SPONTANEOUS CARDIOVASCULAR DISEASE IN THE RAT *

I. Lesions of the Heart

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Relatively little attention has been directed toward naturally developing cardiovascular disease in any species other than man. The contributions of Fox [1] on natural disease in captive wild animals, and of Krause [2] and Wolkoff [3] on the senile changes in the vascular system of domestic animals, serve as outstanding exceptions. Systematic observations of cardiovascular lesions in the common, small laboratory animals have been less often recorded. There are a few sporadic reports such as the description of spontaneous myocarditis in rabbits by Miller,[4] and of medial lesions in the aorta of this same species, originally noted by Ophüls [5] and by Miles.[6]

Even less information is available concerning cardiac diseases to which the rat is naturally susceptible. Löwenthal [7] in 1931 was unable to collect any records of this type of disease in rodents other than those of inflammatory changes in the myocardium. McCay, Crowell and Maynard [8] in a study of the relation of growth to longevity mention that the hearts of old rats are constantly enlarged. A diligent search of the literature has failed to reveal any other reports dealing with this subject although the incidental description of such lesions in control rats used for other purposes may have escaped attention.

The elucidation of this field is of some practical significance. A comparison of spontaneous lesions in animals with those of man may be of value since differences in habits and environment as well as time factors related to life span may shed light on their etiology. It has sometimes been stated (Sherman [9]) that the rat is rather similar to man in its omnivorous food habits and in most aspects of the chemistry of nutrition. It might not be unreasonable to suspect that if these factors play a rôle in the development of

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cardiovascular disease, this species should closely parallel man in this respect.

That distinct specie idiosyncrasies exist in the development of both spontaneous and experimentally produced cardiovascular disease seems fairly well established. Fox [1] describes variations in the types of spontaneous lesions to which different species are vulnerable. Information concerning spontaneous disease may be of importance in evaluating the results of a given experimental procedure. Cowdry and Scott [10] have demonstrated that repeated doses of vitamin D in the monkey do not regularly lead to calcification of arteries, as in the rabbit and rat. Lesions developing in an animal on an experimental regimen are not necessarily the specific result of that procedure even if control animals of a similar age period fail to show them. The procedure may so debilitate and impair the general health of the animal as to precipitate the earlier initiation of lesions that might well have developed spontaneously later in life. The numerous, varied experimental methods that have led to medial degeneration of the rabbit's aorta lose much of their significance when it is appreciated that 87 per cent of all rabbits at 8 months of age exhibit similar lesions (Kesten [11]). The importance of knowing the inherent disturbances and weaknesses of this system in measuring the effects of any experimental procedure is obvious.

For these reasons a systematic study of cardiac disease in a group of 487 rats was undertaken. The animals were derived from an inbred strain of pure albinos of Osborn-Mendel stock and maintained for more than 30 generations in the laboratories of Drs. H. C. Sherman and H. L. Campbell of the Chemistry department of Columbia University.* The influence of diet on longevity in this strain has been previously reported by these authors.[12] From the time of weaning the animals were fed adequate diets of known composition and maintained under constant laboratory conditions until death occurred spontaneously. The group includes 266 females whose average age at death was 746 days, and 221 males averaging 702 days in age at death. The youngest animal to die spontaneously was a female at 79 days, and the oldest also a female at 1124 days. The majority (79.2 per cent) survived longer than

* We wish to acknowledge our indebtedness to Dr. H. C. Sherman and Dr. H. L. Campbell for providing us with the opportunity of autopsying the animals on which this study is based.

600 days and 24 or 4.9 per cent (19 females and 5 males) were older than 1000 days.

The maximum life span of the rat is usually considered to be about 3 years or roughly 1/30th of that of man. At this ratio a period of 100 days in the rat is equivalent to 8.2 years in man. The average age at death in this group of rats would be equal to about 58 years of human life for the male and 61 years for the female. The average age at death for a similar group of humans would compare closely to these figures. The work of McKay, Crowell and Maynard [8] would seem to indicate that the maximum life span in the rat is somewhat longer than 3 years, since they succeeded in sustaining life in 12 out of 106 animals for more than 1200 days. Their oldest rat survived 1421 days. The senile rats of the group reported here showed a high incidence of suppurative infections in the lungs, middle ears, brain and genito-urinary tract. If these had not occurred the survival periods would undoubtedly have been prolonged. Nevertheless, at autopsy even the human subject often shows evidence of infectious processes which may be unrelated to the principal underlying disease. Thus, lobular pneumonia of varying extent is encountered in more than 50 per cent of all human autopsies. It would perhaps be futile to attempt a more accurate comparison of life spans from our present knowledge.

Each animal in the group was subjected to a detailed postmortem examination. Tissues were fixed in Zenker's fluid without addition of acetic acid, embedded in paraffin, sectioned and stained with hematoxylin and eosin. Special connective and elastic tissue stains were employed when required. One complete sagittal anteroposterior section through the entire heart was prepared for microscopic study. This included routinely the left ventricle, interventricular septum, right ventricle, portions of the valve leaflets, auricles and ascending aorta. The following presentation is a description of the pathological findings encountered. The more common lesions are grouped and classified. The incidence and influence of sex and age were determined. The relations of the lesions to each other or to extracardiac changes was explored whenever a possible connection existed. The composition of the diets was analyzed to see if any favored or inhibited the development of these lesions. Reference to diet will be made only when such an influence is indicated.

Endocardium

Intracardiac Thrombosis: In 31 animals (6.4 per cent) thrombi were found in one or more chambers of the heart. 17 of these were females and 14 males. Fairly old rats were particularly susceptible. The average age of the males with cardiac thrombosis at death was 799 days, compared with 702 days for the entire group, and of the females 905 days compared to 746 days for the entire group. In fact, 7 or 29.2 per cent of the 24 animals surviving more than 1000 days showed the lesion. The left auricle was by far the most common site, being involved 20 times. The right auricle was next with 7 cases. Two parietal thrombi had deposited on the wall of the left ventricle overlying areas of myocardial and endocardial fibrosis even though this chamber lacks columnae carneae to form favoring endocardial recesses. Two other thrombi were found loose and detached; one caught in the orifice of the mitral valve, presumably after having broken free from its origin in the left auricle, and one occluding the aortic valve ring.

Only the larger thrombi completely filling the left auricle were recognized grossly. They could be distinguished by their firm inelastic consistence, grayish red color, and by the marked auricular dilatation. The majority of the thrombi in the left auricle were of this type. They apparently obstructed blood flow completely. Usually the thrombi were of recent formation, microscopically showing little or no evidence of organization. They were composed of alternating columns of massed platelets, fragmented leukocytes and intervening deposits of other blood constituents. They were as a rule only loosely attached to the underlying endocardium. In some instances only smaller parietal thrombi were found, most often in pockets of the auricular appendages between musculi pectinati.

Extracardiac infectious processes were neither more severe nor more numerous in this group than in the other animals. Tumor growths were not found in any of the 31 rats. Factors favoring the development of thrombi were usually present in the heart itself. 23 of the animals showed some degree of sclerosis of the coronary arteries, of which 15 were either moderate or severe in degree. 25 showed evidence of myocardial fibrosis, of which 23 were either moderate or severe. As will appear shortly, the incidence of these

two lesions was just as high in animals of a comparable age as in this particular group. Moreover, myocardial fibrosis was rarely encountered in the walls of the auricles. It is therefore difficult to evaluate the rôle of either of these two changes in favoring the formation of intracardiac thrombi.

There was, however, still a third lesion, apparently a chronic inflammation involving chiefly the endocardium of the left auricle, which appeared to play a definite rôle in the precipitation of thrombi in at least some instances by damaging the surface endothelium. Such changes, designated as chronic auriculitis, were found in 8 of the 20 showing thrombi in the left auricle.

Chronic Auriculitis: This lesion was recognized on microscopic examination in 18 (3.7 per cent) rats, 10 females and 8 males. The left auricle alone was involved in 15 cases, twice in conjunction with slight alterations of the left ventricular endocardium and once with minor changes in the right auricle. The lesion attacked a fairly old age group. The average age at death of the females was 819 days, and of the males 758 days. Both sclerosis of the coronary arteries and myocardial fibrosis were extremely common in these animals, but there were 3 in which both of these lesions were entirely absent, so that it can hardly be considered as a secondary extension or complication.

The lesion usually involved the entire endocardial surface of the auricle without extending into the appendage or onto the auricular aspect of the mitral valve leaflets. Microscopically the essential finding was a marked thickening of the endocardial layer. As in man, the left auricular endocardium is normally thicker than the right, although smooth muscle cells cannot be identified. With the development of this lesion, the endocardium increased in thickness 4 to 6 times. The picture varied considerably, depending probably on the stage of development and severity of the process. The endothelial cell lining was usually intact although swollen, basophilic and prominent. Occasionally defects were present and fibrin thrombi were precipitated. The entire endocardium was infiltrated with a variable number of cells. Large numbers of mononuclear wandering cells were frequently encountered, but occasionally lymphocytes, plasma cells or even polymorphonuclear leukocytes predominated. More often there was a mixture of cells including proliferating fibroblasts. The thickening was due in part to the

cellular infiltration but also to an irregular production of connective tissue fibers so that the surface frequently became uneven. In some instances connective tissue proliferation was the conspicuous finding, presumably in arrested or relatively inactive cases. The endocardium did not become vascularized.

The lesion is at once reminiscent of the auriculitis associated with rheumatic heart disease in man, both because of its location and because of its microscopic detail. However, palisading of cells against swollen collagen bands, irregular pyknotic nuclear forms and well defined Aschoff nodules did not occur. In addition, the valve leaflets and perivascular connective tissue of the myocardium were not involved by the specific lesions of rheumatic fever. For these reasons it is impossible to associate it with rheumatic infection. The etiology of the process is completely shrouded although its microscopic characteristics indicate that it is inflammatory in nature. Bacteria were not demonstrable.

Bacterial Endocarditis: The heart valves of the rat were occasionally the seat of bacterial infection which reproduced the local picture of acute bacterial endocarditis in man. 15 such cases (3.08 per cent) are included in this series, 9 in males and 6 in females. The age of these rats at death was widely dispersed, the youngest animal being 138 days and the oldest 1072 days. The average age for the group was well below that of the entire series, being 650 days in the male and 566 days in the female. It is interesting that the incidence in which the individual valves are involved compares closely to that in man.

The mitral valve was attacked 11 times, the tricuspid once, the aortic once, and all three mitral and aortic valves and left auricle once. In one instance the infectious process appeared to originate in the endocardium of the right ventricle, although multiple sections might have revealed a focus in the valve. The microscopic picture was somewhat variable. The less severe lesions centered about the distal ends of the leaflets. More often the entire leaflet was involved, its substance eroded so that only remnants of the valve could be made out. Heavy cellular infiltrations of polymorphonuclear leukocytes and other inflammatory cells occurred regularly. Occasionally abortive attempts at repair were evidenced by accumulations of large mononuclear cells and fibroblastic activity. The damaged surfaces of the valves were covered by bulky

vegetations composed of precipitated fibrin, large bacterial colonies and cellular débris. Often the vegetation filled the entire valve orifice and protruded into the adjacent chambers.

The chief extravalvular finding was metastatic abscesses in the myocardium and kidney, and less often at other sites. The spleen was usually enlarged but not pultaceous. Inasmuch as enlargement of the spleen usually accompanies other infections coexistent in these rats, it is difficult to attribute it solely to the blood stream infection. Gross embolic manifestations were conspicuously absent. In several instances bacteria had gained a foothold in the walls of some of the arteries and had led to arteritis and thrombosis, but infarcts of the spleen and kidney were not seen.

It was not usually possible to establish the exact portal of entry of the bacterial agent. This was due to the presence of too many possible sources rather than too few. Most of the animals had infected ulcerations of the plantar surfaces of the extremities and tail, any one of which might have served as the initial point of infection. In addition, 12 had bilateral suppuration of the auditory bullae, 5 bronchiectatic cavities and abscesses in the lungs, and 2 extensive suppurative endometritis. It must be admitted that infections of the same type and severity were just as frequent in the animals with normal heart valves.

Bacterial cultures were not made so that no accurate information concerning the causative agents can be furnished. Bacteria were, however, readily demonstrable in the vegetations. In most instances they appeared to be Gram-positive cocci, but in two, Gram-negative rods were identified. Although usually the valve leaflets were too completely destroyed to make any positive statements as to their condition previous to infection, there is no reason for assuming that preceding damage existed. Unaffected leaflets in the same hearts showed little or no evidence of alteration.

The normal valve leaflets of the rat are delicate fibrous acellular structures having a little centrally placed elastic tissue and regularly failing to reveal the presence of blood channels. At the base of the aortic valve, cartilaginous plates are embedded so often as to be considered a normal component of the annulus. With advancing age the valves underwent only slight changes. The annulus frequently became larger and often directly continuous with myocardial scars at the base of the heart. Rarely small masses of

calcium were deposited either at the base of the atrioventricular leaflets or in the cartilage of the aortic valve leaflets. The substance of the valve became more solid and compact but remained avascular. Just proximal to the distal end of the mitral valve leaflets on the auricular aspect, and coinciding with what must have been the line of closure, a mound-like eminence of loose edematous connective tissue usually developed. Because of the apparent avascularity of the leaflets, the assumption must be made that bacterial infection occurs as a surface implantation possibly at some minutely damage point on the line of closure. It would probably require technically difficult injection experiments to rule out beyond question the existence of valvular blood channels. Much of the theory that has been expounded to account for the greater frequency of involvement of the mitral valve in man has been based on the more common vascularization of this valve. In the light of the present findings this explanation loses at least some of its validity.

It should be pointed out that in every instance the lesion was that of an acute spreading bacterial infection and never was the picture of subacute bacterial endocarditis in man reproduced. The valvular lesions were too acute and destructive and embolic lesions when they involved the renal glomeruli were frankly purulent.

"*Chronic Endocarditis*": The usual appearance of the heart valves has already been described. In addition to the well defined cases of bacterial endocarditis there were 15 cases in which the heart valves showed minor deviations from the normal. For want of a better term these have been designated as "chronic endocarditis," although the changes were perhaps too minute to be so classified. Never was there obvious deformity or insufficiency of any valve. In 7 the lesions consisted essentially of a mild but definite cellular infiltration of large mononuclear cells into the substance of the mitral valve most conspicuously near the distal end and on the auricular aspect. This was sometimes associated with slight edema of the connective tissue and heaping up of the overlying endothelium. In 4 more, the mitral valve showed, in addition, slight defects in its endothelial surface over which small quantities of fibrin or fibrin-like material were attached. In no instance was there true verruca formation. The tricuspid valve shared these lesions in 3 cases; the mitral and aortic valves were involved together in 1 case. The exact interpretation of these

changes is in doubt. Bacteria were not demonstrable. Possibly they merely represented the results of slightly excessive trauma. The valvular lesions of human rheumatic infection were never very closely simulated.

Myocardium

Fibrosis of Myocardium: One of the most common findings encountered was scarring of the heart muscle. 292 or 59.9 per cent of the animals showed some degree of myocardial fibrosis. Males (65.6 per cent) were more often involved than females (55.3 per cent). The sex difference was more definite when the degree of fibrosis was compared, as in Table I. Here it can be seen that 14.5 per cent of the males and only 6.8 per cent of the females showed severe fibrosis — a ratio of more than 2:1. Fibrosis of the myocardium also occurs more often and severely in the human male than in the female. In the case of the rat, differences in environmental factors cannot be held accountable.

The lesion showed a definite relation to age, tending to involve older animals. The average age at death increased with the severity of the fibrosis in both males and females. In view of the fact that the female rat has a distinct advantage in longevity over the male, the sex difference noted above is even more striking. The average age of the 32 males exhibiting marked fibrosis at death was 805 days and of the 18 females 896 days. The age factor in relation to fibrosis is more apparent from the figures in Table II where the animals are grouped in 100 day intervals. Only 2 males between 500 to 600 days old showed severe fibrosis and none at a younger age. It may be seen that the milder degrees of scarring occurred more often in younger animals, but that animals less than 400 days old almost always had intact myocardia. With advancing age groups, the lesion became more frequent and severe and, in general, the males showed earlier and greater involvement than the female. The impression is gained therefore that fibrosis of the myocardium in the rat depended to a large extent upon age and sex. It appeared to be a progressive lesion having its inception about the 400th day of life and becoming more marked throughout the rest of life. Nevertheless, there was a great deal of individual variation and a good proportion escaped the lesion entirely. Close analogies to human arteriosclerotic heart disease might be drawn,

TABLE I

Incidence of Myocardial Fibrosis

	No fibrosis		Slight fibrosis		Moderate fibrosis		Marked fibrosis		Total with fibrosis	
	No. of animals	Per cent	No. of animals	Per cent	No. of animals	Per cent	No. of animals	Per cent	No. of animals	Per cent
Males	76	34.4	62	28.1	51	23.0	32	14.5	145	65.6
Females	119	44.7	63	23.7	66	24.8	18	6.8	147	55.3
Total	195	40.1	125	25.7	117	24.0	50	10.2	292	59.9

TABLE II

Age Distribution of Myocardial Fibrosis

Age	Sex	No fibrosis		Slight fibrosis		Moderate fibrosis		Marked fibrosis	
		No. of animals	Per cent *	No. of animals	Per cent *	No. of animals	Per cent *	No. of animals	Per cent *
days									
0–400	Male	8	88.9	1	11.1	0	–	0	–
	Female	21	95.5	0	–	1	4.5	0	–
400–500	Male	10	55.6	7	38.9	1	5.6	0	–
	Female	11	100.0	0	–	0	–	0	–
500–600	Male	14	51.9	9	33.3	2	7.4	2	7.4
	Female	9	64.3	4	28.6	1	7.1	0	–
600–700	Male	20	40.0	12	24.0	14	28.0	4	8.0
	Female	15	48.4	11	35.5	4	12.9	1	3.2
700–800	Male	12	21.8	20	36.3	16	29.1	7	12.7
	Female	26	38.8	17	25.4	18	26.9	6	9.0
800–900	Male	9	21.9	10	24.4	7	17.1	15	36.6
	Female	24	36.4	18	27.3	22	33.3	2	3.0
900–1000	Male	2	12.5	3	18.7	9	56.3	2	12.5
	Female	13	36.1	11	30.6	10	27.8	2	5.5
1000–1125	Male	1	20.0	0	–	2	40.0	2	40.0
	Female	0	–	2	10.5	10	52.6	7	36.9

* Per cent of all rats in the same 100 day interval.

not only in chronological relation to life span, but in frequency and sex distribution.

The fibrosis was entirely a microscopic finding. The distribution of the scarring varied somewhat but there were certain vulnerable areas. The left ventricle and interventricular septum were by far the most common sites. The earliest scars were found at the base sometimes extending as tenuous bands from the annulus of the mitral valve or at the tip of the left ventricle. In the latter region the muscle layer is normally quite thin and in advanced cases the entire muscle from epicardium to endocardium was replaced by dense connective tissue. Frequently scars seemed to center about and extend from the adventitia of the larger coronary arteries as they coursed through the muscle layer. At times they were haphazardly distributed. The right ventricle was almost never involved save only at its very base. The auricular muscle remained intact.

For the most part the scarring was partial and rather diffuse, isolated groups of atrophic or hypertrophic muscle bundles being intermingled. Not infrequently fibrous tissue was very finely distributed but widespread, appearing as a generalized increase in loose interstitial connective tissue. Rarely large continuous masses of partly hyalinized, completely acellular connective tissue devoid of muscle fibers had formed. These most closely resembled healed infarcts. True fresh or partly healed infarcts were never found and it seems very doubtful that any of the fibrosis was preceded by necrosis of muscle. Blood pigment and mononuclear cells were usually lacking. The absence of cellular reaction makes it seem most probable that the fibrosis was associated with a slow atrophy of the muscle either due to impaired blood supply or to intrinsic degeneration of the muscle tissue itself. In a few instances cellular reaction was very striking, a variety of leukocytes participating. In these, the evidences of inflammation were so apparent that the lesion might well be classified as chronic myocarditis. This finding occurred so seldom that it is difficult to believe that the more usual type of scarring was the result of a previous inflammatory process which had become arrested. Moreover, the earliest lesions showed no tendency to exhibit an inflammatory character. The specific localizations of the scarring in certain parts of the heart also argues against an infectious background.

As will appear shortly, alterations of the coronary arteries, which would lead to loss of elasticity and hardening if not to complete closure, frequently accompanied the myocardial damage. The weight of the evidence at hand suggests that a process is involved somewhat analogous to arteriosclerotic heart disease in man, namely that with advancing age there is commonly a progressive sclerosis of the coronary arteries interfering with the nutrition of the myocardium and resulting in atrophy and fibrosis. In the rat the vascular lesion is essentially a medial one and never leads to occlusion or thrombosis so that the acute lesions of myocardial infarction do not develop.

Sclerosis of Coronary Arteries: As previously noted, showing close coincidence with fibrosis of the myocardium, the coronary arteries frequently developed changes that consisted essentially of a loss of smooth muscle and a replacement of the media by fibrous tissue. The intima remained thin, the lumen patent, although often reduced to a slit, and the internal elastic lamella although straightened retained its unity. The earliest visible change was a reduction and irregular distribution of smooth muscle nuclei of the media. Some of the muscle cells were hypertrophied and on cross section appeared strikingly vacuolated. Accessory bunches of smooth muscle cells forming imperfect new coats occasionally developed in the adventitia or beneath the intima to give the vessel irregular contours, make it thicker than normal, and encroach somewhat upon the lumen. The adventitia became thickened and densely fibrous. Eventually connective tissue replaced the smooth muscle and this often became hyalinized. Calcification of the media occurred rather infrequently but was noted in 17 cases. It was usually rather scanty, consisting of small deposits in and beneath the elastic lamella. In one instance the calcification was extensive enough to form curved plates within the media, incompletely encircling the entire circumference of the right coronary artery just beyond its point of origin. The intima remained delicate, consisting of a single layer of endothelium closely approximating the subjacent elastic fiber. Lipoid deposits in the intima, similar to atherosclerosis of man, were not observed.

The coronary arteries of the rat's heart are two in number and arise from the sinuses of Valsalva at points similar to the origin of the coronary arteries in man. They also follow the usual distribu-

tion of the human vessels, the right being slightly the larger and supplying portions of the left ventricle posteriorly and the interventricular septum. In the atrioventricular sulci the coronary arteries lie in the subepicardium sometimes embedded in a slight amount of adipose tissue. Subepicardial fat in the rat's heart is always scanty, even in well nourished animals, and is confined to the base of the ventricles. All the branches of the main coronary arteries equivalent to the descending branches in man are definitely intramuscular, although the coronary veins course superficially. The microscopic appearance of the normal coronary artery shows no striking peculiarities. They possess a single internally placed, wavy elastic lamella. Both vessels appeared to be involved with equal frequency and severity by the sclerotic process. The most profound changes were found in the main arteries just beyond their points of origin but the lesion extended well into the intramuscular branches. One exception was a small artery rather constant in position near the base of the anterior leaflet of the mitral valve which often showed marked fibrosis when all other vessels were quite normal.

From Table III it may be seen that, as in fibrosis of the myocardium, the male is more often and severely affected than the female. The incidence of severe coronary sclerosis is again almost 2:1 in favor of the male. Similarly it may be seen that the total incidence of each grade of involvement closely parallels that of myocardial fibrosis by comparing the figures in Table I. 57.7 per cent of all the animals showed some degree of coronary arteriosclerosis, whereas 59.9 per cent showed some myocardial fibrosis.

Table IV shows the age distribution at 100 day intervals and discloses that age plays an analogous rôle in the development of the arterial lesion, as it did in myocardial fibrosis. There is this exception however. The milder degrees of arterial change not infrequently made their appearance in the first 400 days of life. This might be taken as evidence that the vascular lesion antedated the muscular scarring and if any cause and effect relation between the two lesions existed, the arteriosclerosis was responsible for the subsequent myocardial degeneration.

Although the two lesions were closely linked as regards frequency, age and sex distribution, the two did not always parallel each other in individual cases. For example, 16 animals that

TABLE III

Incidence of Sclerosis of Coronary Arteries

	No sclerosis		Slight sclerosis		Moderate sclerosis		Marked sclerosis		Total with sclerosis	
	No. of animals	Per cent	No. of animals	Per cent	No. of animals	Per cent	No. of animals	Per cent	No. of animals	Per cent
Males	86	38.9	51	23.1	59	26.7	25	11.3	135	61.1
Females	120	45.1	72	27.1	58	21.8	16	6.0	146	54.9
Total	206	42.3	123	25.3	117	24.0	41	8.4	281	57.7

TABLE IV

Age Distribution of Sclerosis of Coronary Arteries

Age	No sclerosis		Slight sclerosis		Moderate sclerosis		Marked sclerosis	
	No. of animals	Per cent	No. of animals	Per cent	No. of animals	Per cent	No. of animals	Per cent
days 0–400	25	80.6	4	12.9	2	6.5	0	–
400–500	22	75.9	3	10.3	4	13.8	0	–
500–600	23	56.1	13	31.7	5	12.2	0	–
600–700	41	50.6	20	24.7	13	16.1	7	8.6
700–800	46	37.7	35	28.7	28	23.0	13	10.6
800–900	30	28.0	27	25.2	37	34.6	13	12.2
900–1000	19	38.5	11	21.2	17	32.7	5	9.6
1000–1125	0	–	10	41.7	11	45.8	3	12.5

exhibited moderate or marked fibrosis of the myocardium revealed apparently normal coronary arteries. On the other hand, 10 animals showing moderate or severe sclerosis of the coronary arteries had intact myocardia. It is likely that a more extensive microscopic examination would have yielded a closer correlation in these exceptional cases. Reference to Table V will show that in general there is a fairly close correspondence between the two lesions. 77.5 per cent of the animals showing no myocardial fibrosis had normal coronary arteries and 84 per cent with either moderate or severe fibrosis also had either moderate or severe sclerosis of the coronary arteries. In arteriosclerotic heart disease in man, the severity of the coronary artery lesion usually parallels the degree of myocardial damage but individual cases show just as marked discrepancies as do the two lesions in the rat.

Coincidental extracardiac lesions were analyzed to see if they played a rôle in the development of these lesions but no such relationship was discovered. One lesion of interest in this connection is the pulmonary one consisting of a combination of bronchiectasis and lung abscess and occurring in varying degree in about 75 per cent of the animals. Both by reason of its high incidence and because of the effects of a badly impeded pulmonary circulation, it is conceivable that this lesion might have been a factor in producing cardiac changes. However, distinct hypertrophy of the right ventricle was lacking. Moreover, 26 per cent of the animals showing little or no bronchiectasis had moderate or severe cardiac lesions. On the other hand, 31 per cent showing severe or moderate bronchiectasis had little or no cardiac damage, whereas only 19 per cent with equally severe bronchiectasis exhibited severe or moderate cardiac lesions. The implication of the latter finding is that animals with marked pulmonary lesions may have succumbed before reaching the age in which severe lesions of the heart were prevalent. Furthermore, the pulmonary lesions did not progressively increase in frequency with advancing age so uniformly as the cardiac ones. For these several reasons it seems quite unlikely that the cardiac damage resulted secondarily from impaired pulmonary circulation.

Cardiac Hypertrophy: The size of the heart of the adult rat at spontaneous death varied considerably and was dependent largely on the body size of the animal and the degree of dilatation. Since

TABLE V

Relation of Myocardial Fibrosis to Sclerosis of Coronary Arteries

	No sclerosis		Slight sclerosis		Moderate sclerosis		Marked sclerosis	
	No. of animals	Per cent	No. of animals	Per cent	No. of animals	Per cent	No. of animals	Per cent
No fibrosis	151	77.5	34	17.5	8	4.1	2	1.0
Slight fibrosis	39	31.2	53	42.4	26	20.8	7	5.6
Moderate fibrosis	13	11.1	31	26.5	63	53.9	10	8.5
Marked fibrosis	3	6.0	5	10.0	20	40.0	22	44.0

the weight of the animal was influenced by the state of nutrition and the amount of postmortem dehydration, the ratio of body weight to heart weight was not a reliable criterion of hypertrophy. The weight of the heart was not recorded and estimations of hypertrophy were based on total size, ventricle wall thickness and the microscopic diameters of the individual fibers. It is admitted that these criteria are largely subjective and liable to error. 173 rats, 86 males and 87 females, were considered to have definite hypertrophy of the heart in some degree, an incidence of 35.5 per cent. Although the sexes were about equally involved, the enlargement in the male was often more striking. Hypertrophy was rarely detectable before 500 days of age and became more frequent with advancing age thereafter. The left ventricle was the chief site, so that this chamber often occupied a disproportionately large part of the entire organ. The increase in muscle was usually associated with some degree of dilatation.

Microscopically the muscle bundles were increased in diameter. This was more striking at the base of the left ventricle rather than at the apex, and often hypertrophic fibers were unevenly distributed, leaving many groups of muscle bundles unchanged. The structure of the hypertrophic fiber was essentially unaltered. The nuclei were not strikingly enlarged but were irregular and hyperchromatic. The interfibrillar substance was not increased.

Many of this group showed a coincidental myocardial fibrosis and changes in the coronary arteries. This is not surprising when it is recalled that similar age groups are involved. The total incidence of hypertrophy was much lower than that of the other two lesions, so that it was not an inevitable accompaniment of the latter. In fact, many of the most severely fibrotic hearts were quite small. 43 per cent of the animals with no hypertrophy did have some degree of fibrosis. Hypertrophy without fibrosis was seen in only 14, about 8 per cent of all those without fibrosis. The inference may be drawn that hypertrophy often accompanied fibrosis and coronary arteriosclerosis but was by no means a constant concomitant. Hypertrophy in the absence of other myocardial changes, although uncommon, also occurred. As in man, enlargement of the heart not infrequently accompanied chronic renal disease. 24 per cent of the hypertrophy group also had relatively severe renal lesions.

Infrequent Myocardial Lesions: Suppurative infections were extremely common in the entire series. The most usual sites were the auditory bullae, lungs, uterus, cranial cavity, kidneys, prostate and liver. In a few instances bacteria had gained entrance into the blood stream and given rise to metastatic foci. The heart was one of the most common sites for such secondary abscesses, being involved 17 times. The abscesses were usually small, fresh, well demarcated, often multiple and without an encapsulating membrane. They consisted of disintegrating polymorphonuclear leukocytes and centrally placed clumps of bacteria.

Aside from these definite foci of infection, it was not unusual to find a few scattered cells infiltrated into the interstitium and often in the adventitia of blood vessels. Occasionally these were grouped together in submiliary collections so as to resemble somewhat the appearance of Aschoff nodules, although in no instance was the reproduction very exact. Such lesions did not occur in conjunction with endocardial or pericardial changes. These cellular aggregates exhibited much individual variation. In 21 cases the resemblance to human Aschoff bodies was fairly close. They were apt to occur in hearts that were considerably scarred but could also be found in otherwise normal areas. Occasionally the cells in them were large basophilic and had hyperchromatic, heavily rimmed nuclei with prominent nucleoli. Swollen fragmented collagen bundles were usually lacking and never very definite.

The series includes 17 cases of leukemia in which infiltrations of leukemic cells into various organs were common. The heart was infrequently invaded but twice there were small collections of leukemic cells penetrating the interstitial and subendocardial tissue.

Relatively few malignant growths were observed in the present series and most of these were of connective tissue origin rather than epithelial. Only one, an osteosarcoma of undetermined origin had metastasized to the heart.

Pericardium

Acute Suppurative Pericarditis: The pericardial sac was infected with bacteria 11 times, an incidence of 2.3 per cent. Five times the resultant inflammation was confined to small areas, usually at the base of the heart over the auricular epicardium, but in the others the entire surface was covered by thick layers of fibrinopurulent

exudate. Bacteria were readily demonstrable. Granulation tissue was seen at times in the deeper layers of the exudate. In 9 of these 11 there was extensive pulmonary and pleural infection, and in several direct extension to the pericardium could be traced. The remaining 2 had suppurative lesions elsewhere which might have served as an initial source. The underlying myocardium and the endocardium were unaltered.

Acute pericarditis in rodents caused by infection with a streptothrix has been described by Berberich and Nussbaum [13] in hemorrhagic septicemia, and with a diplococcus by Seifried.[14] The pericardial infection was the result of extension from pulmonary foci.

Chronic Pericarditis: The pericardium of the rat appears to be subject to a specific inflammatory process which because of its distinctive histological appearance seems to be a disease entity. The picture was that of a low grade, persistent inflammation attended by the infiltration of lymphocytes, plasma cells, large mononuclears and the local proliferation of mesothelial cells and fibroblasts. The epicardium was thickened and densely cellular. The overlying mesothelium was swollen, basophilic, heaped up in villus protrusions and even stratified. Often the infiltrating cells were lined up in palisade formation parallel to the surface and lodged against long acellular bands of eosinophilic collagen. Such palisades were often multiple. In more severe cases the surface mesothelium was disrupted and minute quantities of a fibrinoid substance were deposited in the exposed surface. Occasionally eosinophils were fairly numerous but neutrophilic polymorphonuclear leukocytes were always infrequent.

The process was not uniformly distributed and was usually most advanced in crevices and sulci about the auricular appendages or in the atrioventricular groove. Less often it involved the entire epicardium, but unevenly, with mound-like eminences irregularly scattered. The normal parietal pericardium was apparently so delicate as to be invariably ruptured on removing the chest plate. At any rate it was not often detected. Pericardial adhesions, which from the nature of the lesion might be reasonably expected, were not encountered. Grossly the epicardial surface was described as smooth but milky gray and translucent.

The lesion was not confined to the percardium but frequently involved both pleural cavities and the peritoneum. In the latter

it was most often seen over the capsules of the spleen and liver, around the pancreas, mesentery and posterior peritoneal surface. The microscopic appearance was always essentially the same. In neither pleura nor peritoneum did obliterative adhesions form. Aside from the serous surfaces, no other tissues were involved. The process may be considered to be essentially a polyserositis.

29 such cases were recognized, an incidence of 6 per cent. In 13 all three serous cavities were involved and in the remainder the pericardium alone was attacked. One striking feature was its decided preponderance in female rats, there being only 5 males to 24 females in the group. The lesion was found in comparatively old rats. The age at death of the females was 878 days and of the males 735 days.

The etiology of this process is completely obscure. Microorganisms were not demonstrated, but cultures were not taken. Intracellular inclusion bodies were not encountered. It bore no definite relation to any other disease process. Suppurative infections, although common, were not more so than in the other animals. Three of the rats had inflammatory changes in the left auricular endocardium previously designated as auriculitis. Since both lesions were relatively infrequent (6 per cent and 3.7 per cent), this association is perhaps higher than might be expected. Myocardial fibrosis and cardiac hypertrophy were not more marked than in animals of similar age periods. Because of its distribution and chronicity, one is reminded of Pick's disease in humans, but arrested cases with calcification, hyalinization and obliterating adhesions were never observed. Occasionally several cubic centimeters of clear pale fluid were contained within the peritoneal cavity, but larger accumulations were lacking.

A similar disease, possibly etiologically related, has been described in guinea pigs by Steinmetz and Lerche [15] and more recently by Roth.[16] A Gram-labile bacillus was recovered from the lesions but an etiological relationship has not been definitely established. The guinea pig disease differs from that in the rat in that it is acute and fatal.

Summary

The pathological manifestations of cardiac disease in a group of 487 inbred albino rats maintained on adequate diets and under

constant laboratory conditions over their entire life span are described. The animals were not subjected to experimental manipulation and the disorders encountered must be considered to have evolved from spontaneous causes and under natural circumstances. The rat's heart proved to be quite susceptible to a variety of disease processes, some of which are distinctive and peculiar to this species, while others have their counterpart in man. A simple tabulation of each type of lesions described and its incidence follows:

Endocardium

Intracardiac thrombosis	6.4%
Chronic auriculitis	3.7%
Acute bacterial endocarditis	3.4%
"Chronic endocarditis" (without valvular insufficiency)	3.4%

Myocardium

Fibrosis of myocardium	59.9%
Sclerosis of coronary arteries	57.7%
Sclerosis of coronary arteries with calcification	3.7%
Hypertrophy of myocardium	35.5%
Abscess of myocardium	3.7%
"Interstitial myocarditis"	4.3%
Leukemic infiltration	0.4%
Secondary osteosarcoma	0.2%

Pericardium

Acute suppurative pericarditis	2.3%
Chronic pericarditis	6.0%

Both the lesions classified as chronic auriculitis and chronic pericarditis are apparently peculiar to the rat, although the latter lesion may be related to a similar condition occurring in guinea pigs. They consist essentially of long-standing, low grade inflammatory changes. In neither case was the responsible etiological agent identified. The pericardial lesion appears to be merely one expression of a generalized disturbance of the serous surfaces.

All the other processes described resemble in part at least those recognized in man. The acute bacterial infections certainly differed in no essential manner. Intracardiac thrombosis, chiefly of the left auricle, is somewhat similar to the auricular appendage thrombosis in the diseased human heart. In the rat, however, the thrombus usually filled the entire auricle and occurred as a terminal event, especially in senile animals. Myocardial fibrosis of the left

ventricle is common to both species but in the rat it is less obviously the result of reduced arterial circulation. Changes in the coronary arteries are common and do parallel the myocardial lesions but they never lead to complete or even marked occlusion.

With the exception of the infectious processes, almost all of the changes described make their appearance late in the 2nd year of life and do not attain their maximum incidence until well into the 3rd year. These periods correspond roughly to late middle age and early senescence. Many of the human cardiac conditions have a similar age distribution. In both species the male is somewhat more susceptible to this type of disease than the female.

Notable in the rat by their absence are the intimal atheromas of human coronary artery disease, evidences of myocardial infarction, chronic valvular deformities and rheumatic infection. Slight, apparently non-specific inflammatory changes of the mitral valve and perivascular tissue of the myocardium, which might be erroneously construed if encountered in experimental animals, do occur but are relatively rare.

REFERENCES

1. Fox, Herbert. Arteriosclerosis in lower mammals and birds: its relation to the disease in man. Arteriosclerosis, Cowdry, E. V. The Macmillan Company, New York, 1933, Chapter 6.

   Fox, Herbert. Disease in Captive Wild Mammals and Birds. J. B. Lippincott Company, Philadelphia, 1923.

2. Krause, Curt. Zur Frage der Arteriosklerose bei Rind, Pferd und Hund. *Beitr. z. path. Anat. u. z. allg. Pathol.*, 1922, **70**, 121–178.

3. Wolkoff, Kapitoline. Über Altersveränderungen der Arterien bei Tieren. *Virchows Arch. f. path. Anat.*, 1924, **252**, 208–228.

4. Miller, C. Philip, Jr. Spontaneous interstitial myocarditis in rabbits. *J. Exper. Med.*, 1924, **40**, 543–551.

5. Ophüls, W. Spontaneous arteriosclerosis of the aorta (atheroma) in a rabbit. *J.A.M.A.*, 1907, **48**, 326.

6. Miles, Amy B. Spontaneous arterial degeneration in rabbits. *J.A.M.A.*, 1907, **49**, 1173–1176.

7. Löwenthal, Karl. Kreislauforgane. Anatomie und Pathologie der Spontanerkrankungen der kleinen Laboratoriumstiere, Rudolf Jaffé. Julius Springer, Berlin, 1931, 1–11.

8. McCay, C. M., Crowell, Mary F., and Maynard, L. A. Effect of retarded growth upon the length of life span and upon the ultimate body size. *J. Nutrition*, 1935, **10**, 63–79.

9. Sherman, Henry C. General introduction. Chemistry of Food and Nutrition. The Macmillan Company, New York, 1932, Ed. 4, 3–4.

10. Cowdry, E. V., and Scott, Gordon H. Effect on monkeys of small doses of a concentrated preparation of viosterol. *Arch. Path.*, 1936, **22**, 1–23.

11. Kesten, H. D. Early incidence of spontaneous medial degeneration ("arteriosclerosis") in the aorta of the rabbit. *Arch. Path.*, 1935, **20**, 1–8.

12. Sherman, H. C., and Campbell, H. L. Further experiments on the influence of food upon longevity. *J. Nutrition*, 1930, **2**, 415–417.

Sherman, H. C., and Campbell, H. L. Rate of growth and length of life. *Proc. Nat. Acad. Sc.*, 1935, **21**, 235–239.

13. Berberich, J., and Nussbaum, R. Anatomie und Pathologie der Spontanerkrankungen der kleinen Laboratoriumstiere, Rudolf Jaffé. Julius Springer, Berlin, 1931, 80.

14. Seifried, O. Anatomie und Pathologie der Spontanerkrankungen der kleinen Laboratoriumstiere, Rudolf Jaffé. Julius Springer, Berlin, 1931, 565, 566, 574, 584.

15. Cited by Seifried, O. Anatomie und Pathologie der Spontanerkrankungen der kleinen Laboratoriumstiere, Rudolf Jaffé. Julius Springer, Berlin, 1931, 584.

16. Roth, Hans. Zur Bakteriologie und Pathologie der Fibrinösen Serositis der Meerschweinchen, Bacterium Caviarum Pericarditidis, N. Sp. *Acta path. et microbiol. Scandinav.*, 1934, 11, 335–360.

DESCRIPTION OF PLATES

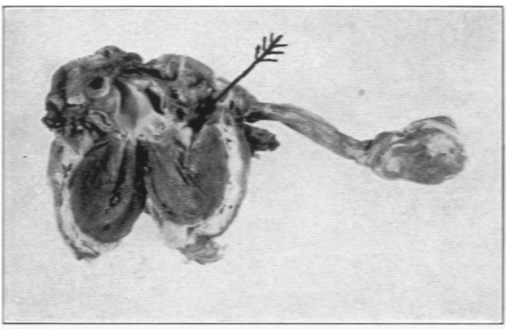
PLATE 37

FIG. 1. Acute bacterial endocarditis and acute suppurative pericarditis. The arrow indicates a ball-like vegetation on the mitral valve leaflets. The epicardium is everywhere coated by a thick layer of fibrinopurulent exudate.

FIG. 2. Cardiac hypertrophy. The variation in size of the heart at death in 2 old rats is indicated. The one on the left is considerably hypertrophied.

FIG. 3. Thrombosis of auricle. The left auricle is completely obstructed by a thrombus loosely united to the underlying intact endocardium. The thrombus still shows distinct platelet columns but the leukocytes at their margins are disintegrating. × 60.

FIG. 4. Chronic auriculitis. The endocardium of the left auricle is irregularly thickened by a dense cellular infiltration of polymorphonuclear leukocytes, lymphocytes and large mononuclear cells. The surface endothelium is swollen. × 460.



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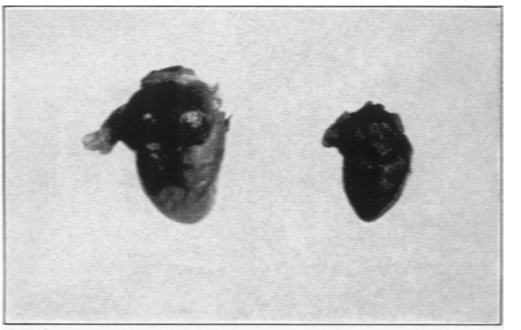

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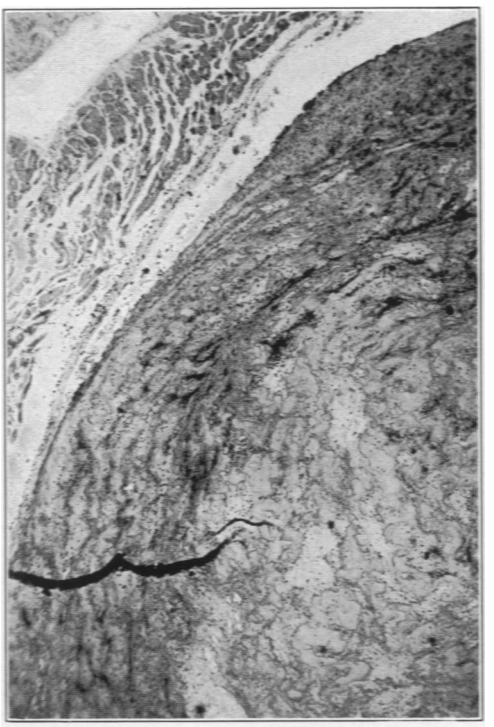

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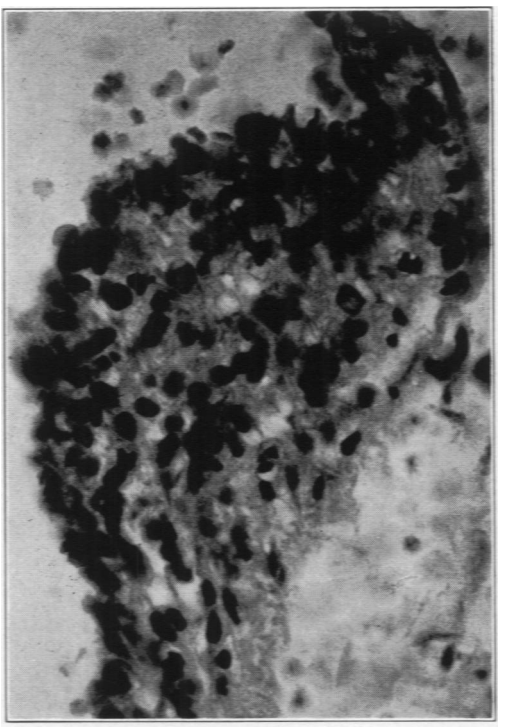

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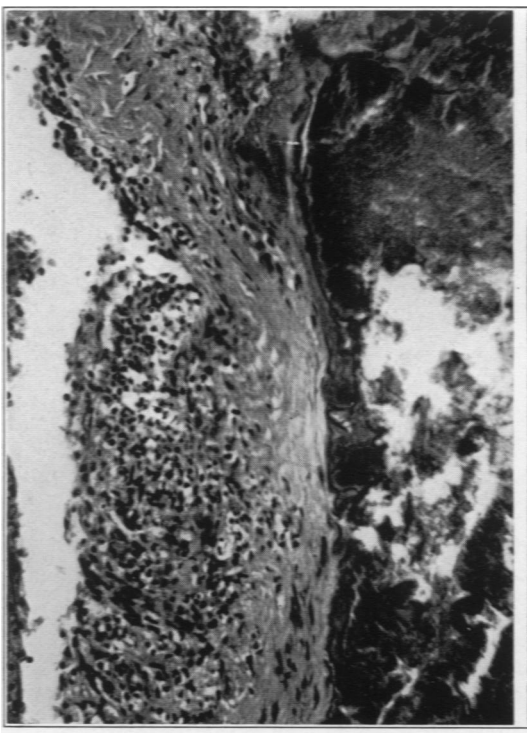
PLATE 38

FIG. 5. Acute bacterial endocarditis. The remains of the mitral valve leaflet are heavily infiltrated with polymorphonuclear leukocytes. The surface is ulcerated and on one aspect a vegetation containing small, deeply staining bacterial colonies is deposited. × 110.

FIG. 6. Calcification of coronary artery. The calcium is in the form of a narrow but continuous band extending around most of the circumference of the vessel. The deposit is confined to the intima and inner aspects of the media. × 60.

FIG. 7. Sclerosis of coronary artery. The lumen is reduced to an elliptical slit lined by intact endothelium. The media is converted into dense acellular collagen. A few strikingly vacuolated smooth muscle cells persist in its outer layers. The adventitia is also thickened by fibrous tissue. × 460.



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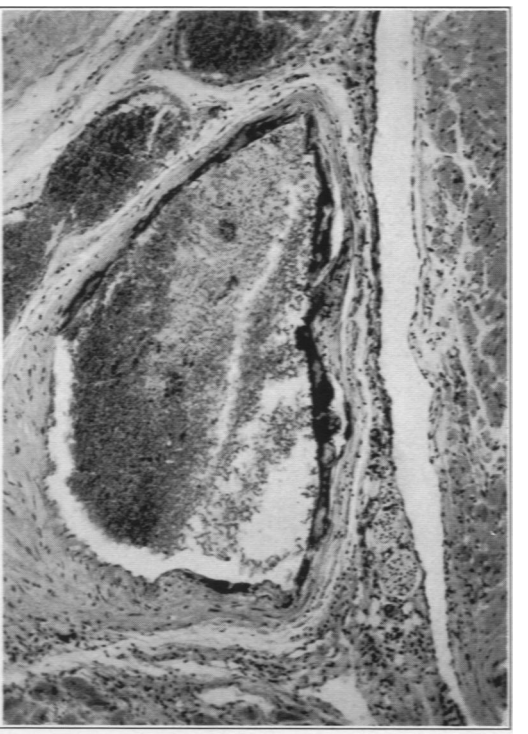

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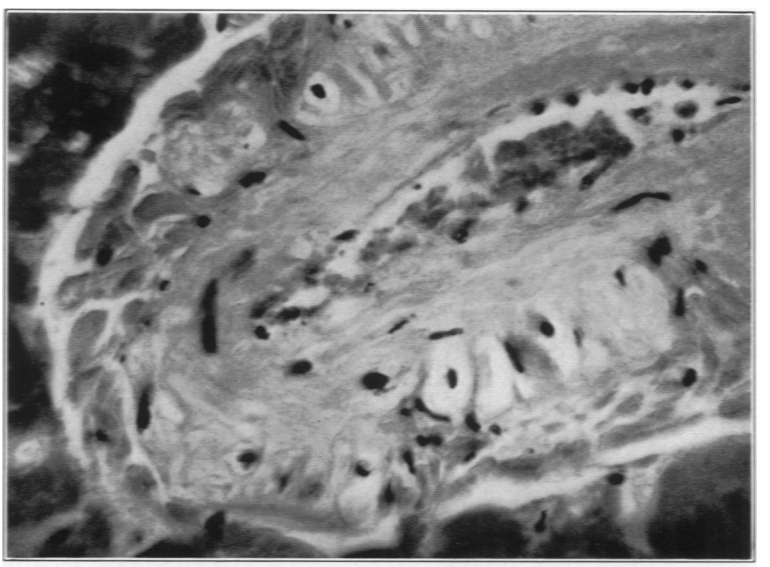

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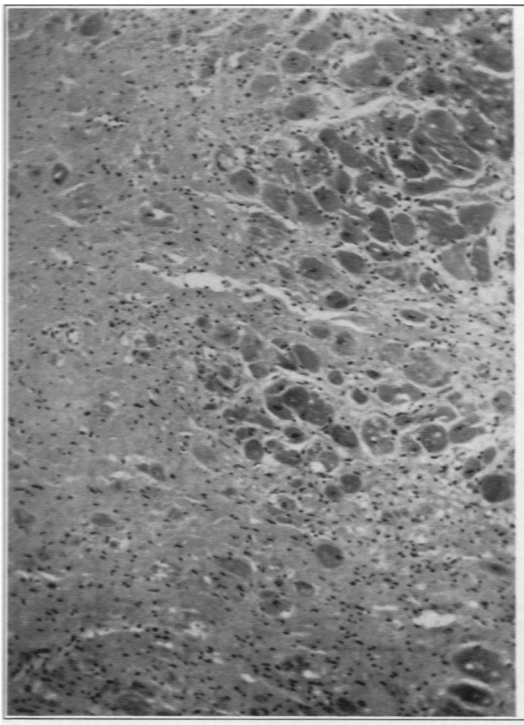
PLATE 39

FIG. 8. Fibrosis of myocardium. The margin of a large, dense, relatively acellular scar is shown where it adjoins and extends between atrophic and hypertrophic muscle bundles. × 110.

FIG. 9. "Interstitial myocarditis." A focal area of cellular infiltration separates adjacent muscle bundles. Lymphocytes, large mononuclear cells and fibroblasts are irregularly dispersed between collagen fibers. × 460.

FIG. 10. Chronic pericarditis. The epicardium is thickened, vascularized and densely infiltrated with a variety of leukocytes. A slight amount of fibrin is deposited at the surface. × 300.

FIG. 11. "Chronic endocarditis." The section is through an area of edematous thickening near the distal extremity of the leaflet. On the auricular aspect there are villus-like irregularities filled with pyknotic and fragmented nuclei. Beyond this point within the leaflet the collagen is swollen, granular, disorganized and deeply eosinophilic. × 300.



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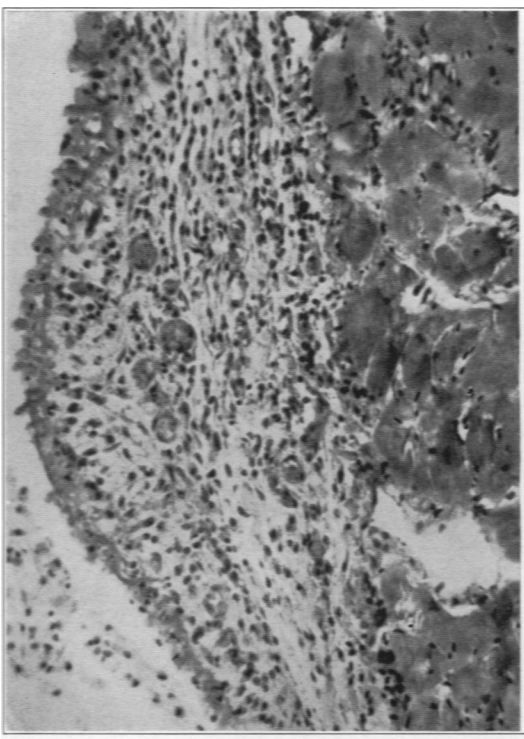

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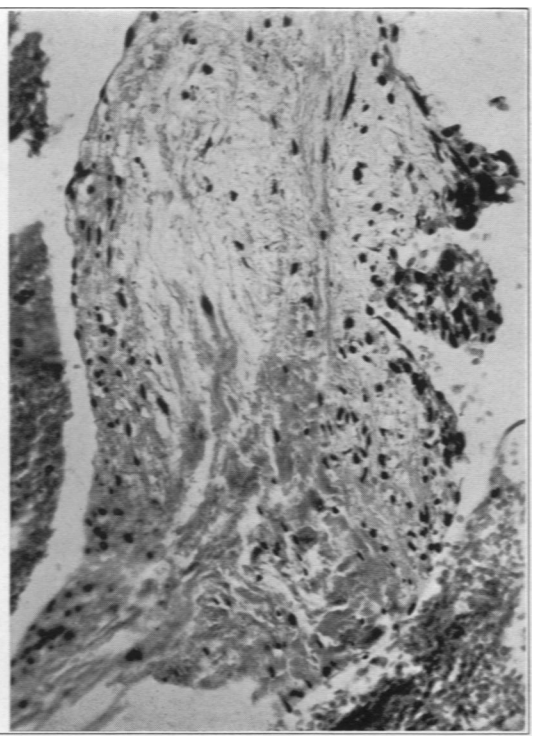

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