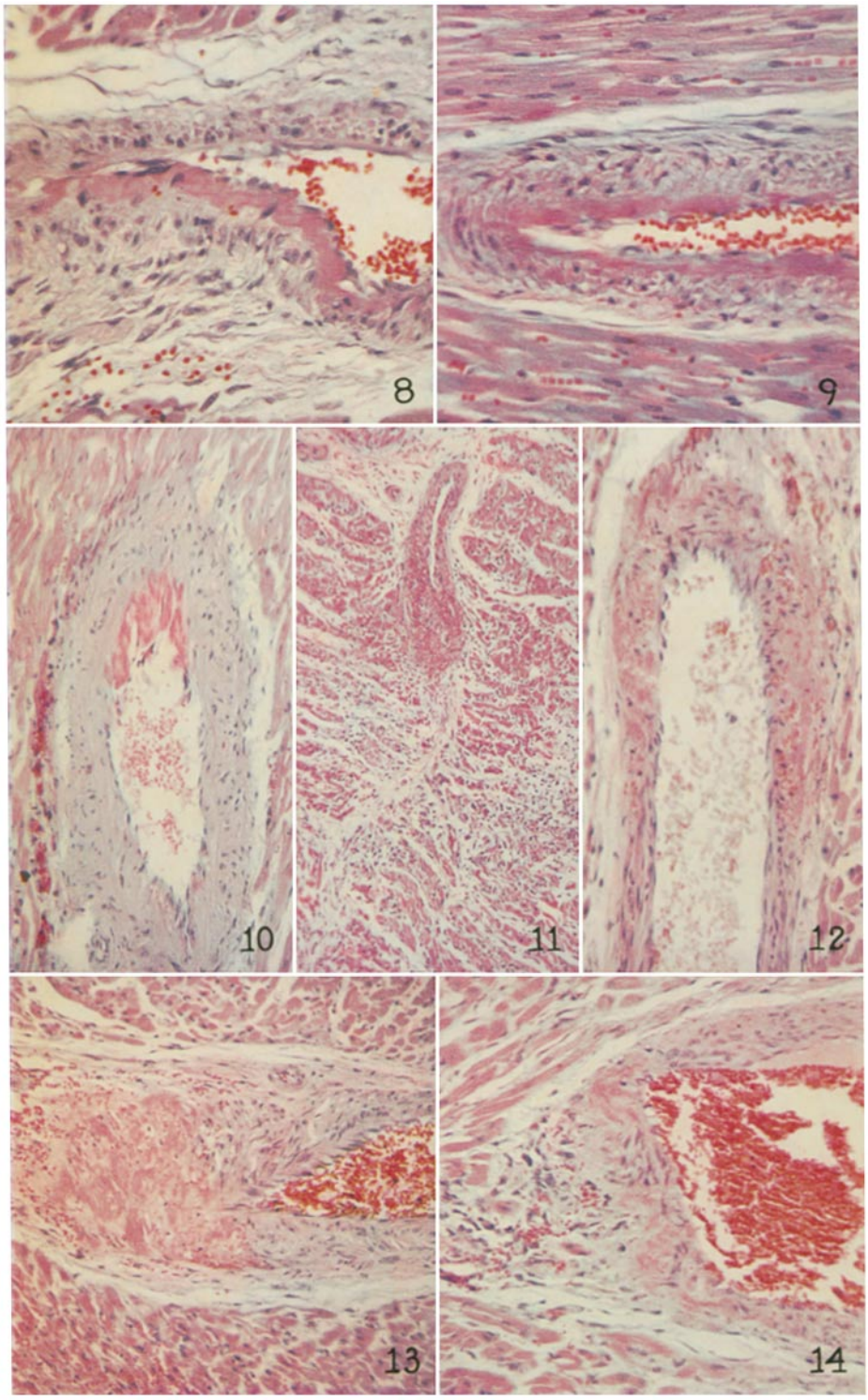

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PLATE 52

FIGS. 15 and 16. Two coronary arteries from the left ventricle of rabbit 28-20, showing accumulations of fibrinoid in adventitia and also outside vessel wall, with perivascular inflammatory reaction. Sacrificed 3 days after toxin. Fig. 15: × 250. Fig. 16: × 150.

FIG. 17. Mitral valve of rabbit 17-84, showing a small deposit of fibrinoid in substance of valve, and increased cellularity of valve. Died 36 hours after toxin. × 250.

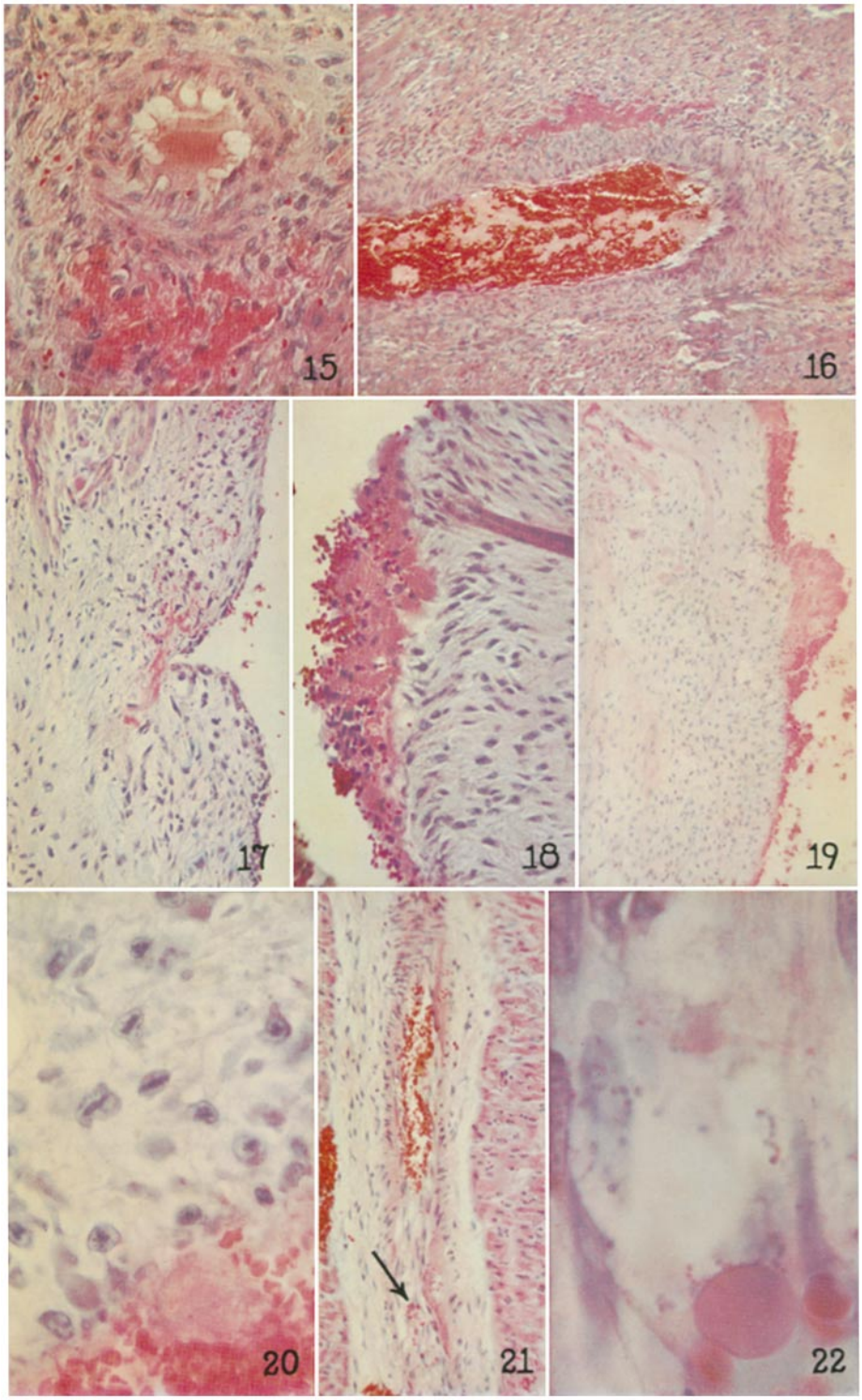
FIG. 18. Aortic valve of rabbit 18-40, with fibrinoid vegetation on surface. Died 40 hours after toxin. × 350.

FIG. 19. Mitral valve of rabbit 29-32, with vegetation on surface. Died 48 hours after toxin. × 50.

FIG. 20. Same valve as in Fig. 19, under higher magnification, to show the appearance of inflammatory cells. × 400.

FIG. 21. A coronary artery from rabbit 18-42, cut in its long axis. In the lower half of vessel the cut is apparently through the intima of the vessel wall. A circular area of eosinophilic material in the wall is marked with an arrow, for reference in Fig. 22. × 150.

FIG. 22. A higher magnification of the area indicated by the arrow in Fig. 21. Note the chains of bodies resembling streptococci. × 900.



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