

SPONTANEOUS INTERSTITIAL MYOCARDITIS IN RABBITS.

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PLATES 20 AND 21.

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During a series of experiments in which attempts were made to transmit to rabbits and guinea pigs a virus assumed to be present in the body fluids of patients suffering with rheumatic fever, certain myocardial lesions were encountered in the inoculated animals. The results of these inoculation experiments have been reported in the preceding communication (1). Although the experiments failed to demonstrate a virus in the materials employed, they brought to light the fact that in the hearts of uninoculated rabbits kept as controls, lesions were present similar to those found in the animals subjected to inoculation. This led to a systematic investigation of the hearts of apparently healthy stock rabbits. The myocardial lesions found in these animals constitute the subject of the present paper.¹

Despite its obvious importance for experimenters with animals no description of spontaneous myocarditis in rabbits could be found in literature. Bell and Hartzell (2) in a paper on spontaneous nephritis in rabbits state that while they did not study the hearts of their nephritic rabbits systematically, they found in three hearts of which many sections were cut "foci of lymphocytes similar to those described by Longcope, and attributed by him to the action of foreign protein." Wright and Craighead (3) in a study of infectious motor paralysis in young rabbits mention that sections of the myocardium of two animals were examined and "a few focal lesions found." In neither instance was the condition given more than the brief mention quoted.

A few spontaneous diseases of rabbits have attracted considerable attention. Spontaneous arterial degeneration has been described by Miles and Johnstone (4), and by Pearce (5); spontaneous nephritis by Ophüls (6), Bell and Hartzell (2),

¹A preliminary report of this study has recently appeared (Miller, C. P., Jr., *Proc. Soc. Exp. Biol. and Med.*, 1923-24, xxi, 521).

and Twort and Archer (7); spontaneous encephalitis by Wright and Craighead (3), McCartney (8), Oliver (9), Levaditi, Nicolau, and Schoen (10), Goodpasture (11), and others.

Material and Methods.

This report is based on the study of the hearts of thirty-four apparently healthy adult rabbits. No animals have been included in this series which, while under observation, manifested any clinical evidences of disease—snuffles, ear canker, diarrhea, cutaneous or subcutaneous infections. It is interesting to note in passing, however, that myocardial lesions identical with those described below occurred in rabbits suffering from snuffles and from rabbit septicemia, but not with appreciably greater frequency than in the animals which constitute the series here reported.

The observations extend over a period of $3\frac{1}{2}$ years and the animals employed were obtained from a variety of sources. The myocardial lesions encountered cannot, therefore, be regarded as peculiar to a special strain of rabbits.

Most of the rabbits were kept under observation for periods ranging from a few days to 2 months. They were weighed twice a week and were periodically examined for clinical evidences of disease. When sacrificed the animals were killed by a blow on the back of the head. A complete autopsy was performed, but only the hearts were prepared for microscopical examination. They were washed in Ringer's solution, examined macroscopically, and fixed in Zenker's fluid. Prior to imbedding, the hearts were cut up into segments which were diagramed in order to facilitate the orientation of the sections. Sections were cut in paraffin and stained with eosin and methylene blue as routine or with Giemsa's stain. Several sections were cut from each block,—so that the walls of all four chambers and usually all of the valves were examined.

RESULTS.

Table I gives the following data about each rabbit: length of the observation period, maximum weight, gross pathological changes found at autopsy, and the essential facts concerning the microscopical appearance of the myocardial lesions, their number, size, and predominating cells.

The longest period of observation was 124 days; and for half of the rabbits it was longer than a week. At the time of autopsy the

animals weighed 400 to 3,200 gm.—the majority between 1 and 2 kilos. The weights have been used as a rough index of the age of the animals as their exact ages were not known. The macroscopic appearance of the viscera was normal in twenty-five of the thirty-four rabbits. Eight, or 23 per cent, suffered from coccidiosis of the liver, but this condition was not regularly associated with the myocarditis. Myocardial lesions were found in twenty, or two-thirds of the animals. They occurred less frequently in the younger animals, and were also somewhat less common in the years 1923 and 1924, when the general condition of the stock rabbits had improved; that is to say, when their general nutrition was better and they suffered less from snuffles and coccidiosis than those examined in previous years.

Description of the Myocardial Lesions.

Of the thirty-four rabbits, twenty, or 60 per cent, showed myocardial lesions. In some hearts they were numerous enough to be found in every section, while in others search through a number of sections was necessary to demonstrate their presence. The lesions were never visible to the naked eye. Upon microscopical examination they were found to be distributed as follows, in order of frequency: papillary muscles, septum, left ventricle wall, right ventricle wall, auricles. No lesion was ever found in the valves or at the bases of valves. Some occurred immediately beneath the epicardium and endocardium (Figs. 4 and 5), but the majority were within the myocardium itself. Most frequently, they lay in the interstitial connective tissue framework, occasionally close to a medium size blood vessel (Figs. 7 and 8).

Microscopically the lesions appeared as collections of cells more or less densely packed, lying between the muscle fibers. In size they ranged from small groups of six to ten cells up to areas the size of a high power field. They were usually elliptical with their long axes parallel to the muscle fibers. Some were diffuse, appearing as cords of cells filling the spaces between a number of muscle fibers.

For convenience in description the lesions may be arbitrarily divided into two groups according to the predominating cell type. Those in the first group were most frequently encountered. They consisted almost entirely of cells with irregularly round nuclei and very little

TABLE I.
Myocardial Lesions in Apparently Healthy Rabbits.

Animal No.	Period of observation.	Maximum weight.	Autopsy findings.	Number.	Myocardial lesions.		
					Size.		Predominant cell.
H-3	86	1,650	Negative.	Several.	Medium.	Lymphocytes; endothelial leucocytes.	
H-27		700	"	Few.	Small.	"	
H-71	77	1,400	"	Several.	" and medium.	endothelial leucocytes.	
H-96	80	1,420	"	" 1	"	"	
K-13	48	940	"	Few. ²	"	"	
K-19	6	1,400	"	"	Medium.	"	
K-33	11	1,640	"	" 3	"	"	
B-84	124	1,570	"	"	Small.	"	
P-19	3	1,220	"	"	"	"	
P-21	2	1,500	"	"	"	endothelial leucocytes; fibroblasts.	
P-608	2	3,270	"	" 4	"	Lymphocytes.	
P-651	8	2,670	"	"	"	endothelial leucocytes.	
P-652	8	1,490	"	"	"	"	
P-653	8	1,660	"	Many. ⁵	" and large.	endothelial leucocytes; fibroblasts; giant cells.	
X-1	2	2,500	"	" 6	"	Lymphocytes.	
K-14	50	1,190	Coccidiosis.	"	"	"	
P-17	1	1,300	"	Few. ⁷	"	"	
P-18	1	2,100	" scarred kidneys.	"	"	"	
P-27	3	1,100	Coccidiosis.	"	"	endothelial leucocytes.	
H-67	53	2,180	Pregnant.	"	"	"	

H-38	900	Negative.	0	0	0
H-62	1,840	"	0		
P-23	1,900	"	0		
P-24	1,300	"	0		
P-25	1,650	"	0		
P-26	1,270	"	0		
P-607	1,970	"	0		
P-629	2,190	"	0		
P-644	1,790	"	0		
			No focal lesions.		
			Area of atrophy and fibrosis.		
X-2	1,870	"	0		
H-9	400	Coccidiosis.	0		
H-28	600	"	0		
B-78	1,990	"	0		
P-20	1,030	"	0		

¹ Subendocardial as well as myocardial lesions. See Figs. 5 and 8.

² See Fig. 1.

³ Subepicardial as well as myocardial lesions.

⁴ See Fig. 7.

⁵ See Figs. 9 and 10.

⁶ See Figs. 2 to 4 and 6.

⁷ Subendocardial as well as myocardial lesions.

or no visible cytoplasm. Some of the nuclei were densely stained and solid; others, rather pale with the chromatin gathered into large granules. When a narrow rim of cytoplasm was visible it was pale blue. Cells were usually packed closely together in an elongated space between the muscle fibers and had in general the character of lymphocytes (Figs. 1 and 5).

The other type of lesion was made up principally of cells with large, pale, elliptical, vesicular nuclei and more or less cytoplasm,—always non-granular (Fig. 2). These cells, which have been known by various names—macrophages, endothelial leucocytes, and macrocytes—were usually less densely packed than the lymphocytes. Lesions of this type were sometimes the size of a high power field, filling the spaces between a number of muscle fibers, and occasionally were found in the vicinity of a medium sized blood vessel (Figs. 7 and 8). The cells in such lesions failed to stain deeply with pyronine in the Unna-Pappenheim stain, thus exhibiting an outspoken dissimilarity to the type cells of an Aschoff body.

While these two types of lesion were found, others were encountered that consisted of mixtures of lymphocytes and endothelial leucocytes in varying proportions (Figs. 4 and 6). Other cells were also occasionally found—mature connective tissue cells, polymorphonuclear eosinophils, polymorphonuclear neutrophils, and plasma cells (Fig. 3) and mast cells. In one rabbit a single large lesion was found containing large giant cells with many nuclei gathered together in the middle of the cells (Figs. 9 and 10).

When muscle fibers ran through a lesion they often showed loss of striation, but no other degenerative change of the parenchymatous elements was noted.

Many of the lesions have been carefully searched for microorganisms. Special stains have been employed to facilitate their demonstration,—prolonged staining with Giemsa, aqueous fuchsin, carbol-fuchsin, and gentian violet,—but no bacteria or protozoa have been seen, nor have any cell inclusion bodies been found.

DISCUSSION.

The lesions described are of interest and importance from several points of view. The rabbit is one of the most commonly used of

laboratory animals, but until recently comparatively little attention has been paid to the spontaneous diseases of this species. Mention has been made of the arterial degeneration, the nephritis, and the meningoencephalitis which previous workers have described. According to Goodpasture (11) and others the last mentioned disease is associated with a minute protozoan organism. It is probable that many other diseases are still unrecognized, and it is possible that certain experimental work on rabbits may need repetition after the spontaneous pathological conditions in this species are more clearly understood. For example, the reports of de Vecchi (12) and of Natali (13) on the production of rheumatic myocarditis in rabbits are assuredly thrown open to criticism. A comparison of their illustrations with those here presented is sufficient to make one wonder whether they observed anything except the lesions occurring in "normal" rabbits.

The work of Longcope (14) on the myocardial lesions produced by the intravenous injection of foreign proteins cannot be so easily dismissed. He describes foci in the hearts of rabbits injected with repeated doses of egg white and of horse serum strikingly similar to the lesions here described in apparently normal rabbits. In a series of controls, however, he found no pathological changes.

The incidence of the myocardial lesions in this series of "normal" rabbits (60 per cent) was higher than among rabbits included in a series of transmission experiments reported in the preceding paper (1) in which about 45 per cent showed a similar myocarditis. This higher incidence may possibly be accounted for by the fact that the present series contained older rabbits, which seem to show this myocarditis more frequently than young ones, and by the additional fact that the series extended over a longer time, as the incidence varied somewhat from year to year. It was higher, for instance, as already stated during 1920, 1921, and 1922 than during 1923 and 1924.

No etiology for the myocardial lesions has been established. It has been said that they occurred in rabbits not suffering from snuffles, coccidiosis, or hydatid disease. But nevertheless they occurred during the years when these diseases were more prevalent among our stock rabbits. Webster (15), who presents evidence indicating that

snuffles is caused by *Bacterium lepi-septicum*, has found that among the stock rabbits at The Rockefeller Institute 40 per cent harbor this organism in their nasal passages without manifesting any clinical symptoms of the disease.

The myocardial lesions may be the result of mild infection, possibly with some diseases with which we are as yet unfamiliar. It is possible that they are due to a variety of causes. The diversity of the histopathological picture suggests this hypothesis.

The failure to demonstrate any microorganisms or cell inclusion bodies in the lesions does not preclude their presence at an early stage in the development of the lesions. For the cell inclusion bodies found in the lesions produced by certain filterable viruses disappear very soon after inoculation and many bacterial and protozoan organisms are destroyed by the inflammatory reaction which they incite.

SUMMARY.

The hearts of thirty-four apparently healthy adult rabbits were carefully studied microscopically. Twenty, or 60 per cent, contained lesions consisting of lymphocytes and endothelial leucocytes in varying proportions, to which were sometimes added polymorphonuclear eosinophils, plasma cells, and fibroblasts in small numbers. The lesions occurred most frequently between the muscle fibers of the papillary muscles and ventricular walls, and occasionally beneath the endocardium and epicardium. No microorganisms or cell inclusion bodies were found in the lesions.

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EXPLANATION OF PLATES.

All sections were stained with eosin and methylene blue.

PLATE 20.

FIG. 1. Lesion composed largely of lymphocytes in a papillary muscle of the left ventricle. Rabbit K-13. $\times 365$.

FIG. 2. Left ventricle wall. Lesion composed almost entirely of endothelial leucocytes. Rabbit X-1. $\times 365$.

FIG. 3. Large lesion close to the endocardium composed chiefly of lymphocytes with a few plasma cells. Rabbit X-1. $\times 365$.

FIG. 4. Wall of left ventricle. Large subepicardial lesion composed of endothelial leucocytes and lymphocytes. Rabbit X-1. $\times 365$.

FIG. 5. Small subendocardial lesion composed entirely of lymphocytes. Rabbit H-96. $\times 365$.

FIG. 6. Interventricular septum. Large lesion composed of endothelial leucocytes and lymphocytes. Rabbit X-1. $\times 365$.

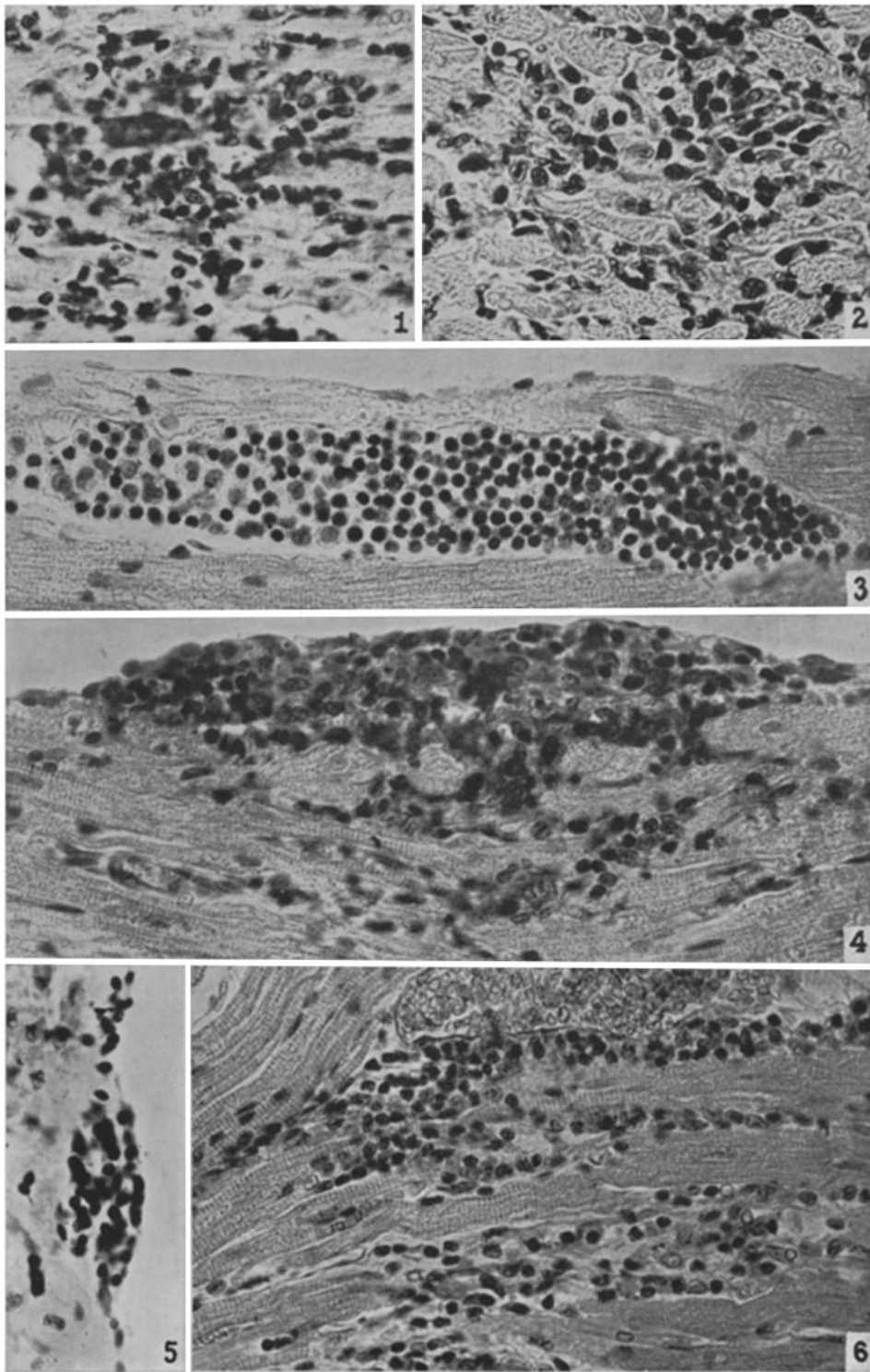
PLATE 21.

FIG. 7. Lesion composed of endothelial leucocytes and lymphocytes, adjoining a vessel. Rabbit P-608. $\times 365$.

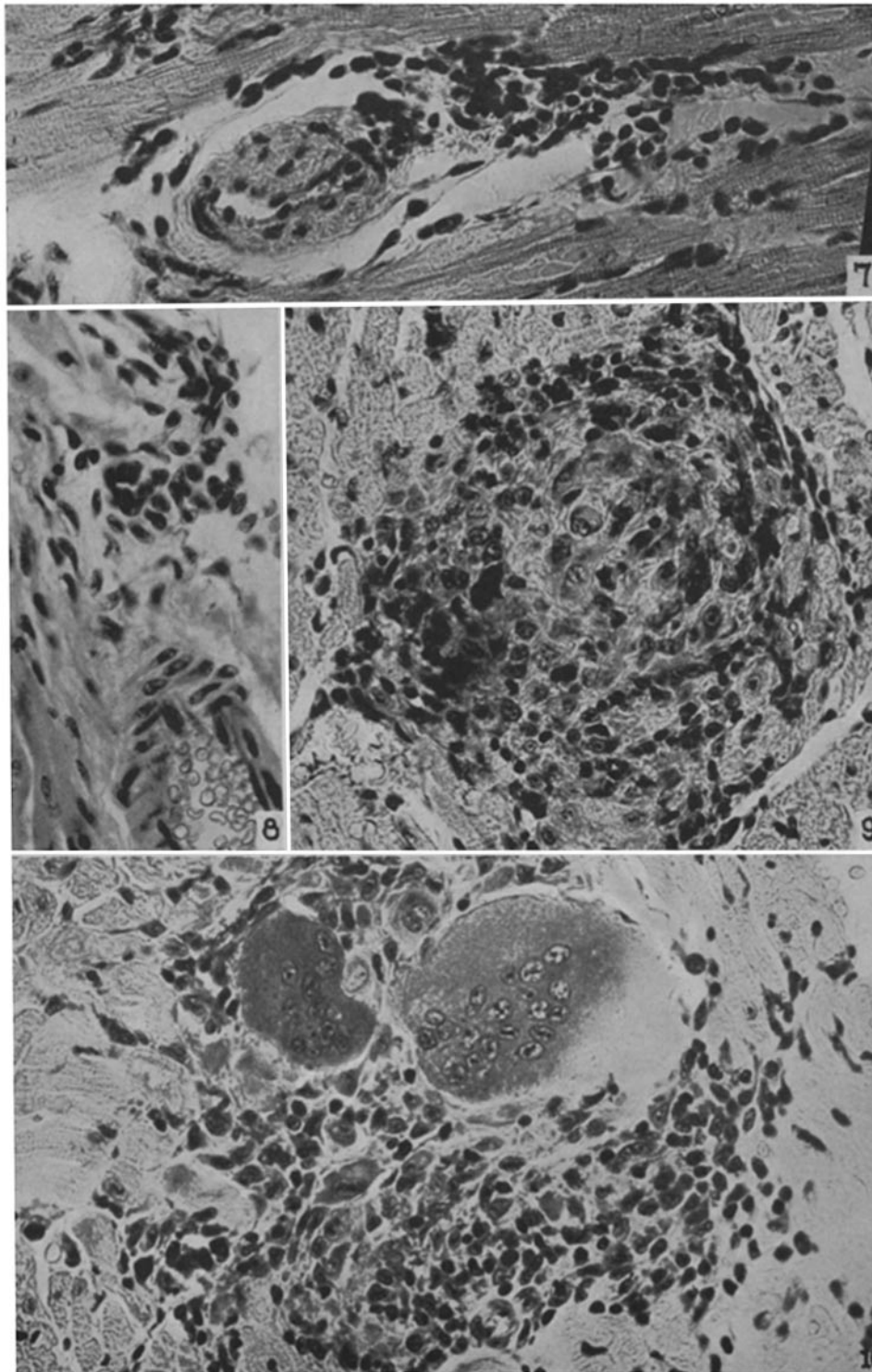
FIG. 8. Small lesion composed of endothelial leucocytes, fibroblasts, and lymphocytes, adjoining a vessel. Rabbit H-96. $\times 365$.

FIG. 9. Interventricular septum. Large lesion composed of endothelial leucocytes, lymphocytes, and a few fibroblasts and polymorphonuclear eosinophils. Rabbit P-653. $\times 365$.

FIG. 10. Another section of the same lesion as Fig. 9 showing two large multinucleated cells. Rabbit P-653. $\times 365$.



(Miller: Interstitial myocarditis in rabbits.)



(Miller: Interstitial myocarditis in rabbits.)