FORCED EXERCISE AND CARDIAC PATHOLOGY IN RATS

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ABSTRACT

FORCED EXERCISE AND CARDIAC
PATHOLOGY IN RATS

by James L. Wilson

This study was designed to determine the effects of an intense exercise program on cardiac muscle in adult rats and to determine if pre-pubertal forced exercise had a protective effect on the heart when the animals were subjected to forced exercise later in life.

One hundred and fifty male albino rats were brought into the laboratory as weanlings and divided randomly into three equal groups. These groups were randomly assigned to the forced, voluntary, and sedentary exercise groups. At thirty days of age, the pre-pubertal training period began. For thirty-five days, the forced group animals were exercised for one-half hour per day by swimming with 2% of their body weight fastened to their tails. The voluntary group could exercise at will in running wheels attached to their cages. The sedentary group remained relatively immobile within standard cages. Following the initial training period all animals were placed in voluntary cages for one hundred and eighty-two days. A second training period of thirty-five days duration followed the voluntary stage and this

post-pubertal or adult training program involved all but ten control animals from each group. In the second training period the animals were forced to swim one-half hour per day with 1-2% of their body weight tied to their tails. The control animals for the groups were returned to their original experimental condition with the exception of the controls in the forced group which were placed in sedentary conditions.

The design described above was duplicated at a later date on forty-two female albino rats. The heart tissues of all animals were studied histologically.

Myocarditis was induced in 29.57% of the male animals forced to exercise later in life. The pathological changes were independent of the pre-pubertal condition. Cardiac lesions were found in 10% of the male control animals. This pathology ranged from a very mild perivascular lymphocytic infiltration to a severe myocardial necrosis and polymorphonuclear leucocytic infiltration. No pathological changes were found in the female animals.

On the basis of the evidence presented, pre-pubertal forced exercise does not appear to play a role in the prevention of myocardial damage later in life. The detrimental effects of heavy exercise later in life in the male animals which was unexpectedly independent of the pre-pubertal experimental conditions warrants further investigation.

FORCED EXERCISE AND CARDIAC PATHOLOGY IN RATS

By

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PREFACE

In the preparation of this thesis, many people devoted much time and effort. I would especially like to thank Drs. R. E. Carrow, W. D. Van Huss, and W. W. Heusner of the Michigan State University faculty. I would also like to thank the staff of the Human Energy Research Laboratory at Michigan State University for their assistance in this study.

In addition to the above people, I would like to thank the National Institute of Health, for without its financial assistance this thesis would not have been possible.

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CHAPTER I

INTRODUCTION

It has been the contention of many research workers and educators that physical activity has beneficial effects on circulo-respiratory efficiency. In an earlier study (10) involving exercise of adult male rats (227 days), gross inspection of the hearts aroused speculation that pathology was present. The present study was designed to investigate these findings in more detail using appropriate histological techniques.

Cardiac lesions have been studied in several laboratory animals (1-6). However, none of these reports have dealt with physical exercise, and more specifically, none have attempted to show what the early effects of exercise might have later in life. Therefore, the current study was undertaken to determine if there is any protective value of early exercise on cardiac pathology in later life, and to determine the effects of an intensive exercise program later in life. Specifically, the necrotic and fibrotic foci, indicative of cell degeneration and scar tissue formation, were to be searched for.

The subject of this study is currently of great public interest. With the promotion of this interest through

the President's Council on Physical Fitness, the YMCA, and numerous local agencies, the effect of fitness programs on their middle-aged participants is extremely important. Generalizations from this animal study to human physiological phenomena would be difficult and are not to be implied. However, research in this area can assist in the elimination of many perplexing problems confronting the field of exercise physiology and can provide clues for further research on man.

CHAPTER II

METHODS

One hundred and fifty male albino rats were received in the laboratory as weanlings (25 days) and placed in individual cages (5" x 5" x 12"). No animals were removed from the study on the basis of pre-experimental activity, as had been done in the initial pre-puberty study (10). At thirty days of age, the rats were randomly assigned to three equal groups.

Group A was a sedentary group and remained relatively inactive within their cages. Group B, the voluntary group, had running wheels attached to the sides of their cages, and could exercise under their own volition. Group C was the forced group. They were kept in cages of the type used for Group B, but were forced to exercise for one-half hour per day by swimming in individual tanks with 2% of their body weight tied to their tails. The rats had free access to commercial rat food (Wayne Lab Blocks) and water at all times.

From thirty to sixty-five days, the pre-pubertal stage in albino rats, the groups remained as stated above. Following the 65th day, the 182 day adolescent period was

marked by the three groups being placed in voluntary-exercise-type cages. The second training program which included all animals except ten animals from each group followed. These ten animals were used as controls and returned to conditions characteristic of their pre-pubertal training period, with the exception of those from the forced group. These animals were placed under sedentary conditions. The remaining rats in each group were forced to exercise by swimming in individual tanks for one-half hour per day with 1-2% of their body weight tied to their tails. (Figure 1)

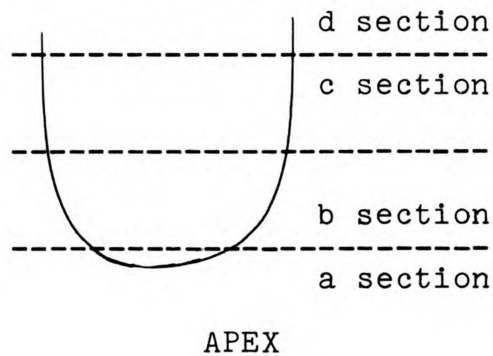
All animals, having expired during the training period, or sacrificed at its completion, were subjected to the experimental method shown in Figure 1. The intact body was weighed. The hearts were excised immediately by severing the blood vessels at their junction with the heart. The heart was weighed in whole, dissected in transversely sectioned quarters (Figure 2), and fixed in 10% formalin. The tissues were dehydrated in graded concentrations of alcohol, embedded in paraffin, and sectioned at 7 microns. Sections from each quarter were stained with hematoxylin-eosin and Mallory's Trichrome stain (for connective tissue) and examined under the microscope.

At this time, another pre-pubertal study was conducted in our laboratory using female albino rats. The same experimental design was used, except that the

Figure 1.--Experimental Method

	Pre-Puberty Training Pd. I	Post-Puberty	Training Pd. II
Sedentary (S) 50 Rats	35 days	182 days (V)	35 days - 40 rats (SVF)
			10 rats (SVS)
Voluntary (V) 50 Rats	35 days	182 days (V)	35 days - 40 rats (VVF)
			10 rats (VVV)
Forced (F) 50 Rats	35 days	182 days (V)	35 days - 40 rats (FVF)
			10 rats (FVS)

Figure 2.--Heart Sectioning Method



post-pubertal voluntary exercise period was 160 days rather than 182 days. Forty-two animals were used and all were subjected to the same histological examination as the males.

CHAPTER III

RESULTS

Myocardial lesions were observed in 34 of the 115 rats that were exposed to the second training period. This involved 12 forced, 10 voluntary, and 12 sedentary animals (Tables 1 and 2). The lesions ranged from a slight perivascular lymphocytic infiltration to advanced myocardial necrosis and polymorphonuclear leucocytic infiltration (Plates 1 and 2). In certain small foci, the muscle was entirely replaced by mononuclear and polymorphonuclear leucocytes and plasma cells.

The lesions were classified by groups (Table 1).

The lesions were also classified as being either mild or severe and are tabulated in Table 2 by location and degree of involvement.

Using a Chi Square statistical analysis, its was calculated that there was no significant difference in the frequency or severity of necrosis between the three experimental groups.

The animals of all three groups were grouped together as experimental animals in the second exercise period since no difference between groups was found. In these animals 29.6% exhibited cardiac necrosis. This

Table 1.--Pathology by Groups

	SVF	SVS	VVF	VVV	FVF	FVS
Number in Group	38	10	38	10	39	10
Number with Lesions	12	0	10	2	12	1

Table 2.--Location and Severity of Lesions

Heart Section	SVF		SVS		VVF		VVV		FVF		FVS	
	sev.	md.	sev.	md.	sev.	md.	sev.	md.	sev.	md.	sev.	md.
a only	4	0	0	0	1	1	0	0	0	0	0	0
a & b	1	1	0	0	2*	0	0	1	4	1	1*	0
b only	0	1	0	0	1	3	1	0	4*	0	0	0
b & c	0	2	0	0	0	1	0	0	0	1	0	0
c only	0	0	0	0	1*	0	0	0	0	0	0	0
a & b & c	2*	1**	0	0	0	0	0	0	1	1	0	0

*Animals with lesions in right and left ventricles.
**Animals with lesions in right ventricle.
All other lesions were in the left ventricle.

group was then compared with the controls which were found to have cardiac necrosis in 10% of the animals. The groups when compared using the Mann-Whitney U-Test were found to be different at the .06 level of probability. Since no hypothesis of this nature had been drawn up and no alpha error set, no conclusions are warranted. However, the results are highly suggestive of cardiac damage as a result of intensive exercise later in life and certainly warrant further investigation.

CHAPTER IV

SUMMARY AND CONCLUSIONS

The experimental production of cardiac necroses has been observed by many investigators (5, 6, 9). Foci of destruction, usually of microscopic size, appear regularly in the ventricular myocardium of corticoid-sensitized rats after application of a variety of physical stresses (restraint, exposure to cold and heat, forced exercise, surgical trauma, etc.) (6). The forced exercise program in the adult rats in the current study was considered to be an extremely stressful physical experience. Therefore, cardiac lesions were expected in the experimental animals. The pathology observed in the present study was very similar in structure to that induced in the aforementioned research.

The current investigation was designed to study the protective value of pre-pubertal forced exercise against cardiac necrosis induced as the result of a post-pubertal forced exercise regimen. Within the forced voluntary and sedentary exercise groups, no significant differences were observed in either the total incidence or severity of the process of necrosis. The conclusion is

warranted that pre-pubertal forced exercise did not have a protective value on the heart for post-pubertal forced activity.

The animals remaining sedentary in later life showed only 10% necrosis compared to a 29.57% involvement in exercised rats. The pathology tended to be more localized and more severe in the forced group animals as opposed to the voluntary and sedentary groups. This revelation is contrary to the above hypothesis. The great majority of animals suffered lesions in the apical region of the heart, specifically in the left ventricle.

Of the 42 female animals examined, none showed any heart pathology. Perhaps this could be related to the continued voluntary exercise period during the post-pubertal voluntary exercise period. In albino rats, the female is approximately nine times as active in voluntary activity as the male (11). If exercise does have a protective value, it might be based on the amount of regular physical activity.

Recommendations for future research in this area would be to repeat this particular study with more rigid controls and more varied staining techniques. During the voluntary exercise period, these animals only had the basic drive to exercise, then in adulthood, they were confronted with a severe exercise regimen. Additional sedentary and forced exercise groups with these

conditions carried through the voluntary exercise period used in the current study would help clarify the situation.

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APPENDIX A

BASIC DATA--EXPERIMENTAL ANIMALS

Animal No.	Body Weight	Heart Weight	$\frac{\text{Heart Wt.}}{\text{Body Wt.}} (10^{-3})$
F-4	600 gms.	2.0880 gms.	3.408
F-5	600	1.7054	2.842
F-6	450	1.5137	3.364
F-8	546	1.5542	2.828
F-11	581	2.2865	3.935
F-12	550	1.9160	3.484
F-13	573	1.6815	2.935
F-14	430	1.4110	3.281
F-15	599	1.9688	3.287
F-16	572	1.7692	3.093
F-17	588	1.8404	3.130
F-18	603	1.6134	2.676
F-19	572	1.9270	3.369
F-20	511	1.8830	3.685
F-21	490	1.5160	3.094
F-25	593	2.0515	3.460
F-26	597	1.8844	3.156
F-27	554	1.7570	3.171
F-29	599	1.8370	3.067
F-30	516	1.6889	3.273
F-31	516	1.5969	3.095
F-32	558	1.6480	2.953
F-33	480	1.4698	3.062
F-34	462	1.8129	3.924
F-35	488	1.7480	3.582
F-36	519	1.4706	2.834
F-40	474	1.4792	3.121
F-41	610	1.5994	2.622
F-43	530	1.4812	2.795
F-44	530	2.0030	3.779
F-45	520	1.4580	2.804
F-46	632	1.7635	2.790
F-47	571	1.6577	2.903
F-49	530	1.7283	3.261
F-50	502	1.4770	2.942
F-55	573	2.0790	3.628
F-58	600	1.5872	2.645
F-59	572	2.0436	3.573
F-60	581	2.5500	4.389

Animal No.	Body Weight	Heart Weight	$\frac{\text{Heart Wt.}}{\text{Body Wt.}} \cdot (10^{-3})$
MEAN	565.6	1.9511	3.452
V-4	535	1.8570	3.471
V-5	528	1.4715	2.787
V-6	568	3.7150	6.540
V-8	450	1.6783	3.730
V-11	420	1.4961	3.562
V-12	532	2.0920	3.932
V-13	610	1.9435	3.186
V-14	579	2.0937	3.616
V-15	544	1.7558	3.228
V-16	510	1.7575	3.446
V-17	584	1.4655	2.509
V-18	570	1.8421	3.232
V-19	554	1.6102	2.906
V-20	417	1.2981	3.113
V-21	528	1.4442	2.735
V-25	500	1.9020	3.804
V-26	528	1.5000	2.841
V-27	526	1.8847	3.583
V-29	500	2.0737	4.147
V-30	428	1.4938	3.490
V-31	492	1.5854	3.222
V-32	550	2.0750	3.773
V-33	546	1.8944	3.470
V-34	510	1.4325	2.809
V-35	456	1.3177	2.890
V-36	573	1.6202	2.828
V-40	452	1.4390	3.184
V-41	512	1.5490	3.025
V-43	565	2.0103	3.558
V-44	450	1.5665	3.481
V-45	528	1.5591	2.953
V-46	570	1.8161	3.186
V-49	458	1.5306	3.342
V-50	556	1.7190	3.092
V-55	513	1.6844	3.283
V-58	488	1.3609	2.789
V-59	601	1.6851	2.804
V-60	590	1.7049	2.890
MEAN	547.2	2.0234	3.865
S-4	528	1.5282	2.894
S-5	498	1.5830	3.179
S-6	497	1.6298	3.279
S-8	512	1.6692	3.260
S-11	460	1.4829	3.224

Animal No.	Body Weight	Heart Weight	$\frac{\text{Heart Wt.}}{\text{Body Wt.}} \cdot (10^{-3})$
S-12	549	1.5544	2.831
S-13	462	1.5054	3.258
S-14	558	1.8867	3.381
S-15	482	1.5310	3.176
S-16	580	1.5386	2.653
S-18	532	1.5621	2.936
S-19	460	1.4860	3.230
S-20	575	1.7404	3.027
S-21	502	1.5511	3.090
S-25	471	1.6320	3.465
S-26	617	1.6743	2.714
S-27	594	1.6703	2.812
S-29	536	1.7248	3.218
S-30	574	1.7421	3.035
S-31	488	1.5532	3.183
S-32	546	1.6770	3.071
S-33	566	1.5546	2.747
S-34	559	1.7606	3.150
S-35	480	1.5407	3.210
S-36	538	1.5615	2.902
S-40	438	1.4219	3.246
S-41	466	1.3690	2.983
S-43	524	1.6531	3.155
S-44	469	1.4958	3.189
S-45	566	1.6939	2.993
S-46	432	1.3860	3.208
S-47	602	1.7401	2.891
S-49	570	1.7534	3.076
S-50	668	1.8147	2.717
S-55	550	1.7090	3.107
S-58	495	1.6035	3.239
S-59	634	1.8929	2.986
S-60	564	2.1040	3.730
MEAN	561.0	1.9954	3.556

APPENDIX B

BASIC DATA--CONTROL ANIMALS

Animal No.	Body Weight	Heart Weight	$\frac{\text{Heart Wt.}}{\text{Body Wt.}}(10^{-3})$
F-2	607 gms.	1.6680 gms.	2.748
F-7	599	1.8895	3.154
F-9	581	1.9798	3.408
F-10	516	1.3165	2.551
F-22	558	1.4441	2.588
F-37	484	1.4032	2.899
F-42	523	1.4913	2.851
F-48	574	1.9236	3.351
F-51	551	1.6331	2.964
F-52	606	1.5986	2.638
V-2	556	1.5584	2.803
V-7	622	1.5371	2.471
V-9	564	1.5401	2.731
V-10	572	1.4594	2.551
V-22	488	1.5296	3.134
V-37	560	1.5063	2.690
V-42	584	1.6703	2.860
V-48	584	1.7482	2.993
V-51	672	1.7230	2.564
V-52	562	1.6784	2.986
S-2	510	1.3950	2.735
S-7	554	1.7346	3.131
S-9	639	1.6814	2.631
S-10	638	1.7428	2.732
S-22	564	1.4016	2.485
S-37	593	1.5590	2.629
S-42	522	2.0274	3.884
S-48	568	1.5154	2.668
S-51	582	1.6631	2.858
S-52	596	1.6680	2.799

APPENDIX C

BASIC DATA--PATHOLOGY

Code No. & Ht. Section	Animal No.	Lesion Location	Degree of Severity	Comments
2b	V-19	LV (Ec)	S	Localized - one area only
3a,b	F-19	LV (Ec,Mc)	S	Several foci
5b	F-4	LV, RV (Ec)	S	Local - one area in each ventricle
6b	F-15	LV (Ec,Mc, Epc)	S	Local - one foci in each area
8a	V-6	LV (Mc)	S	Several diffuse foci
12a	S-14	LV (Ec)	S	Local - one area
b		LV, RV (Ec,Mc,Epc)	S	Local - several area
c		LV (Ec)	S	Diffuse - large area
15b	F-29	LV (Ec)	S	Local - one area
16b	F-16	LV (Ec)	S	Diffuse - large area
30b	S-5	LV (Ec)	Md	Local - one small area
32b	S-27	LV (Ec)	Md	Local - one small area
c		RV (Ec)	Md	Local - one small area
35a	S-49	LV (Ec)	S	Local - one area
b		LV (Mc)	S	Local - one area

Code No. & Ht. Section	Animal No.	Lesion Location	Degree of Severity	Comments
37a	F-45	LV (Ec)	S	Diffuse - one area
b		RV (Mc)	S	Diffuse - one area
41c	V-8	LV, RV (Ec)	S	Local - one area
42b	V-52	LV, RV (Ec)	Md	Local - one small area
47a,b	F-21	LV (Ec)	Md	Local - one small area
50a,b,c	S-60	RV(Ec)	S	Local - one area
54 a,b	S-43	LV (Ec)	Md	Local - one small area
68c	S-34	LV (Ec)	Md	Local - one small area
74b	V-34	LV (Ec)	S	Local - one area only
75a	S-45	LV (Ec)	S	Local - one area
94b	V-35	LV (Ec)	Md	Local - one small area
97a	S-32	LV (Ec)	S	Local - one area
99b	F-48	LV (Mc)	S	Local - one area
102a	V-15	LV (Mc)	Md	Local - one small area
103b	V-49	LV (Mc)	Md	Local - one small area
107b,c	F-51	LV (Mc)	Md	Local - one small area
117b,c	F-42	LV (Mc)	Md	Local - one small area

Code No. & Ht. Section	Animal No.	Lesion Location	Degree of Severity	Comments
122a,b	F-5	LV (Ec)	S	Local - one area
125a,b	V-50	RV (Mc)	S	Diffuse - one area
126b	S-19	LV (Ec)	Md	Local - one small area
129b	F-46	LV (Ec)	S	Local - one area
134a	S-47	LV (Ec)	S	Local - one area
135b	V-36	LV (Ec)	Md	Local - one small area
137a	S-21	LV (Ec)	S	Local - one area
143b	V-9	LV (Ec)	S	Local - one area
144b	F-36	LV (Ec)	S	Local - one area
145b,c	V-21	LV (Mc)	Md	Local - one small area

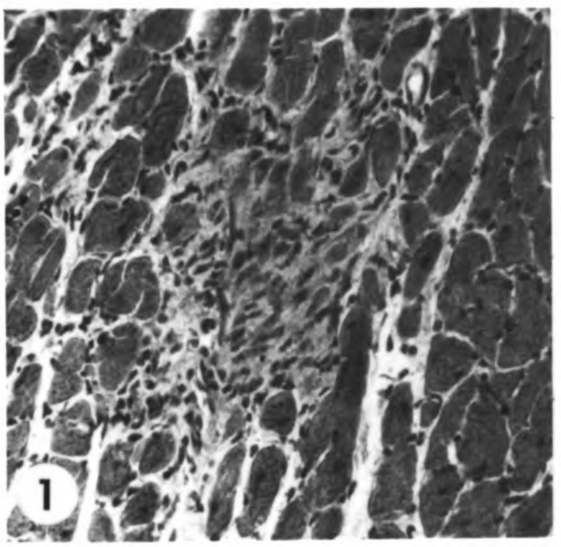
Figure 1.--Rat myocardium of nine hour live-in anxiety study. Hematoxylin and eosin. X 165.

Figure 2.--Same area as Figure 1, demonstrating lack of succinic dehydrogenase activity. SDH stain. X 180.

Figure 3.--Myocardium of rat involved in injection study showing diverse damage. Hematoxylin and eosin. X 205.

Figure 4.--Same area as Figure 3, demonstrating lack of beta-hydroxybuterate dehydrogenase. B-OH DH stain. X 180.

Figure 5.--Same area as Figures 3 and 4 demonstrating lack of succinic dehydrogenase activity. SDH stain. X 195.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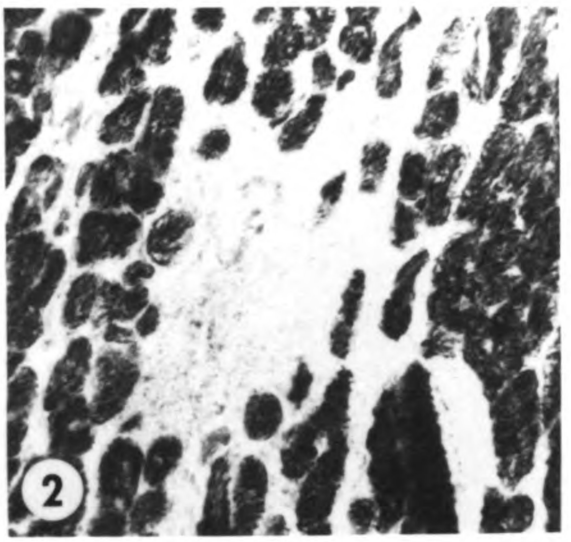
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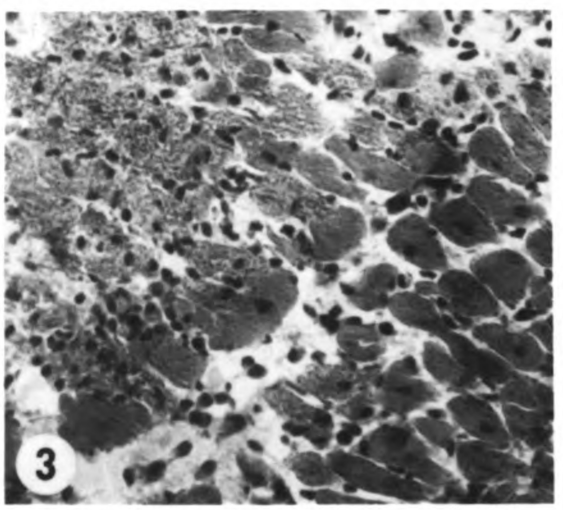
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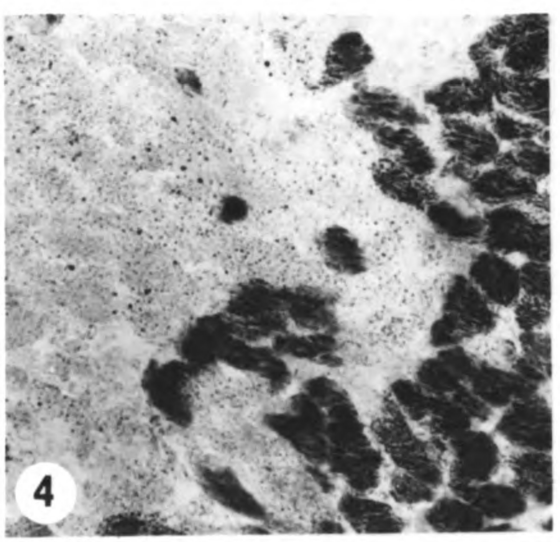
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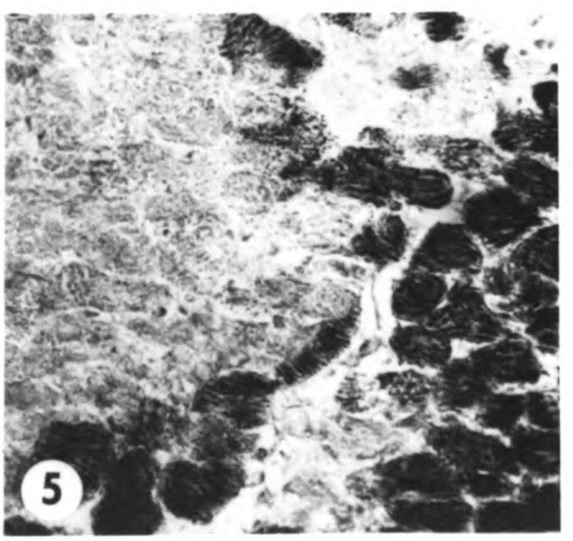
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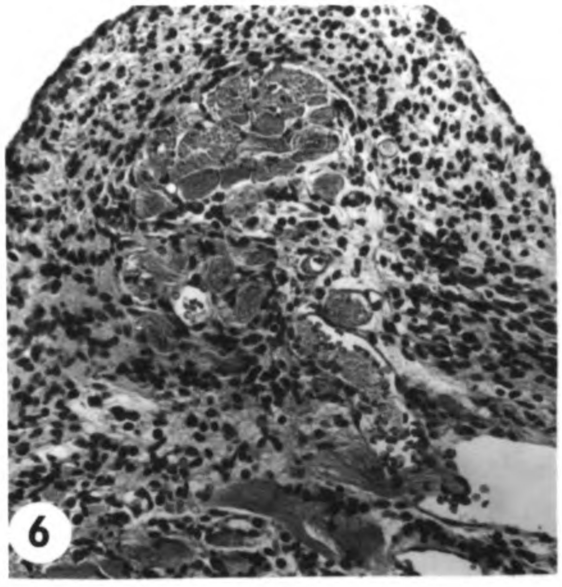
Figure 6.--Diverse damage in endocardium (left ventricle) of rat in Wilson's study. Hematoxylin and eosin. X 180.

Figure 7.--Recent heart damage in myocardium of rat from Wilson's study. Hematoxylin and eosin. X 180.

Figure 8.--Scar tissue in myocardium of rat from Wilson's study. Hematoxylin and eosin. X 165.

Figure 9.--Older scar tissue in endocardium of rat from Wilson's study. Hematoxylin and eosin. X 185.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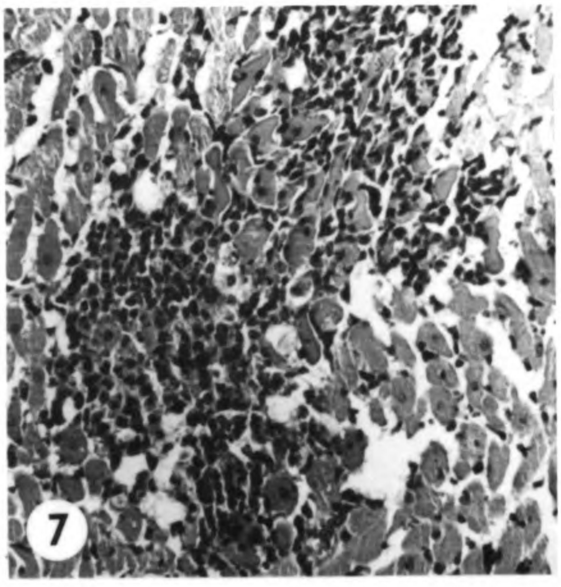
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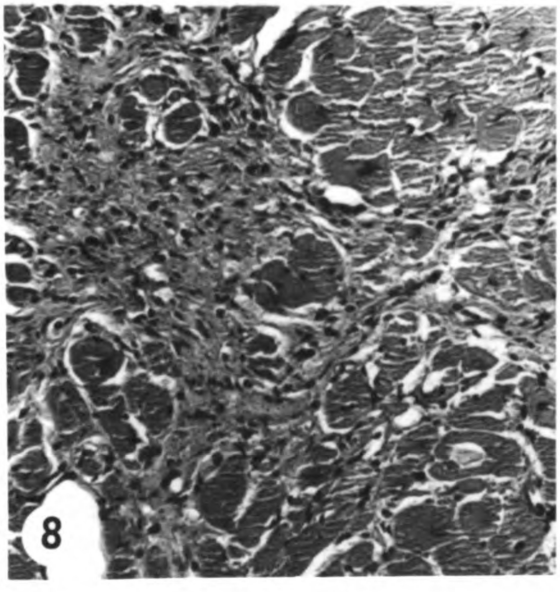
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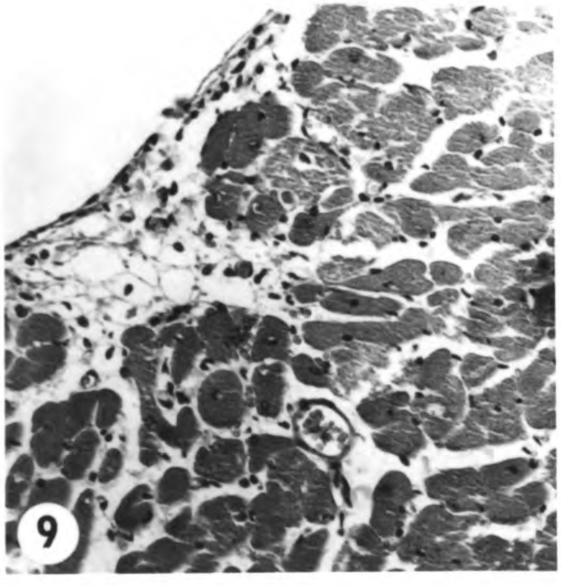
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