

INDUCTION OF CARDIAC LESIONS, CLOSELY RESEMBLING
THOSE OF RHEUMATIC FEVER, IN RABBITS FOLLOWING
REPEATED SKIN INFECTIONS WITH GROUP A
STREPTOCOCCI

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PLATES 37 TO 42

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Elucidation of the rôle of group A streptococci in the pathogenesis of rheumatic fever might be furthered if a host alteration closely simulating this disease could be induced in laboratory animals infected with these microorganisms; but to date efforts in this direction have failed. This failure possibly stems from one or more factors: (1) lower animals may be incapable of developing the disease; (2) the streptococci employed may have been unable to induce the characteristic host alterations; (3) the experimental conditions may have been unsuitable.

Because a spontaneous disease closely resembling rheumatic fever has not been found in lower animals, its experimental induction might be impossible. The streptococci usually pathogenic for animals belong to serological groups other than A, whereas group A streptococci are chiefly pathogenic for man, and in so far as is known this group comprises the only streptococci that induce the respiratory infections preceding rheumatic fever; the host-parasite relationships among lower animals and streptococci may not be reflected in a rheumatic fever-like state. In experimental streptococcal infections, single strains have usually been employed; but valid data indicate that successive group A streptococcal infections in one person are probably caused by different serological types (1, 2). Rheumatic fever, moreover, occurs among patients in an age period and under conditions which make it probable that they had experienced one or more previous streptococcal infections.

In investigating possible relationships between rheumatic fever and various states of altered reactivity induced experimentally in animals infected with streptococci, workers in this laboratory observed the following phenomena: (a) focal infections of rabbits with viridans, group A or C streptococci resulted in the development of clear cut cutaneous and general hyperreactivity to the homologous infecting strain (3, 4), which was markedly enhanced by frequently repeated minute intracutaneous inoculations (5); (b) intravenous injections of living viridans streptococci (6), or of heat-killed vaccines of group A or C streptococci (5) induced in rabbits a state of diminished cutaneous reactivity

* This work was done during the tenure of a Life Insurance Medical Research Fellowship.

to inoculation with homologous strains; (c) these immunized animals simultaneously showed cutaneous hyperreactivity to strains belonging to heterologous groups (4, 7); (d) some rabbits immunized intravenously or subcutaneously with heat-killed vaccines of one type of group A streptococci developed decreased skin reactivity to intracutaneous inocula of the same type, but simultaneously showed greater than normal cutaneous reactivity to minute intracutaneous inocula with heterologous types, and the same often held true when the preliminary immunization was induced by repeated skin infections with a strain of a very rabbit-virulent group A streptococcus (8), and the state of cutaneous hyperreactivity was brought out far more clearly with high dilutions of the challenging inocula than with low dilutions; (e) rabbits resting 2 or more months from immunization with viridans, group A or C streptococci became cutaneously and systemically hyperreactive to the homologous strain of streptococci previously injected (7). The following information was also available: Rheumatic fever patients develop type-specific antibodies to the group A streptococcus inducing the nasopharyngeal infection preceding a rheumatic fever attack (1, 2); rheumatic fever patients, as a rule, were found to be hyperreactive to viridans and group A streptococcal nucleoproteins (9) and to group A streptococcal vaccines (10). It was later assumed that this hyperreactive state was induced by recurring focal (nasopharyngeal) infections with a succession of different serological types of group A streptococci.

These observations suggested that a rheumatic fever-like state might be induced in animals by successive focal group A streptococcal infections, each caused by a serological type heterologous to those previously employed. This communication reports the first testing of this hypothesis.

Methods

Because it was desirable to test a relatively large group of animals, and as considerable information was available concerning the reactivity of rabbits to streptococcal infections, this species was chosen. New Zealand Reds and a cross-breed designated hare brown, all bred in the Rockefeller Institute, were usually employed; occasionally chinchilla and other varieties were tested. All rabbits were fed approximately 560 gm. of Rockland rabbit ration pellets within each week. Animals with skins which mostly remained bare for considerable periods after close clipping were preferred; and such clipped sites were used primarily; but after repeated inoculations it sometimes became difficult to find very suitable skin, and coarsely hairy areas had to be inoculated.

The group A streptococci employed all exhibited matt or mucoid colony forms in 18 to 24 hours growth on moist rabbit blood agar; and were shown to produce large amounts of type-specific M protein in Todd-Hewitt broth made with neopeptone. Mostly they had only moderate virulence for rabbits, but even in this respect there was considerable variation, both among the various types employed and in different subcultures of single strains. Efforts to increase virulence by rabbit passage have been only moderately successful.

Sixteen to 20 hour Todd-Hewitt neopeptone broth cultures were serially diluted in tenfold steps with broth, and the inocula in 0.1 cc. volume, containing between 10^{-8} and 10^{-2} cc. of the original culture, were injected into closely clipped skin of right and left gluteal, lumbar, thoracic, or shoulder areas. Ten times more cocci were injected on the right side than on the

eft. In the original groups of animals, the 2nd to 4th successive focal cutaneous infections ere set up in the same well healed, but scarred, gluteal sites. Subsequently, because of re-

TABLE I
Rabbit 70-58—New Zealand ♀

Infections					Course of infections				
Date	Streptococcus type	Inocula	Skin site	Skin reaction	Date	Weight	ESR	ASO units	Remarks
1946					1946	kg.	mm./hr.		
7/17	1	10 ⁻⁸	Gl	N	7/17	3.0			
8/28	11	10 ⁻⁸	Gl	Initially >N; <N in few days	8/19	3.1			
10/ 8	17	10 ⁻⁸	Gl		10/30		1		
11/12	13	10 ⁻⁴	Gl		11/12	3.8		<25	
11/15	13	10 ⁻⁴	Gl		11/16		1		
11/19	13	10 ⁻⁸	Gl		11/25		1	25	
11/25	13	10 ⁻⁸	Gl		11/28		1	25	
1947					1947				
1/13	3	10 ⁻⁴ , 10 ⁻⁸	Gl;	Th—>N	1/10	3.7			
					1/18		1	25	
					1/20	3.8			
4/18	19	10 ⁻⁴ , 10 ⁻⁸	Gl;	Sh—>N	4/16	4.0			
					4/25		1	50	
5/28	17	10 ⁻⁸ , 10 ⁻⁸	Gl;	Lu—<N	5/27			25	
					6/ 7		1	25	
9/16	1	10 ⁻⁴ , 10 ⁻⁸	Gl;	Th—>>N	9/19				*
					9/22				Anorexia
					9/25		130	400	"
					9/26		47	>1000	"
					9/27		76		" ‡
					9/29				Died§

N, average cutaneous response of normal control rabbits to intracutaneous inoculation with streptococci.

Gl indicates gluteal; Lu, lumbar; Th, thoracic; Sh, shoulder.

* Erythema over right knee.

ESR, erythrocyte sedimentation rate (Westergren).

‡ Negative blood culture.

§ Autopsy blood culture negative.

ASO, antistreptolysin O titer.

E.S.R. of normal rabbits, 1 to 2 mm. per 1 hour and 2 to 4 mm. per 2 hours.

sults recorded below, each reinfection, usually with a type of streptococcus not previously injected, included 4 areas: right and left scarred gluteal skin sites and a right and left skin site least likely to have been locally inflamed in previous infections.

All skin lesions were measured daily until recession was demonstrated. The condition of the rabbits was observed; and their weights were recorded at suitable intervals. Blood was obtained from the ear veins for serum which was refrigerated and later tested for antistreptolysin O and antistreptokinase content, and for precipitin reactions with extracts of homologous and heterologous types of group A streptococci. These data furnished a rough index of the serological responses to the infections. During the earlier experiments erythrocyte sedimentation rate determinations (Westergren method) were made once or twice during the

TABLE II
Rabbit 71-77—Hare Brown ♂

Infections					Course of infections					
Date	Streptococcus type	Inocula	Skin site	Skin reaction	Date	Weight	ESR	WBC 1000	ASO units	Remarks
1947					1947	kg.	mm./hr.			
5/ 8	19	10 ⁻⁴ , 10 ⁻⁶	Gl;	Sh—>2 other norms	5/8	2.7				
6/20	1	10 ⁻⁴ , 10 ⁻⁵	Gl;	Lu—<<N	6/20				<25	
9/16	1	10 ⁻⁴ , 10 ⁻⁵	Gl;	Th—<<N						
11/ 6	London	10 ⁻³ , 10 ⁻⁴	Gl;	Th—=N	11/ 4				<25	
					11/19		3		75	
1948					1948					
2/ 6	3	10 ⁻³ , 10 ⁻⁴	Gl;	Th—<N	1/16			1 7.7	25	
					2/ 6	3.4				
					2/14	2.8	152	10.8	600	Anorexia, marked, cardiac irregularity* died*

* Negative blood culture ante- and postmortem.

fortnight following the inoculations; but later both erythrocyte sedimentation rate determinations and leucocyte counts were made twice or thrice weekly until they were approximately normal or until the animal died or was sacrificed

Where indicated, blood cultures were made from living rabbits with blood obtained from ear veins and placed both in Todd-Hewitt neopeptone blood broth and on rabbit blood agar plates. Blood obtained postmortem from the inferior vena cava of all dead rabbits was similarly cultured; and streptococci recovered were identified serologically.

During the first 4 to 5 months' experimentation it was found that successive monthly to bimonthly inoculations with streptococci of different serological types into the same gluteal skin sites usually induced progressively smaller local lesions than those which followed similar inoculation in comparable skin of normal controls; but in their previously uninfected (*e.g.*

thoracic) skin the same sized inoculum almost invariably induced greater local inflammation than in their multiply infected gluteal skin or in the thoracic skin of normal controls (Table I;

TABLE III
Rabbit 71-80—Hare Brown ♀

Infections					Course of infections						
Date	Streptococcus type	Inocula	Skin site	Skin reaction	Date	Weight	ESR		WBC 1000	ASO units	Remarks
							1 hr.	2 hr.			
1947					1947	kg.	mm.				
5/28	6	10 ⁻³ , 10 ⁻⁴	Gl; Lu	>2 other norms	5/28	3.4				700	
					6/7	2.7					
6/20	1	10 ⁻⁴ , 10 ⁻⁵	Gl; Th	<<N	6/20	2.8				<25	
9/16	1	10 ⁻⁴ , 10 ⁻⁵	Gl; Th	<N	9/30		1			100	
11/6	London	10 ⁻³ , 10 ⁻⁴	Gl; Sh	=N	11/3					100	
					11/14	3.3					*
					11/18	3.2	1			75	†
1948					1948						
2/6	3	10 ⁻³ , 10 ⁻⁴	Gl; Th	<N	1/19	3.4	1	16.2		25	
					2/16	3.4	4	19.3		50	
					2/19		2	14.2		75	
					2/25		3	13.5		25	
					5/26	3.5					
9/21	9	10 ⁻³ , 10 ⁻⁴	Gl; Th	=N	9/21	3.5	1	18.0			
9/22	9	10 ⁻³ , 10 ⁻³	Lu		9/22			22.5			
					9/23		5	11		<25	
					9/24	3.1	7	16	18.9	<25	
					9/26	2.9					
					9/27	2.7	29	56	30.1	50	
					9/28	2.7	33	54	21.8	50	Anorexia
					9/29		49	80		75	Diarrhea
					10/1	2.3	45	88	19.5	200	
					10/2	2.4				100	Sacrificed§

* Guards hind legs.

† Negative blood culture.

§ Autopsy blood culture negative.

January, 1947). It was, therefore, obvious that new areas were requisite to obtain a rough approximation of an animal's cutaneous reactivity to successive inoculations. Furthermore, because of this finding that succeeding streptococcal skin inflammation was likely to be more

intense in previously uninfamed skin than in scarred sites, it seemed possible that streptococci might survive longer and effect more sustained local infection in fresh skin than in scarred areas; and that from the larger inflammatory zones more toxic material might be elaborated and absorbed than from the small lesions.

Variations in the inoculation procedures are illustrated in Tables I, II, and III. Occasionally, as in November, 1946 (Table I), or in September, 1948 (Table III), repeated intracutaneous inoculations with the same type of streptococcus were given within a few days in attempts to enhance the infectious stimulus of lowly virulent strains. In other instances, as with type 1 (Tables I, II, and III), the same type was reinjected after a relatively long interval; but generally each successive inoculation in a given animal was with a type heterologous to those previously used to infect a given animal.

Autopsies were performed as soon after death as possible; those on sacrificed rabbits were carried out immediately after exitus, usually effected with intravenous sodium nembutal. Tissues were fixed in Zenker-acetic acid, and sections cut from paraffin blocks were stained with hematoxylin and eosin, Giemsa, Weigert-hematoxylin and eosin, Masson's trichrome, Mallory's aniline blue, and where indicated with Gram-Weigert and malachite green-acridine red (11), a technique applicable to Zenker-fixed tissues, whereas the Unna-Pappenheim methyl green-pyronine technique requires alcohol fixation.

RESULTS

After sustaining 2 to 10 infections with streptococci of different serological types within 3 to 20 months, some rabbits sickened and showed various combinations of the following signs and symptoms: elevated erythrocyte sedimentation rates for 1 to 2 weeks; leucocytosis; anorexia; weight loss; postexertional dyspnea; occasional transient pulmonary rales; tachycardia; and in a few instances, definitely irregular cardiac rhythm. Many of these rabbits recovered; a portion were sacrificed within 10 to 14 days following their last infection while exhibiting definite symptoms, leucocytosis, and elevated erythrocyte sedimentation rates higher than were occurring in normal controls; in several rabbits, however, a severe illness developed following the last streptococcal infection and terminated fatally, whereas some of the normal controls survived the same streptococcal infection. A few in the fatal group died within 2 to 5 days following the last infection (even though normal controls in some instances survived) and in all except one of these rapidly fatal cases, streptococcal bacteremia was established at autopsy. In about half of a group of rabbits dying spontaneously between 6 and 14 days after the last infection, streptococcal bacteremia was demonstrated at autopsy; in the other rabbits of this group, however, streptococci could not be cultured from the blood either before death or at autopsy.

In the hearts of the successively infected rabbits which had sickened and succumbed, and of those sacrificed while sick, there have been found on microscopic examination focal alterations in the connective tissue framework in blood vessel adventitia, valves, mural endocardium, epicardium, and in the myocardial interstitium. Many collagen fibers in these sites are swollen; some are intensely eosinophilic, others stain poorly; some swollen collagen fibers stain entirely, whereas others stain in patchy fashion like fibrin with both Masson's trichrome and Mallory's connective tissue techniques. Arranged about and interspersed

in fields of swollen "fibrinoid" collagen are nodular collections of large, irregularly shaped cells, often with abundant, finely granular basophilic cytoplasm which takes a smudgy red color with the malachite green-acridine red stain. Often these cells have very indistinct outlines; some have long streamer-like cytoplasmic processes which gradually fade into the contiguous areas. The vesicular nuclei, single or multiple, are variously shaped, and have sharply defined membranes. Clumping of chromatin often leaves the rest of the nucleus clear. Some nuclei are pyknotic. Cells with multiple, centrally placed nuclei, 2 to 10 in number, occur in greatest profusion in the mitral and aortic sulci and rings and in the endocardium (Fig. 3). The lesions also contain many cells of the Anitschkow myocyte type, and occasionally small round cells and polymorphonuclear leucocytes, both pseudo- and true eosinophiles. The sites of predilection for the occurrence of these nodular granulomata in most hearts are endocardial, subendocardial, and blood vessel adventitia and paraadventitia. These adventitial lesions are by no means limited to the roots of the valves, but at times are conspicuously present throughout the hearts, particularly in the left ventricle and interventricular septum.

In some hearts the granulomata occur in the loose myocardial interstitium unassociated with arteries or veins but, in most instances, with capillaries. In agreement with Gross (12) the latter are designated "myocardial granulomata" to distinguish them from granulomata associated with other cardiac structures. Interstitial valvulitis, most marked in the middle of the cusps, has occurred commonly in the mitral and aortic valves and also in the right auriculoventricular valve; these areas beneath the line of closure show edema of varying intensity and cellular components like those in the submiliary nodules. Marked proliferation of mitral and aortic valvular endocardial and subendocardial cells occurs in several hearts to create many layered palisades containing numerous multinucleated giant cells dispersed in swollen or "fibrinoid" collagen (Figs. 1 and 2). These lesions are occasionally limited to the sulci, but are also found often on both surfaces of the valve and of the chordae tendineae (Fig. 10). At times the most superficially palisaded cells have apparently undergone necrosis and conversion into acellular material that stains like fibrin. The latter phenomenon was most marked in rabbits dying spontaneously within 2 weeks after the final infection. On no valves were there seen, macroscopically, rows of fine verrucae along the lines of closure. In the gross, however, the mitral valve of several rabbits showed along the line of closure a row of fine discrete opalescent elevations usually more marked on the aortic leaflet. Microscopically these elevations consist of interstitial edema and valvulitis which in some instances are more intense than in the neighboring tissues. Occasionally larger fine, firm white nodules projecting from the surface of the valve were visible. Foci of frankly "fibrinoid" collagen¹ are seen in auricular (Fig. 12) and ventricu-

¹ The expression "frankly fibrinoid collagen" indicates that the altered collagen stains unequivocally like fibrin with Mallory's connective tissue and Masson's trichrome techniques.

lar epicardium in association with proliferated epicardial and subepicardial elements. These patches of epicarditis are microscopic in size; and no extensive plastic pericardial exudate has been detected in the gross.

Granulomata in the compact paravascular connective tissue differ in architectural configuration from the "myocardial granulomata" in the looser tissue between muscle bundles. There are submiliary granulomata closely resembling the coronal (Fig. 6), reticular (Figs. 4 and 7), and mosaic (Figs. 8, 9, and 11) types of Aschoff bodies described by Gross in human rheumatic hearts (12); and in the left ventricle and interventricular septum of a few rabbit hearts several myocardial granulomata are often seen in a low power field; but in no rabbit dying spontaneously or sacrificed within 2 weeks after final infection, have there been found well developed polarized or fibrillar types of granulomata. Gross considered the peculiarly shaped and arranged cells in such Aschoff bodies to represent terminal metamorphosis of the rheumatic granuloma cells into fibroblasts. Damage to myocardium adjacent to granulomata has been prominent, and has ranged from swelling and vacuolation of the myofibers and vesiculation of their nuclei to complete dissolution and replacement by scar. Occasionally apparent fusion of neighboring granulomata combined with extensive adjacent myocardial destruction and connective tissue replacement has resulted in macroscopically visible lesions in stained sections of left ventricle and papillary muscles.³

The coronary arterial system is variously altered. Fairly commonly there is marked intimal hyperplasia and elastification involving chiefly small arteries and arterioles. A well developed intimal musculoelastic hyperplastic lesion occurs in several rabbits. In the hearts of two rabbits there is found marked ramification of fibrinoid material throughout or surrounding the wall of a small artery or capillary (Fig. 5). Interspersed in and arranged about the extension of this intensely eosinophilic material into the tissue adjacent to the vessel are granuloma cells of the type found in the previously described rabbit granulomata. This vascular lesion closely resembles that described in rheumatic human hearts by Pappenheimer and Von Glahn (13). Panarteritis of the so called "allergic" or periarteritis nodosa type is conspicuously absent in the hearts of all intracutaneously infected rabbits. Verrucous and polypoid endarteritis are occasionally present. In the intima and immediately subadjacent media of the aorta near its root there occasionally is seen a lesion comparable with that in the valve sulci and closely resembling that described by Pappen-

³ A striking increase in size of the adrenal glands occurred in rabbits dying, or sacrificed while sick, following the last of several intracutaneous streptococcal infections. Microscopically hyperplasia, hypertrophy, and necrosis of fascicular zone cells are seen. There is striking correlation between the degree of macroscopic enlargement of the fascicular zone of the adrenal cortex and the occurrence of myocardial granulomata. Detailed data concerning these observations will be presented shortly.

heimer and Von Glahn (13) in human rheumatic aortitis. Occasionally there are foci of clearly defined fibrinoid collagen in the adventitia of the root and first portion of the aorta. These lesions will be illustrated later.

Neither bacteria nor any structures resembling inclusion bodies have been seen in the above described lesions stained according to Gram-Weigert or Giemsa techniques. There has, moreover, been no calcification of the myocar-

TABLE IV

Rabbit groups	No.	Bacteremia	Acute rheumatic fever-like cardiac lesions	Myocardial scars or healed arteritis of rheumatic type
1. Normal rabbits.....	8	—	0	0
2. I.v. vaccine (group A or C streptococci)....	8	—	0	0
3. Dying 1-18 days after 1 i.v. infection.....	20	20	0*	0
4. Sacrificed 1 and 4 mos. after 1 i.v. infection..	2	0	0	0
5. Sacrificed within 1 mo. after 1 i.c. infection..	4	0	0	0
6. Dying within 2 wks. after 1 i.c. infection....	13	12	0	0
7. Dying 3 wks. after 1 i.c. infection.....	1	1	1‡	0
8. Sacrificed 10 to 21 days after last of several i.c. infections..	37	0	7	8
9. Dying 2 to 5 days after 2nd i.c. infection....	3	2	3§	0
10. Dying 8 to 14 days after last of 5 to 9 i.c. infections.....	3	0	3	2
11. Dying 5 to 9 days after last of 2 to 9 i.c. infections.....	7	7	6	3
12. Dying several weeks after last of several i.c. infections.....	4	0	0	3
Totals.....	110	42	20	16

i.v., intravenous. i.c., intracutaneous.

* The hearts of two rabbits dying 12 and 18 days, respectively, after one i.v. inoculation show an acute exudative and necrotizing arteritis.

‡ Interstitial valvulitis marked; vascular adventitial and interstitial foci of lymphocytes and plasma cells and occasional foci of young mesenchymal cells in adventitia without demonstrable alteration of collagen; no necrotizing arteritis.

§ Slight interstitial valvulitis only.

dial lesions, a very conspicuous phenomenon in experimental myocarditis induced with filterable viruses.

Controls.—The tissues of rabbits of the same stock and breeds, both normal and subjected to various experimental procedures have been examined at intervals during the investigative period in order to learn whether comparable lesions were occurring in such control animals, for it should be recalled that a peculiar myocarditis was described by Loewe and his coworkers (14) among stocks of rabbits injected with various materials as well as among uninoculated

controls. These workers ascribed these lesions to a spontaneous epidemic in their stock. As indicated in Table IV, enough controls have been examined to eliminate fairly certainly the possibility that such an epidemic existed among the animals we used. The small focal lesions described by Miller (15) in rabbits' hearts have not been encountered among our present stock of experimental animals or controls; hence it seems probable that the cardiac lesions that developed in our animals bear no relationship to previously described spontaneous rabbit myocarditis.

Inspection of Table IV indicates that the cardiac lesions forming the basis for this report have developed only in rabbits that had undergone multiple, successive cutaneous infections with group A streptococci of different types. In most of the animals showing these lesions, ante- and postmortem blood cultures were negative, and with bacterial stains no bacterial cells could be seen in the lesions; hence it seems improbable that the fresh tissue alterations were due to a direct action of streptococcal cells at the site of injury. In some of the animals, there was evidence of terminal streptococcemia, but even so those animals dying acutely with streptococcemia following their first cutaneous infection, or from intravenous inoculation with streptococci have not developed these submiliary granulomata. Similar negative results were found in rabbits repeatedly immunized intravenously with heat-killed group A or C streptococcal vaccines, as well as in those sacrificed after one intracutaneous inoculation. It seems, therefore, that those finally dying with bacteremia following the last of multiple skin infections developed these cardiac lesions (in which repeated bacterial stains have been negative) because the final insult affected tissues peculiarly conditioned by previous focal infections. It seems quite possible that in this group of rabbits, the bacteremia was, in fact, a terminal event.

DISCUSSION

The cardiac granulomata described, which in many respects bear such a striking histopathological similarity to those of human rheumatic fever, have been encountered only in animals that had undergone multiple, successive cutaneous infections with group A streptococci of several different types. It, therefore, seems probable that the relatively long experimental period and the reconditioning that the animals' tissues underwent as a result of several focal infections with different types of group A streptococci were important factors in the pathogenesis of these lesions. In certain respects this experimental procedure follows the pattern encountered in rheumatic fever patients: they have successive infections with different types of group A streptococci, and these infections are usually focalized in the upper respiratory tract and accessory tissues. Because it was impractical to infect rabbits' throats and sinuses repeatedly, and because successive focal infections appeared hypothetically to be important, the rabbits' skin was selected for the repeated insults.

The carditis developed, moreover, following infections with the same microorganisms that have been repeatedly proven to occur in the infections that precede attacks of rheumatic fever in man. This unique sequential relationship could not be demonstrated until Lancefield's system of classification of streptococci was available (16). In rabbits made hyperreactive to viridans, group A and group C streptococci by repeated focal infections and then shocked with intravenous inoculations of homologous streptococci, cardiac lesions of this type were not encountered (17).

It seems expedient to compare the carditis herein described in rabbits with that in animals of the same species with serum disease or subjected to repeated parenteral injections of foreign protein. This will be the subject of a later communication; but available evidence seems to indicate that the over-all histopathological picture in the rabbits repeatedly infected with streptococci bears closer resemblance to that of human rheumatic carditis than does experimental serum disease carditis. The fatal termination within 6 to 14 days, of an illness developing after the last of several focal infections is a phenomenon which, to our knowledge, has not been recorded in rabbits repeatedly injected and shocked with foreign protein.

Among the random samples of rabbits subjected to the described experimental procedure, only a small portion have developed these cardiac lesions. It seems pertinent to mention that only a small proportion of human beings in this geographical area develop rheumatic heart disease, and today an even smaller proportion develop polyarthritis rheumatica. Among subjects who have recovered from previous attacks of rheumatic fever and in rheumatic families, the incidence is considerably higher. There has been no attempt to select specially susceptible stock among the animals used in these experiments.

On the basis of evidence derived from the experiments here reported and from studies of rheumatic fever in man, it seems justified to assume that similar host-streptococcus relationships may be operative and requisite in the pathogenesis of these cardiac lesions in rabbits and rheumatic carditis in man.

SUMMARY

Cardiac lesions, closely resembling those found in rheumatic fever, have developed in rabbits that sickened following multiple, successive skin infections with several serological types of group A streptococci.

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BIBLIOGRAPHY

1. Kuttner, A. G., and Lenert, T. F., *J. Clin. Inv.*, 1944, **23**, 151.
2. Rothbard, S., Watson, R. F., Swift, H. F., and Wilson, A. T., *Arch. Int. Med.*, 1948, **82**, 229.

3. Andrewes, C. H., Derick, C. L., and Swift, H. F., *J. Exp. Med.*, 1926, **44**, 35.
4. Böhmig, R., and Swift, H. F., *Arch. Path.*, 1933, **15**, 611.
5. Derick, C. L., Hitchcock, C. H., and Swift, H. F., *J. Exp. Med.*, 1930, **52**, 1.
6. Swift, H. F., and Derick, C. L., *J. Exp. Med.*, 1929, **49**, 883.
7. Böhmig, R., *Z. Hyg. u. Infektionskrankh.*, 1933, **115**, 406.
8. Hartman, T. L., and Swift, H. F., unpublished observations.
9. Swift, H. F., Hitchcock, C. H., and Derick, C. L., *Proc. Soc. Exp. Biol. and Med.*, 1928, **25**, 312.
10. Swift, H. F., Derick, C. L., and Hitchcock, C. H., *Tr. Assn. Am. Physn.*, 1928, **43**, 192.
11. Hitchcock, C. H., and Ehrich, W., *Arch. Path.*, 1930, **9**, 625.
12. Gross, L., and Ehrlich, J. C., *Am. J. Path.*, 1934, **10**, 467.
13. Pappenheimer, A. M., and Von Glahn, W. C., *Am. J. Path.*, 1927, **3**, 583.
14. Loewe, L., and Lenke, S. E., *J. Exp. Med.*, 1940, **71**, 89.
15. Miller, C. P., Jr., *J. Exp. Med.*, 1924, **40**, 543.
16. Lancefield, R. C., *Harvey Lectures*, 1940-41, **36**, 251.
17. Böhmig, R., *Verhandl. deutsch. path. Ges.*, 1934, **27**, 166.

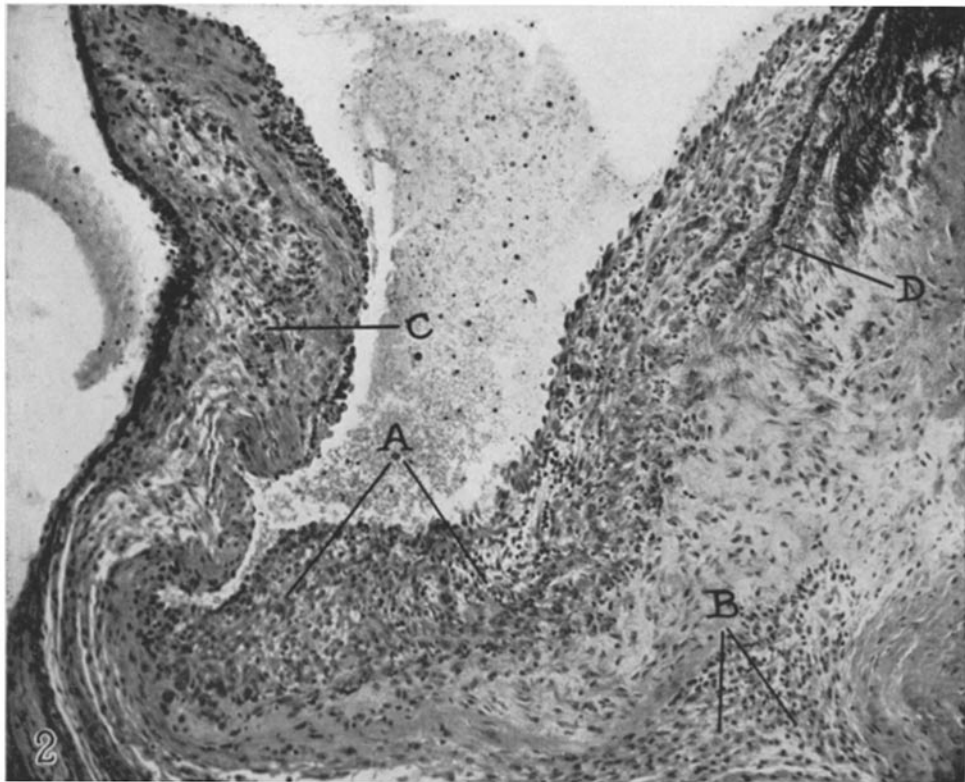
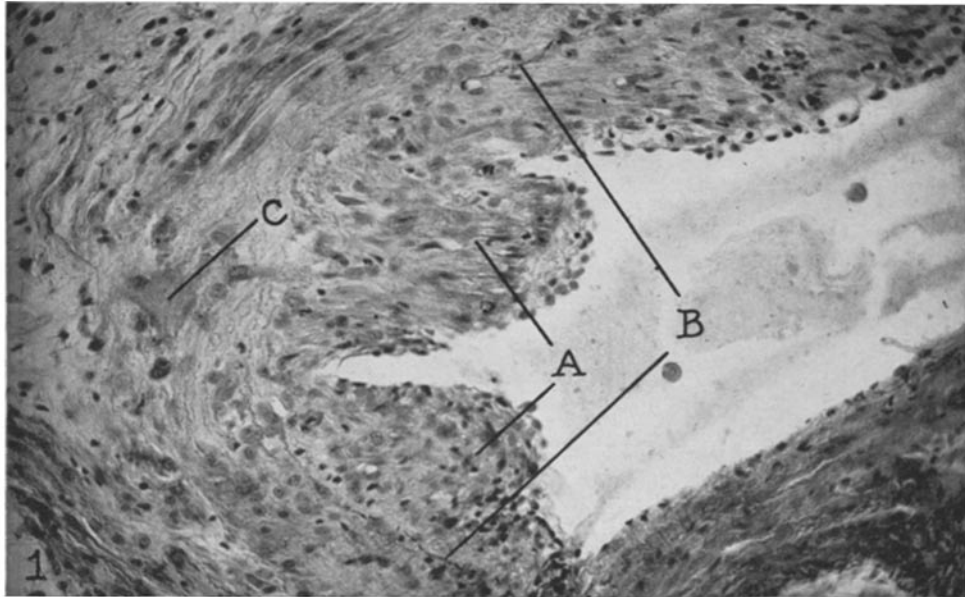
EXPLANATION OF PLATES

The photographs were made by Mr. Julian Carlile and Mr. Richard Carter.

PLATE 37

FIG. 1. Rabbit 73-13, sacrificed 15 days after last of 4 infections; no bacteremia at autopsy. *A*, polypoid endo- and subendocardial proliferation (palisade) in mitral sulcus; *B*, external elastic lamella; *C*, focus of frankly fibrinoid collagen. Weigert-hematoxylin and eosin. $\times 195$.

FIG. 2. Rabbit 71-77 (see Table II),—died 8 days after last of 5 infections; no bacteremia ante- or postmortem. *A*, extensive endo- and subendocardial proliferation (palisade) in aortic pocket; *B*, inflammation in annulus; *C*, aortic interstitial valvulitis; *D*, root of aorta. Weigert-hematoxylin and eosin. $\times 116$.

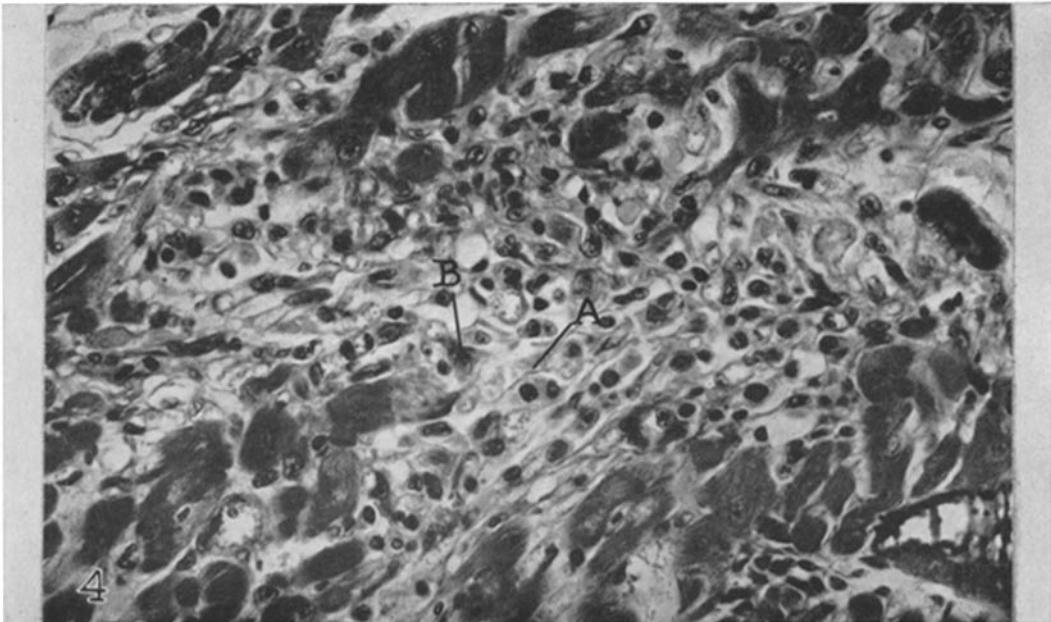
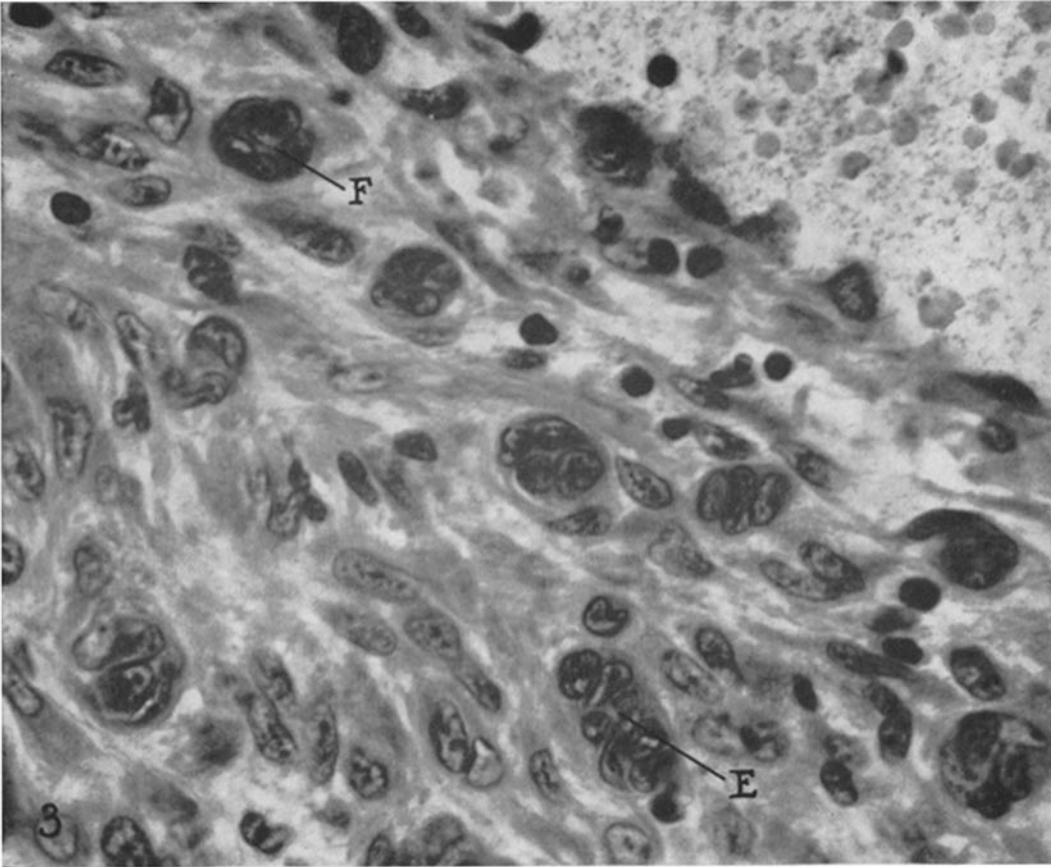


(Murphy and Swift: Induction of cardiac lesions)

PLATE 38

FIG. 3. Rabbit 71-77,—higher magnification of *A*, Fig. 2; numerous mono- and multinucleated cells, some with bizarre shaped nuclei, basophilic cytoplasm, and indistinct cytoplasmic outline; *E* and *F*, cells with 8 nuclei. Hematoxylin and eosin. $\times 886$.

FIG. 4. Rabbit 71-77,—reticular myocardial granuloma, interventricular septum. *A*, swollen collagen fibers forming interlacing network; collagen framework which assumes a direction roughly parallel with the myocardial bundles; *B*, cell with abundant cytoplasm; necrosis of adjacent myofibers. Weigert-hematoxylin and eosin. $\times 395$.

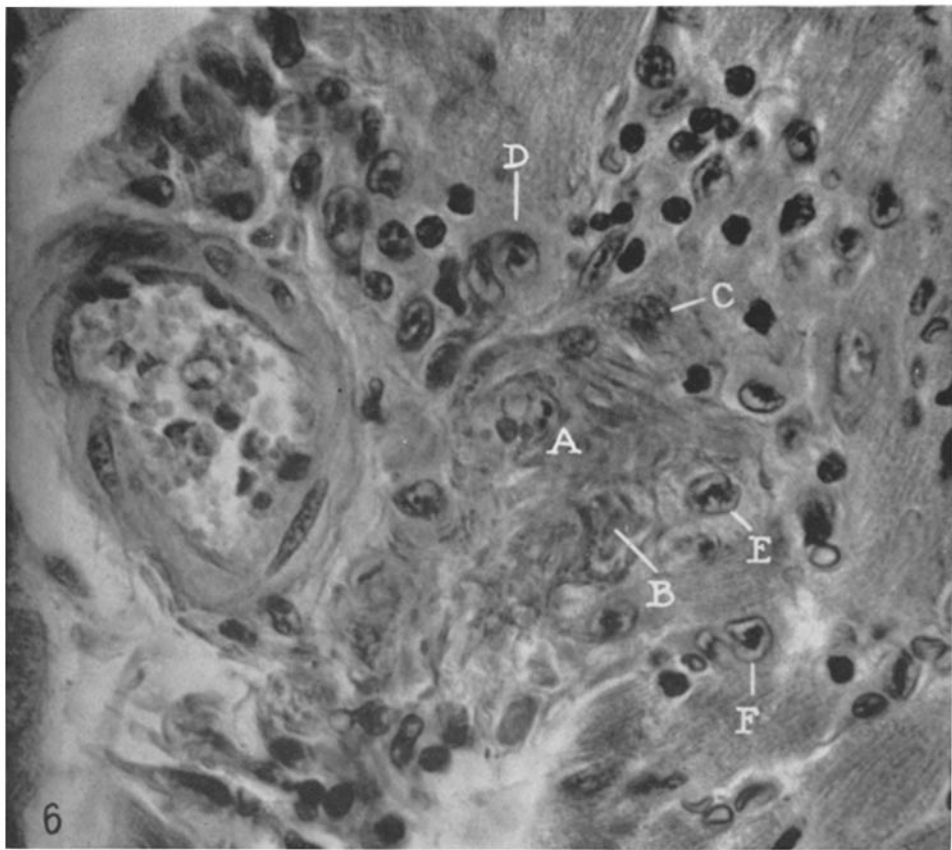
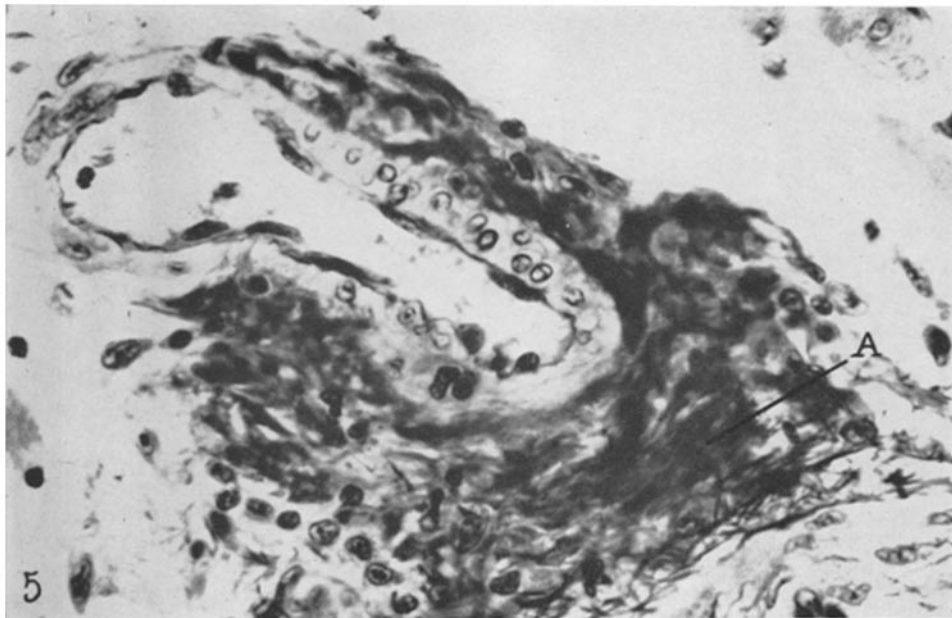


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PLATE 39

FIG. 5. Rabbit 71-77,—artery in left ventricle. *A*, frankly fibrinoid collagen, bordered by granuloma cells, in adventitia and paraadventitia; panarteritis nodosa absent. Weigert-hematoxylin and eosin. $\times 743$.

FIG. 6. Rabbit 71-77,—adventitial and paraadventitial coronal granuloma in interventricular septum. *A*, center of focus of frankly fibrinoid collagen; *B* and *C*, indistinct cell masses; *D*, cell with 3 nuclei; *E*, cell with fibrocytoid nucleus; *F*, cell with owl-eyed nucleus; many cells have indistinct cytoplasmic outlines; panarteritis nodosa absent. Hematoxylin and eosin. $\times 861$.

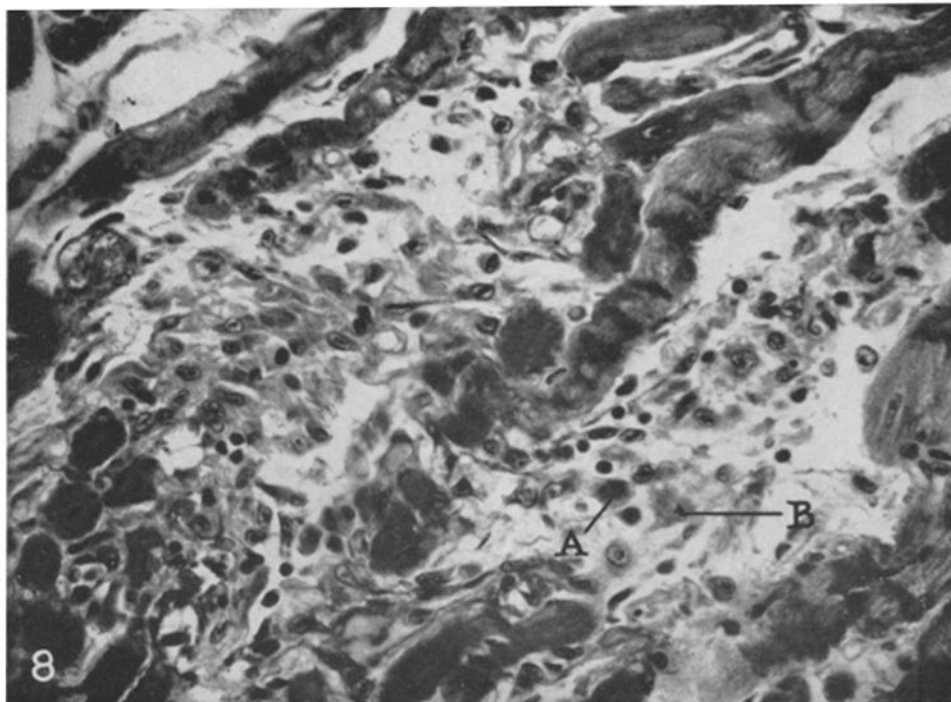
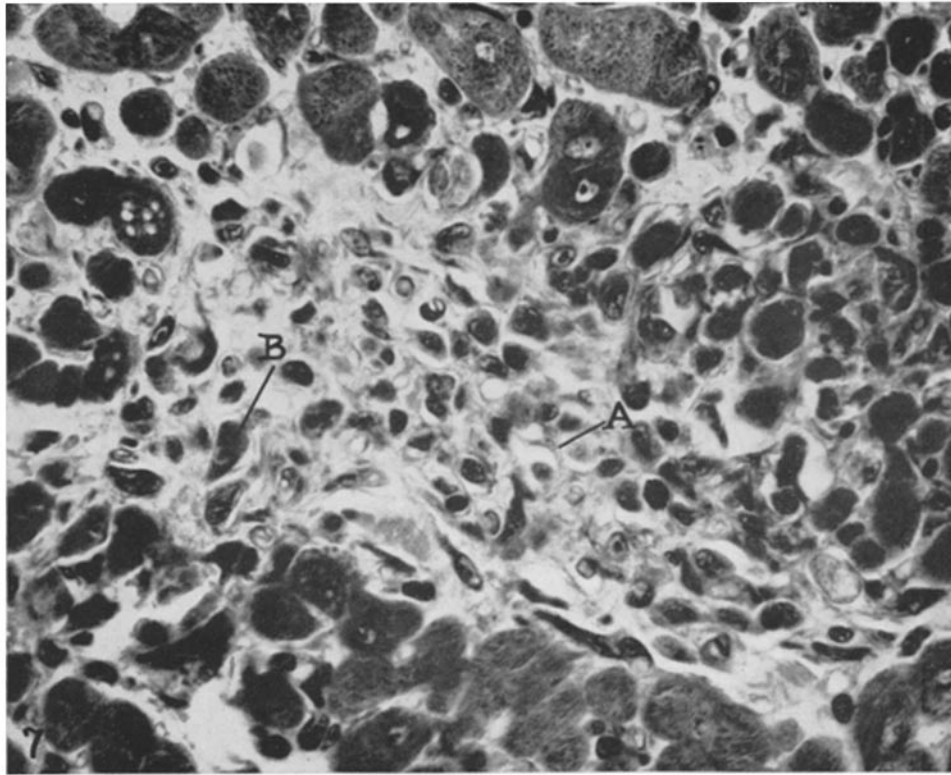


(Murphy and Swift: Induction of cardiac lesions)

PLATE 40

FIG. 7. Rabbit 71-80 (see Table III),—sacrificed 10 days after last of 6 infections; autopsy blood cultures negative; reticular myocardial granuloma in interventricular septum. Cells interspersed in interlacing network of (A) swollen collagen fibers; B, cell with abundant basophilic cytoplasm; vacuolation of nuclei and cytoplasm of adjacent myofibers. Giemsa stain. $\times 621$.

FIG. 8. Rabbit 71-80,—two mosaic myocardial granulomata in left ventricle; granuloma cells lodged between collagen masses. A, cell with 3 nuclei; B, cell with pyknotic nucleus and abundant raggedly outlined cytoplasm; most cells have indistinct cytoplasmic outlines; disintegration of adjacent myofibers. Hematoxylin and eosin. $\times 404$.

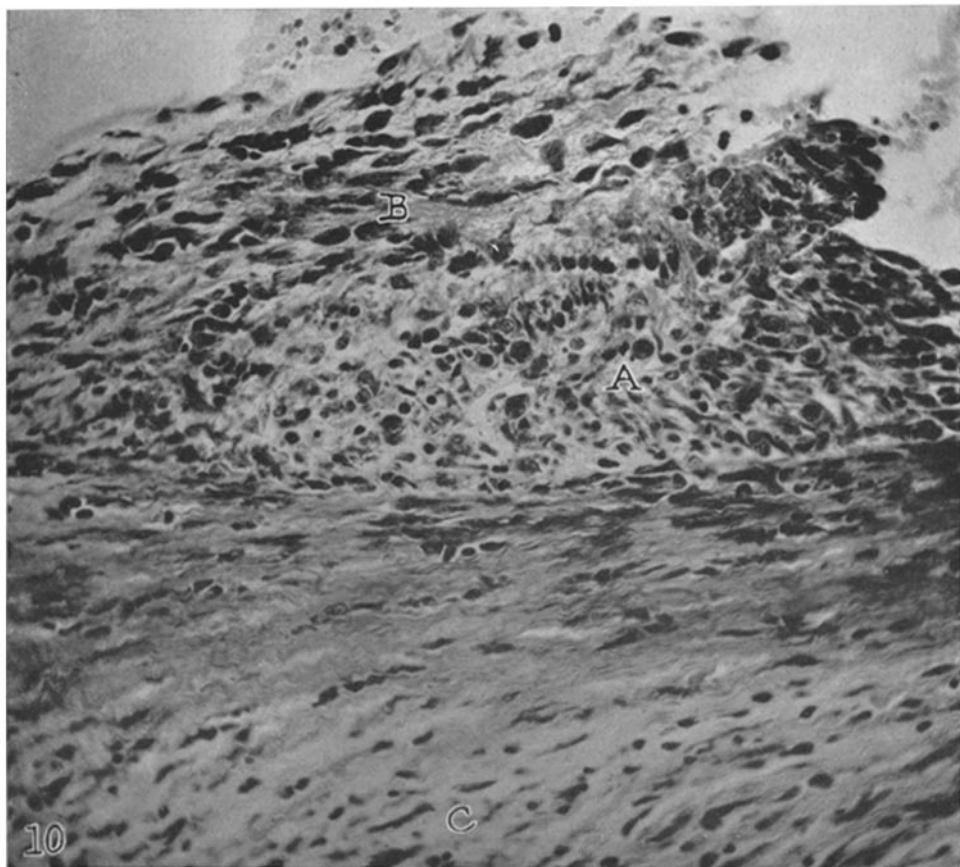
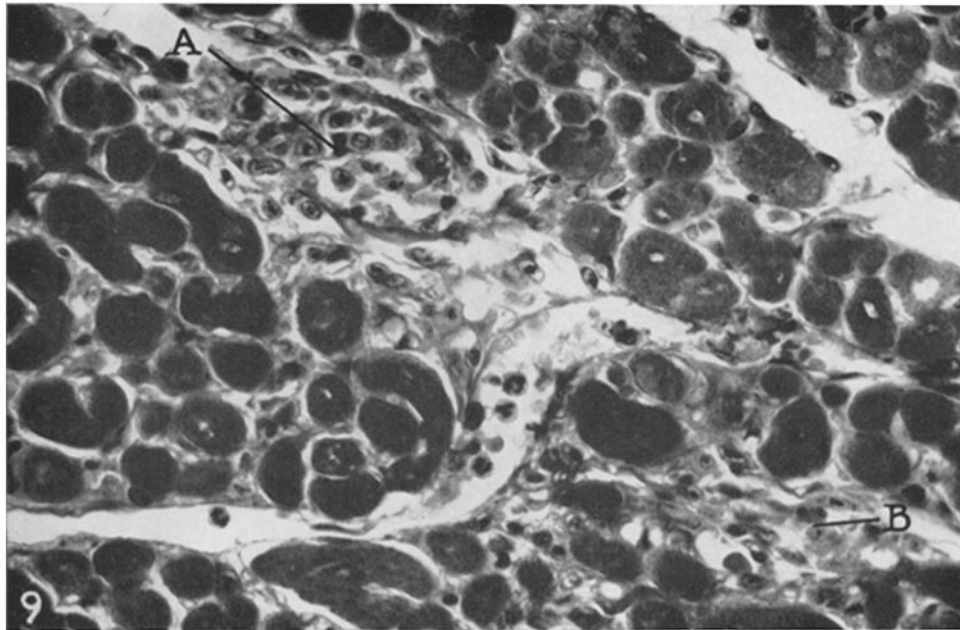


(Murphy and Swift: Induction of cardiac lesions)

PLATE 41

FIG. 9. Rabbit 71-80 (see Table III),—left ventricle; *A* and *B*, 2 mosaic myocardial granulomata; foci of frankly fibrinoid collagen in granuloma *A*. Hematoxylin and eosin. $\times 465$.

FIG. 10. Rabbit 71-77,—endocardial nodule on chorda tendineae at mitral leaflet attachment. Numerous multinucleated cells surrounding *A*; many cells with basophilic cytoplasmic streamers surrounding *B*; interstitial inflammation, *C*. Giemsa. $\times 255$.

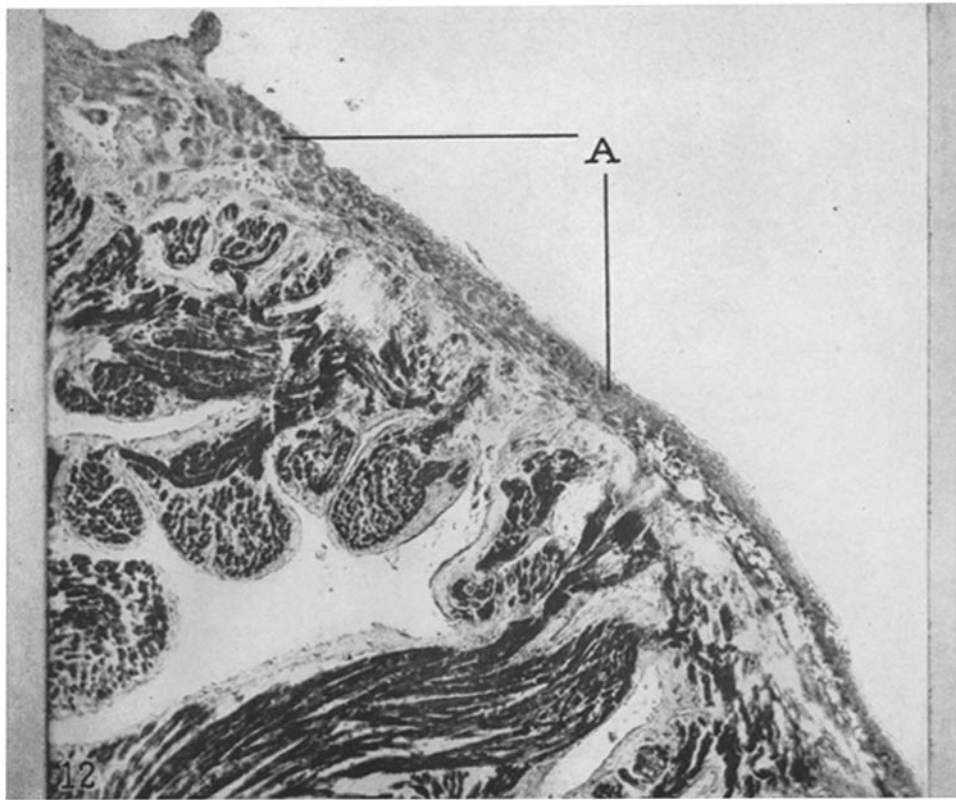
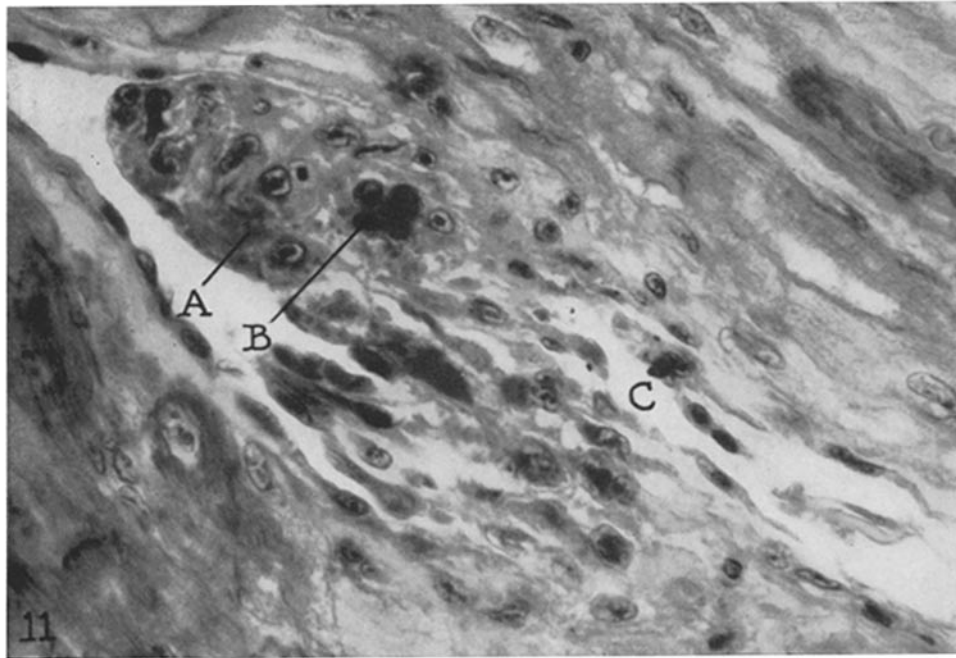


(Murphy and Swift: Induction of cardiac lesions)

PLATE 42

FIG. 11. Rabbit 70-58 (see Table I),—died 13 days after last of 8 infections; negative blood cultures 2 days prior to and at autopsy. Left ventricle; mosaic nodular granuloma arising from thin walled vein; granuloma cells lodged between frankly fibrinoid masses (*A*); cell at *B* has 2 nuclei; several cells with owl-eyed nuclei; axially arranged cells surrounding *C* have streamers of cytoplasm; dissolution of adjacent myofibers. Masson trichrome stain. $\times 659$.

FIG. 12. Rabbit 70-71,—sacrificed 16 days after last of 8 infections. Left auricle; *A*, epi- and subepicardial collagen converted into frankly fibrinoid material; Mallory aniline blue stain. $\times 127$.



(Murphy and Swift: Induction of cardiac lesions)