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Production of Acute Rheumatic-like Heart Lesions in Mice.*

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Klinge[1] and others[2,3] have reported that rabbits subjected to sublethal anaphylactic shock develop cardiac lesions resembling those seen in human rheumatic fever. More complete and convincing evidence to that effect was submitted more recently by Rich.[4-6] These investigations support strongly the thesis that rheumatic fever, as well as certain diseases related to it, is a reaction to parenteral contact with foreign protein to which the tissues have been sensitized. In this laboratory we have recently been engaged in studies of protein hypersensitivity in mice and it was suggested by one of us (Hall) that their hearts be examined histologically for rheumatic-like lesions. The present report is concerned with the production of acute cardiac lesions resulting from 4 parenteral injections of foreign protein.

Methods. The whites were separated from fresh hen eggs, diluted with an equal volume of distilled water, brought to a pH of 7.0 to 7.2, mixed to disrupt the small mucinous sacs, filtered through cloth, and diluted as necessary with distilled water. Refiltration was often required to remove the slowly precipitating mucins of slight solubility. Dilutions of 1 to 8 or higher were used intravenously and 1 to 2 intraperitoneally. All injections were made in volumes of 1 ml. Solutions were used within 2 to 6 hours after preparation, being refrigerated during that time and warmed to approximate body temperature just before injection. Mice of the Olitsky, "typhoid resistant" strain were raised in the laboratory.

Several experiments were conducted using 3 intraperitoneal injections, given at 2-day intervals, followed by a 4th either intraperitoneally or intravenously on the 4th, 7th, 10th, 14th, or 21st day after the 3rd dose. Survivors were held for 7 days, sacrificed, and the tissues fixed in formalin. Serial sections of the heart were cut at 6 μ and stained with phosphotungstic acid, hematoxylin and triosin. Another experiment was conducted using one intraperitoneal dose followed by 3 intravenous injections (Experiment 30 C) given 8, 15, and 22 days after the first dose. Finally, a similar experiment was run where the first 3 doses were intraperitoneal and the 4th intravenous (Experiment 30 D). Mice which died or were moribund as a result of the 4th dose were immediately autopsied and their tissues handled as described above. In Experiments 30 C and D, no more than one hour elapsed between the 4th dose and autopsy. Illustrations shown below were taken from 5 of the mice in Experiments 30 C and D. Mice were 6 to 8 weeks old when autopsied. Tissues from untreated mice of similar age were examined.

Results. Lesions resembling many of those seen in acute rheumatic carditis in man and those described by Rich in rabbits were observed in all experimental animals. The untreated mice, which may be assumed to be spontaneously exposed from time to time to various foreign proteins, had minimal findings which none the less might be looked upon as "rheumatic stigmata:" they showed

* Aided by the Mayer Fund of the Department of Experimental Medicine.

1 Klinge, F., *Ergeb. d. allg. Path.*, 1933, **27**, 1.

2 Vaubel, E., *Ziegler's Beitr.*, 1932, **89**, 374.

3 Hall, E. M., and Anderson, L., *Am. Heart J.*, 1943, **25**, 64.

4 Rich, A. R., and Gregory, J. E., *Bull. Johns Hopkins Hosp.*, 1943, **73**, 239.

5 Rich, A. R., and Gregory, J. E., *Ibid.*, 1944, **75**, 115.

6 Rich, A. R., *Proc. Inst. Med. Chicago*, 1945, **15**, 270.

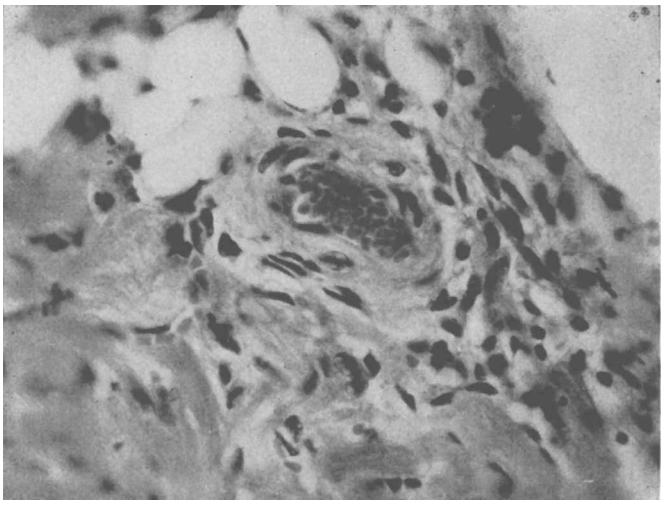

FIG. 1.
Sub-epicardial arteriole.

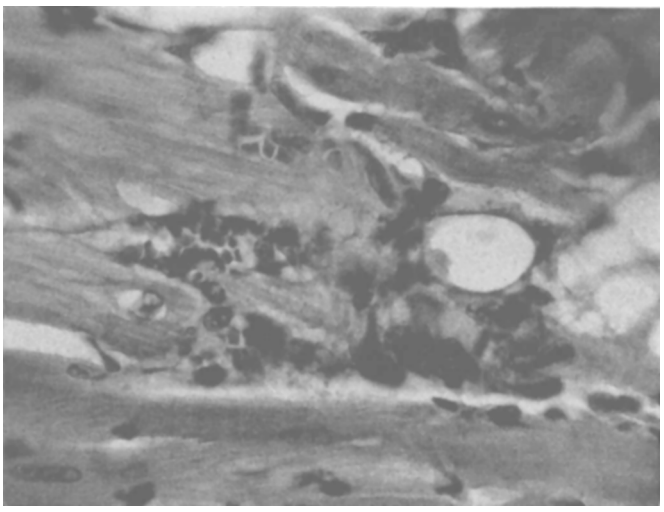

FIG. 2.
Hydropic degeneration of myocardial fibrils.

small and scattered areas of minor collagenous degeneration of the smaller coronaries, inconspicuous perivascular infiltrations, and moderate numbers of Anitschkow myocytes in those areas of greatest mechanical strain (*e.g.*, in the intervalvular septum).

The experimental hearts exhibited innumerable highly focal areas of acute vascular and perivascular degeneration of the reticular and collagenous connective tissue, with essentially complete fibrinoid degeneration of the arteriolar wall, as illustrated in Fig. 1. Fig. 2 illustrates the hydropic degeneration and disruption of myocardial fibrils. Fig. 3 shows a perivascular cellular infiltration in the wall of the left atrium. A low magnification of a moderately large infiltration near the base of a papillary muscle is shown in Fig. 4, the cellular constituents and degenerated central area being shown in higher magnification in Fig. 5. A verrucous lesion on the superior surface of a mitral leaflet is shown in Fig. 6. Such verrucae were seen frequently. Fig. 7 shows an acute verrucous

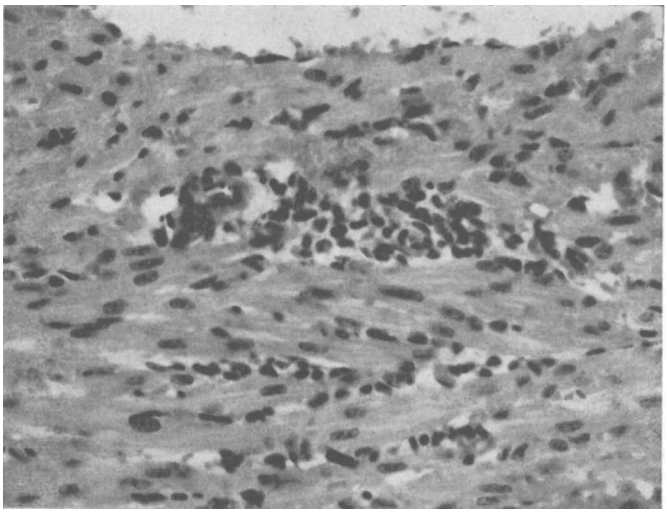

FIG. 3.
Perivascular infiltration in wall of left atrium.

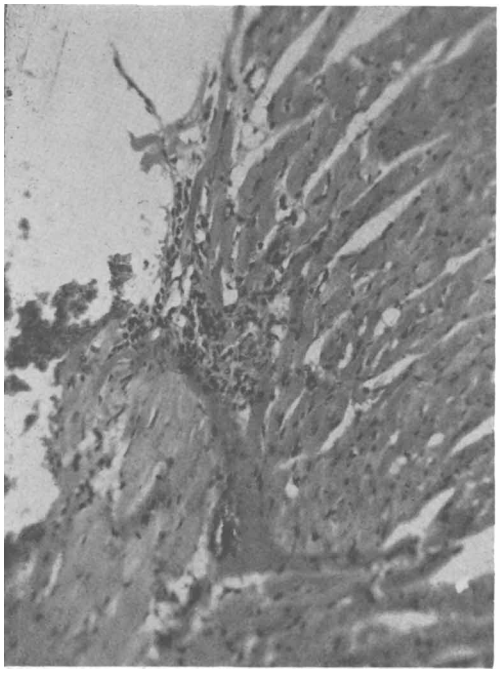

FIG. 4.
Infiltration near base of papillary muscle.

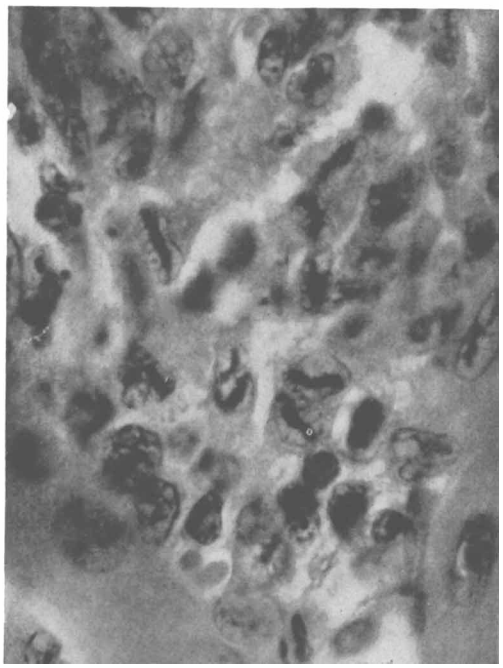

FIG. 5.
Cellular constituents in base of papillary muscle.

excrescence on the left septal wall just below the aortic valve. Fig. 8 shows the aorta and origin of the left coronary, with the septum and left ventricular wall and lumen below, and a high cut through 2 of the aortic cusps near their commissure. The pathologic fusion of these aortic cusps is seen in higher magnification in Fig. 9. A giant cell and degeneration of the collagenous and reticular tissue are evident, and while not apparent

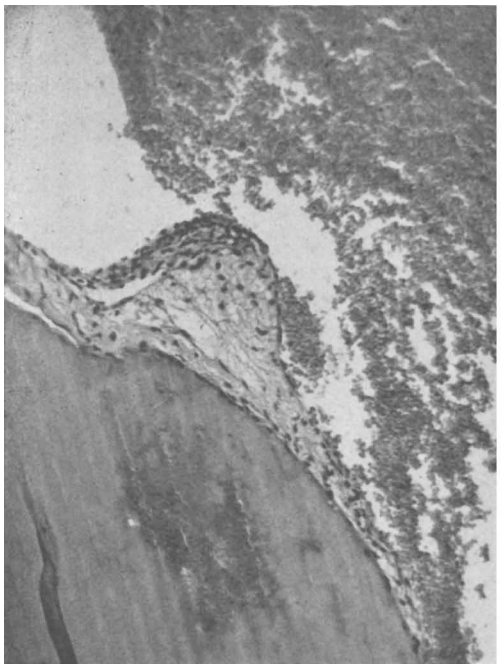

FIG. 6.
Verruca on superior surface of mitral leaflet.

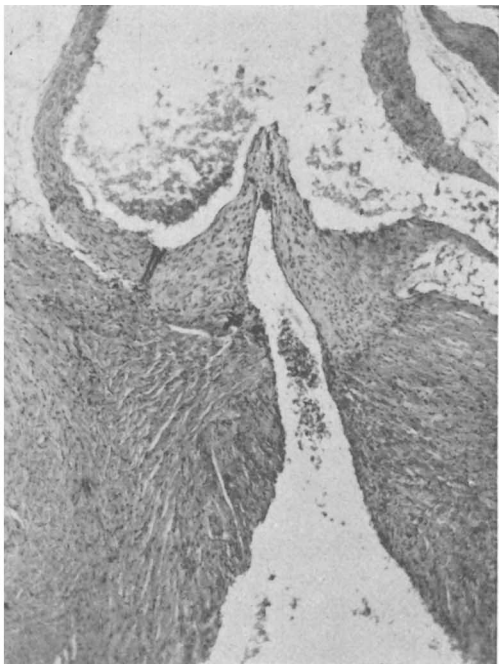

FIG. 8.
Fusion of aortic cusps near commissure.

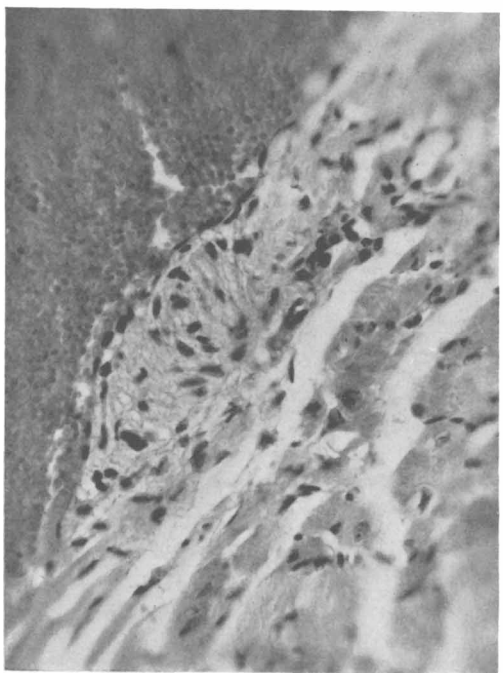

FIG. 7.
Verruca on left septal wall below aortic valve.

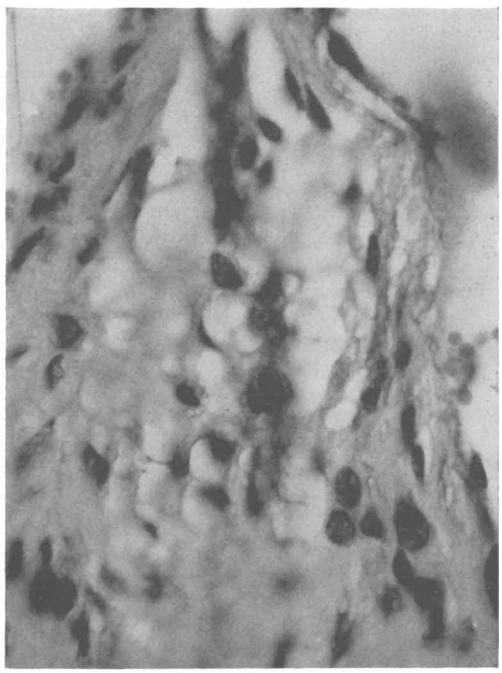

FIG. 9.
Higher magnification of commissural fusion seen in Fig. 8.

in the reproduction, 4 other cells resembling giant cells were also seen at this point of fusion.

Discussion. The material presented above indicates that cardiac lesions may be produced in mice by parenteral readministration of foreign protein, and that they tend to simulate many of those reportedly found in rabbits under similar circumstances. They bear a striking resemblance to the lesions occurring in acute human rheumatic carditis.

It should be recalled that the mice illustrated in this report received only 4 doses of antigen in 22 days. Assuming, for lack of evidence, that the first injection was not injurious, and knowing that the animals were autopsied within one hour after the last dose, it would appear likely that most of the tissue changes must be ascribed to the 2nd and 3rd doses. The injury, therefore, could not easily have been of more than 2 weeks' duration in any animal. Furthermore, it was noted that animals receiving only a single "shocking" dose, and autopsied a week later, generally had much less florid lesions than those found in the hearts illustrated above. This suggests that repeated shocking doses may be more than additive and indicates that the preponderance of the lesions illustrated were not of more than one week's duration.

Further studies are in progress to define more fully the nature, variety, and incidence of these acute lesions and to investigate their production with other antigens. Studies are also in progress in which the mice are given opportunity to develop older, reparative lesions, and these with superimposed fresh ones. In addition, since it is theoretically impossible to obtain animals that have never been subjected to any foreign protein, the most careful investigation of untreated animals is desirable: the spontaneous occurrence of minimal rheumatic-like cardiac lesions in mice is too suggestive of human rheumatic fever to be overlooked or regarded merely as a small impediment to experimental work. Pathologic changes have also been observed in other organs of the mice reported here, and these will be separately described.

As has been pointed out recently by Weiser, Golub, and Hamre,[7] anaphylactic reactions in mice are slow to develop and prolonged in course by comparison with other animals. As noted by Perry and Darcy,[8] mice are relatively refractory to histamine, and unexpected relations between histamine and antihistamine drugs in mice have been reported by Mayer and Brousseau.[9] We know of no effort in mice or other animals, however, to induce cardiac lesions of the type described here with histamine. On the basis of current evidence, it is not entirely unlikely that these changes are not produced by the clinical anaphylactic reaction itself, for we have observed no relation between the severity of the clinical reaction at the time of protein injection and the severity of the pathological lesion resulting from it at a later date. For instance, mice which received all of their doses by the intraperitoneal route exhibited either no clinical reaction to the injection or had only the slightest and most questionable reaction. The cardiac lesions in these mice, however, were at least as advanced as in those inoculated repeatedly by the intravenous route and which developed acute and severe symptoms of cardiovascular and respiratory embarrassment with each succeeding dose. We are therefore tempted to conclude that some biochemical reaction occurs in the tissue which results in damage to them: if that primary reaction is induced by a sufficiently sudden access of antigen to the vascular tree, then frank clinical evidence of anaphylaxis may result, but if the antigen reaches the cardiovascular system more gradually, then there may be no clinical anaphylaxis but just as much fundamental tissue damage in the end.

While it is tempting to refer to the observed changes in mice as "rheumatic carditis," this would appear to remain unwarranted until it is shown first that the advanced lesions of myocardial scarring and valvular stenosis may be similarly produced; second that le-

[7] Weiser, R. S., Golub, O. J., and Hamre, D. M., *J. Inf. Dis.*, 1941, **68**, 97.

[8] Perry, S. M., and Darsie, M. L., Jr., PROC. SOC. EXP. BIOL. AND MED., 1946, **63**, 453.

[9] Mayer, R. L., and Brousseau, D., PROC. SOC. EXP. BIOL. AND MED., 1946, **63**, 187.

sions of other tissues correspond to those of human rheumatic fever and its related diseases; and third, some or most of the clinical and laboratory findings of the human disease are reproduced in animals. Until such time they might be called either "protein carditis," or "rheumatic-like carditis." If, however, one were to hypothecate that human rheumatic fever is the result of parenteral contact with foreign protein to which the tissues have been sensitized, one might expect that hypothesis to be reasonably compatible with the known relation of the streptococcus to the disease, for it would indeed be difficult to find a source better than the streptococcus of foreign protein which customarily, frequently, and intermittently gains access to the more intimate tissues and vascular system of man.

Summary. Acute rheumatic-like heart lesions have been produced in mice by parenteral injection of egg white on repeated occasions. Minimal lesions may also occur in untreated mice, possibly as a result of spontaneous sensitization and shocking by natural contact with protein. There was no apparent relation between the severity of clinical anaphylaxis and the severity of the pathologic changes in the heart.

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On Some Biological Characteristics of Streptomycin B.

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Fried and Titus[1] recently described the isolation, from streptomycin concentrates, of a new entity which they called streptomycin B. Chromatographic fractions described by these authors were made available for biological studies. The materials used for the present work were rich in streptomycin B and from Craig countercurrent distribution data[2] were estimated to contain not more than 10-15%, by weight, of streptomycins other than B. For purposes of comparison, studies with a highly purified preparation of streptomycin, free of streptomycin B, were included in the investigations. In this paper the term "streptomycin" will refer to the preparation containing no streptomycin B, while "streptomycin B" will refer to the above described chromatographic fractions rich in this latter material.

I. *In vitro studies.* The minimal inhibiting concentrations (M.I.C.) of streptomycin and streptomycin B for 5 strains or species of bacteria were determined in yeast beef broth, and for 2 species of mycobacteria in Kirchner's medium modified* to contain albumin and Tween 80 as in media described by Dubos and Davis.[3] The M.I.C. values thus determined are shown in Table I.

The importance of the type of culture medium used for such studies is clearly shown by the comparisons in Table II where addi-

[1] Fried, J., and Titus, E., *J. Biol. Chem.*, 1947, **168**, 391.

[2] Titus, E., and Fried, J., *J. Biol. Chem.*, 1947, **168**, 393.

* Modified Kirchner's synthetic medium for growth of *M. tuberculosis:*

Na_2HPO_4	3 g
KH_2PO_4	4 g
$MgSO_4$	0.6 g
Sodium citrate	2.5 g
Iron ammonium citrate	0.05 g
Asparagin	5.0 g
Glycerine	20 ml
Distilled water	1000 ml
Tween 80	0.05%

Autoclave at 15 pounds for 20 minutes.

Solution of human serum albumin sterilized by filtration added to 0.1%.

[3] Dubos, R. J., and Davis, B. D., *J. Exp. Med.*, 1946, **83**, 409.