Effects of Exercise and Anemia on Coronary Arteries of Small Animals as Revealed by the Corrosion-Cast Technique

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With the technical assistance of Robert Q. Tener

The possibility that lack of physical exercise may be an important contributory factor in the etiology of coronary occlusion has been discussed by many workers. It is generally agreed that the disease is the end result of the interplay of many variables, and it is, therefore, extremely difficult to evaluate the relative importance of each. Certainly, in such a study as that of Morris et al. on workers in the London transport system, many subtle differences in activity pattern may have contributed to the final result.

The observation that coronary occlusion may occur in patients with comparatively slight atherosis of the aorta, or even of the coronaries, and that very extensive atherosis of the coronaries may occur without occlusion suggested to us the possibility that the configuration of the coronary tree itself may be an important determinant of the incidence or prevention of occlusive disease. Moreover, it seemed possible that an adaptation might occur in the coronary tree in response to exercise and that such an adaptation might be the basis for the suggested decrease in vulnerability of the exercised heart to myocardial infarction.

Most studies of the effect of adaptation to exercise on the circulation of the heart in experimental animals have been concerned with capillary-to-muscle-fiber ratios estimated in histological sections. The most recent of these, that of Hakkila, contains a summary of the older literature and a statement of the current majority view: the capillary-to-fiber ratio in the hypertrophied heart is the same as it is in the normal heart, but the increase in fiber diameter results in fewer capillaries per field. It is difficult to see how this finding can be related to the hypothesis that exercise protects the heart from occlusion.

In the present study an attempt was made to examine the effects of exercise, and of other experimental manipulations, on vessels much larger than capillaries, for it seemed to us that important changes in the coronary tree could occur as a result of exercise that might not be detected by histological analysis. Therefore, vinyl acetate corrosion casts were prepared in the manner to be described, and the effects of chronic exercise, anemia, and certain other procedures were studied with this technique in rats, ducklings, and guinea pigs.

Materials and Methods

Animals
Rats were obtained from Albino Farms. Guinea pigs were purchased from a local supplier. Anemic ducklings and age controls were supplied through the courtesy of Drs. Martin P. Schulman and Dan Richert, of the Department of Biochemistry. We are indebted to these colleagues not only for the ducklings, but also for calling our attention to the striking cardiac hypertrophy and dilation they observed as a result of feeding an iron-deficient diet in the course of their experiments conducted for other purposes.

Diets
Rats were fed Purina chow. Guinea pigs were fed a commercial pellet ration supplemented with fresh lettuce and carrots. The normal ducklings were fed a complete starter mash, while the anemic ducklings were fed a milk diet for three weeks before they were killed.
Equipment and Materials

For spontaneous running experiments, standard activity cages equipped with Veeder counters were used. The guinea-pig exercise experiments were done on an 18-stall mechanically driven treadmill with rotating nylon brushes at the rear of each running stall. The treadmill was operated by means of a General Electric Thymatrol drive that permitted infinite variability of speed and calibration in feet of traverse per minute. For swimming experiments, a sink approximately 6 × 2½ × 2½ feet was used. A Cahn electromagnetic balance was used for weighing vinyl casts. Preliminary experiments demonstrated that a 12% solution of vinyl acetate in acetone was the optimum concentration for injection. However, slight variations were found from one bottle to another, and it was observed that after a bottle had been open for some time, evaporation occurred and changed the concentration. Therefore, all injections in each experiment were made from the same batch of plastic solution within the same day. Owing to the variability in results with different batches of vinyl acetate solution, and to the fact that these experiments were not all done by the same operator, no day-to-day comparisons are possible. Analyses are presented only of those experiments that were completed in one morning by one experimenter using as consistently uniform a technique from animal to animal as he could.

Since the polymer sets almost immediately on contact with water, it is essential to inject the plastic solution quite rapidly and with considerable force. When this is done, filling occurs as far down the tree as the 40- to 50-μ internal-diameter level, as estimated in histological sections.

Procedure for the Rat

The animal was injected intraperitoneally with pentobarbital sodium, 60 mg/Kg of body weight. A midline abdominal incision was made and the thoracic cage cut bilaterally in the midaxillary line. The attachment of the diaphragm to the anterior thoracic wall was severed and the sternal plate retracted anteriorly. Heparin (100 units) was injected into the inferior vena cava. The thoracic and abdominal viscera were retracted to expose the thoracic aorta, and a ligature, ready to tie, was placed around it near the beginning of its descent. A syringe containing about 5 ml of vinyl acetate solution just drawn from the dispensing bottle was connected with a sharp 18-gauge needle, the aorta entered, and the waiting ligature tied tightly around it (fig. 1). Immediately, injection was begun. The heart was seen to dilate, and the red plastic solution could be seen to fill the branches of the coronaries on the anterior surface of the heart. From the instant the red material was seen in vessels at the apex, no further pressure was applied on the syringe, and a mosquito clamp was applied to the aorta between the needle tip and the heart. The heart often beat several times after the completion of the injection and then stopped.

The polymer was allowed to set for at least 10 minutes. Then the heart was removed, blotted, weighed, and placed in a 10% potassium hydroxide solution at room temperature for 24 hours. This was sufficient for digestion of all of the tissue from the cast. Following this, the cast was rinsed in cold tap water, dried on paper toweling, and weighed with the intraventricular injection mass undisturbed (figs. 1 and 2). The difference between the initial, aggregate wet weight and the weight at this stage was considered to be the wet weight of the heart.
The coronary casts were severed as close to their aortic origin as possible and weighed on a Cahn electromagnetic balance, an instrument set in this instance at 10 mg capacity and 0.005 mg sensitivity. Weighings were made to the nearest 0.01 mg.

The authors are aware that it is hazardous to draw conclusions from experiments of this type unless there is some estimate of perfusion pressure. In a number of experiments on normal (i.e., unexercised) rats, attempts were made to inject the plastic solution through a three-way stopcock system that was connected with a mercury manometer, but these approaches did not prove to be feasible for routine use. Moreover, we noted that there was some variability from animal to animal in the amount of red vinyl solution that leaked out of the ends of vessels that had been cut in opening the thoracic cage. Therefore, rather than attempt to ensure equal perfusion pressures in all groups, we elected to perform "blind" experiments, i.e., the operators did not know to which experimental group any animal belonged until after the coronary casts had been prepared and weighed. This design effectively produces a random distribution of the perfusion pressure error through the groups, and thus makes the method useful for comparative purposes.

Results

Spontaneous Running in the Male

In a preliminary experiment, casts were prepared as described above of the coronary vessels of eight control male rats and four similar rats that had run from 0.5 to 1.5 miles per day in Wahmann activity cages over a period of 36 days. The results are shown graphically in table 1. Even with this small group of experimental animals, there was a readily demonstrable weight difference in the coronary casts. However, since there was a significant degree of cardiac hypertrophy, there was no difference in the milligram weight of the cast per gram of heart. Therefore, in this experiment, the apparent increase in the size of the coronary tree just compensated for the cardiac hypertrophy. There was no obvious correlation between the weight of the cast and the exercise history of individual rats.

Spontaneous Running in the Female

A similar experiment was done on five exercised female rats and nine "sedentary" controls (table 1). Although the female rats housed in activity cages showed higher mean activity levels than did the males, there was no detectable cardiac hypertrophy. There was, however, an increase in coronary-cast weight in the exercised group, and this was signifi-
CORONARIES AND EXERCISE

Table 1
Effects of Various Exercise Programs on Heart Weight, Coronary-Cast Weight, and Cast/Heart Ratio In the Rat (Means and Their Standard Errors are Given)

<table>
<thead>
<tr>
<th>Expt.</th>
<th>Sex</th>
<th>Exercise history</th>
<th>No. of rats</th>
<th>Terminal body wt., Gm.</th>
<th>Heart/body wt., per cent</th>
<th>Cast wt., mg.</th>
<th>Cast/heart wt., mg./Gm.</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>M</td>
<td>Control</td>
<td>8</td>
<td>295 ± 5.7</td>
<td>0.27 ± 0.01</td>
<td>4.65 ± 0.053</td>
<td>5.79 ± 0.49</td>
</tr>
<tr>
<td></td>
<td>M</td>
<td>Spontaneous running 5 weeks</td>
<td>4</td>
<td>302 ± 12</td>
<td>0.35 ± 0.01</td>
<td>6.25 ± 0.132</td>
<td>6.02 ± 0.25</td>
</tr>
<tr>
<td></td>
<td></td>
<td>P</td>
<td></td>
<td>&lt;0.01</td>
<td>&lt;0.01</td>
<td>&lt;0.01</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>2</td>
<td>F</td>
<td>Control</td>
<td>9</td>
<td>226 ± 8.6</td>
<td>0.50 ± 0.02</td>
<td>3.10 ± 0.18</td>
<td>1.38 ± 0.09</td>
</tr>
<tr>
<td></td>
<td>F</td>
<td>Spontaneous running 5 weeks</td>
<td>5</td>
<td>219 ± 5.1</td>
<td>0.53 ± 0.02</td>
<td>3.94 ± 0.28</td>
<td>1.81 ± 0.17</td>
</tr>
<tr>
<td></td>
<td></td>
<td>P</td>
<td></td>
<td>&lt;0.05</td>
<td>&lt;0.02</td>
<td>&lt;0.05</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>3</td>
<td>M</td>
<td>Control</td>
<td>8</td>
<td>371 ± 17.3</td>
<td>0.42 ± 0.02</td>
<td>5.5 ± 0.43</td>
<td>3.5 ± 0.018</td>
</tr>
<tr>
<td></td>
<td>M</td>
<td>Swimming 30 min. b.d., 11 weeks</td>
<td>8</td>
<td>356 ± 11.2</td>
<td>0.49 ± 0.02</td>
<td>7.1 ± 0.27</td>
<td>4.1 ± 0.016</td>
</tr>
<tr>
<td></td>
<td></td>
<td>P</td>
<td></td>
<td>&lt;0.05</td>
<td>&lt;0.02</td>
<td>&lt;0.05</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>4</td>
<td>M</td>
<td>Control</td>
<td>9</td>
<td>415 ± 12.1</td>
<td>0.42 ± 0.01</td>
<td>4.7 ± 0.09</td>
<td>2.80 ± 0.18</td>
</tr>
<tr>
<td></td>
<td>M</td>
<td>As in 3, followed by 8 weeks rest period</td>
<td>9</td>
<td>499 ± 10.9</td>
<td>0.41 ± 0.02</td>
<td>6.3 ± 0.37</td>
<td>3.85 ± 0.29</td>
</tr>
<tr>
<td></td>
<td></td>
<td>P</td>
<td></td>
<td>&lt;0.05</td>
<td>&lt;0.01</td>
<td>&lt;0.01</td>
<td>&lt;0.01</td>
</tr>
</tbody>
</table>

Since there was no hypertrophy, the exercised rats showed a higher value for the cast-weight/heart-weight ratio, suggesting that, in this case, the apparent increase in size of the coronary tree resulted in a greater degree of vascularization per unit mass of myocardium. Again, with such a small number of animals, it was impossible to detect a relationship between the extent of running activity and the increase in size of the coronary casts for the individual rats.

Swimming
For use in this experiment, 16 male rats were selected by a preliminary adaptation process. Initially, they were placed in a swimming tank for 10-minute periods, and the swimming time was increased gradually by 5-minute increments to 30 minutes twice per day for five days and once on the sixth day. A few animals showed some evidences of fatigue after the 15-minute swim periods, and these were eliminated from the experiment. After successful adaptation to the 30-minute swim periods, all of the animals were able to perform this amount of exercise without obvious distress. This was not a maximal work load for these animals. The swimming-tank water was maintained at 30 C. In order to eliminate possible effects of immersion and subsequent drying, the nonswimmer controls were briefly dipped into the pool after each exercise period. All animals received the standard laboratory chow ration and water ad libitum and were maintained in a constant-temperature room at approximately 78 F. At the end of 10 weeks on this regimen, the animals were coded by one individual and submitted by him to two other workers who proceeded to prepare coronary casts as already described.

The results of this experiment are given in table 1. The exercised animals showed a significant increase in coronary-cast weight and a cardiac hypertrophy of borderline significance. The increase in cast-weight/gram of heart was also of borderline significance.

Another group of 18 rats had been treated in all respects as had those used in the preceding experiment. However, after 10 weeks...
of swimming, the nine swimmers of this group reverted to "sedentary" status, and the controls were no longer immersed in the swimming tank. About eight and a half weeks after the end of the exercise program, all of the rats were injected with vinyl acetate and coronary casts were prepared. The results are shown in table 1. It will be seen that there is no difference in either body weight or heart weight between the groups. There is a difference in cast weight, significant only at the 0.05 per cent level of confidence. However, the animals with a history of exercise followed by rest showed a highly significant increase in cast/heart ratio.

Other Experiments in the Rat

In one experiment seven rats were injected daily with 400 μg. of L-thyroxin for 12 days. This was enough to raise the mean oxygen consumption of the group from the control level of 150 ml./100 cm.²/hr. to 235 (P < 0.01). The mean body weight of the thyroxin-injected animals was significantly smaller than that of the saline-injected controls, 319 versus 376 Gm. The mean absolute heart weights of the two groups did not differ, but the heart/body weight ratio of the hormone group was 0.625 as opposed to 0.457 (P < 0.01). The coronary-cast weight/heart-weight ratios of the two groups were the same: 3.34 ± S.E. 0.258 and 3.23 ± S.E. 0.033, respectively.

In another experiment, an attempt was made to discover whether or not chronic treatment with a coronary vasodilator drug would result in a demonstrable effect on the coronary arterial cast. Accordingly, groups of eight rats were fed pentaerythrityl tetranitrate (Peritrate) at two different levels: group A at 240 mg./Kg. of diet and group B at 480 mg./Kg. of diet. After two weeks of feeding, all animals were injected with vinyl acetate solution and casts of their coronary arteries prepared. Table 2 reveals no significant differences in any of the parameters studied.

Although no significant effects were seen in these experiments, together they constitute a methodological control of the ones reported above.

Anemia in the Duckling

Our colleagues, Drs. M. P. Schulman and D. A. Richert, placed newly hatched ducklings on an iron-deficient diet—i.e., cow's milk—because they were interested in studying the metabolism of nucleated erythrocytes in vitro. When they autopsied their animals after three weeks on the iron-deficient ration, they discovered enormous cardiac hypertrophy and dilation in the anemic animals. In addition to calling our attention to this condition, Schulman and Richert prepared a set of five anemic ducklings and six normal ducklings that had been fed a complete ration. Coronary casts were prepared of the hearts of all 11 ducklings. Representative results of one of the pairs are shown in figure 3. The data summarized in table 3 reveal that the hearts of the anemic ducklings were 37 per cent heavier than were those of the controls, while the coronary-cast weight was 57 per cent heavier. While the cast/heart weight ratio data show a trend in the direction of a larger figure for the anemic animals, the results are not statistically significant. Figure 3 reveals that the diameter of the larger bore vessels is larger in the case of the anemic animals, and that homologous vessels of smaller bore are similarly enlarged. This can be seen best in examining the small right atrial branch, which
projects downward from the right coronary cast of each animal. In this photograph (fig. 3) a general impression is obtained of richness of arborization in the cast on the left and comparative sparseness of branching of the one on the right.

Guinea-Pig Experiments
A group of 13 guinea pigs was trained to run on a motor-driven treadmill at a speed of approximately one foot/second. At the end of a one-week adaptation period, the animals were able to run from 1,000 to 2,000 feet twice daily. Suitable controls were maintained in small cages. After 52 days of running, the animals were killed and coronary casts were prepared as described above. No significant cardiac hypertrophy was observed, and no increase in cast size was seen in the exercised guinea pigs. However, a study of the casts prepared from these animals was of interest because the majority of them (9 of 13 of the exercised animals and 9 of 12 of the controls) showed definitely demonstrable right-to-left side anastomoses of the coronaries. There was no correlation between the presence of such anastomoses and the antecedent exercise history of the animals. One typical cast showing such an anastomotic connection between a branch of the right and a branch of the left coronary artery is shown in figure 4.

This interesting finding stimulated us to inquire about the possible functional significance of intercoronary anastomoses of the type described. Therefore, a group of five guinea pigs was anesthetized, their hearts exposed, the most prominent coronary branch on the anterior surface of the heart was identified, and the animal heparinized. A nylon ligature was tied tightly around the vessel near its origin and the vinyl plastic material was immediately injected into the aorta with the beating heart under direct inspection. In all cases, the ligated vessel was seen to fill from below, and the plastic could be seen to approach and reach the site of the previously applied ligature. In two instances, the ligature remained on the vessel cast even after digestion in potassium hydroxide; one of these casts is shown in figure 5.

Discussion
It is rather surprising that, in spite of many recent clinical and epidemiological observations that suggest a relationship between lack of physical exercise and the incidence of myocardial infarction, little experimental work has been reported in which an attempt has been made to elucidate possible mechanisms of this relationship. Eckstein, who showed that the stimulus of exercise enhanced the development of collateral circulation after coronary-branch ligation in the dog, has approached the problem at the physiological level.

If, for heuristic reasons, one accepts the concept that physical inactivity can predispose to coronary occlusion, there are a number of possible mechanisms that could be operative. The most obvious of these is the prevention of obesity and of what can be loosely...
Table 2

Lack of Effect of Pentaerythritol Tetranitrate Feeding for Two Weeks on Coronary Casts of Rats

<table>
<thead>
<tr>
<th>Group</th>
<th>Diet</th>
<th>No. of rats</th>
<th>Mean daily food intake, Gm./day</th>
<th>Terminal body wt., Gm.</th>
<th>Heart/body wt., per cent</th>
<th>Cast wt., mg.</th>
<th>Cast/heart wt., mg./Gm.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>Ground chow</td>
<td>7</td>
<td>13.4</td>
<td>223</td>
<td>0.393</td>
<td>3.67</td>
<td>4.10</td>
</tr>
<tr>
<td>A</td>
<td>Same + 240 mg./Kg.</td>
<td>8</td>
<td>13.1</td>
<td>213</td>
<td>0.420</td>
<td>3.42</td>
<td>3.78</td>
</tr>
<tr>
<td>B</td>
<td>Same + 480 mg./Kg.</td>
<td>8</td>
<td>13.9</td>
<td>218</td>
<td>0.414</td>
<td>3.84</td>
<td>4.30</td>
</tr>
</tbody>
</table>

Figures in parentheses are standard errors. No statistically significant differences.

Table 3

Effect of Iron Deficiency on Hematocrit, Heart Weight, and Coronary-Cast Weight in the Duckling

<table>
<thead>
<tr>
<th>No. of animals</th>
<th>Body wt.</th>
<th>Hematocrit % cells</th>
<th>Heart weight Gm.</th>
<th>Coronary-cast weight, mg.*</th>
<th>Cast/heart wt. mg./Gm.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>285 ± S.E. 29</td>
<td>41.4 ± 1.64</td>
<td>2.35 ± 0.17</td>
<td>8.4 ± 0.65</td>
<td>3.5 ± 0.32</td>
</tr>
<tr>
<td>Anemic</td>
<td>224 ± S.E. 15</td>
<td>20.6 ± 1.24</td>
<td>3.22 ± 0.96</td>
<td>13.2 ± 1.48</td>
<td>4.0 ± 0.13</td>
</tr>
</tbody>
</table>

* n = 12 for controls and 10 for anemic, respectively. Cast of each vessel was weighed individually.

referred to as an unfavorable blood-lipid pattern. In Morris's transport-worker study, in fact, it was discovered in retrospect that the girth of the comparatively susceptible bus drivers was significantly wider than that of the "protected" conductors at the time they assumed their jobs.\(^\text{12}\) However, there is no general agreement among experimental workers on the effects of exercise on such parameters as the serum cholesterol level and the extent of atheroma formation in the aorta and in coronary vessels.\(^\text{13-16}\) Moreover, Morris and Crawford have recently given evidence to support the view that exercise does not significantly reduce the extent of atheromatosis of the coronary vessels.\(^\text{17}\) A dissociation of atheroma formation and lumen occlusion in two different groups of autopsy specimens has also been pointed out.\(^\text{18}\)

One important potential factor is the configuration of the coronary arterial tree itself and the role that this may play in either susceptibility to or protection from coronary occlusion. The evidence presented herein generally supports the view that the configuration of the vascular tree can be modified by exercise. The results of the experiment with the ex-swimmers suggest that the modification may be true enlargement of the capacity of the coronary tree or increased distensibility or a combination of the two. Of course, it is not possible to state from this type of evidence whether or not there has been an actual proliferation of vascular tissue, an increase in vascular cell mass.

The demonstration of intercoronary anastomoses in the guinea pig confirms the fact of the existence of such vessels, which have been observed in many species, including man.\(^\text{19-22}\) It is unfortunate that the work load imposed on the guinea pigs in this study was not sufficient to produce cardiac hypertrophy, for the absence of differences between the coronary arterial casts of the exercised and

\[\text{Circulation Research, Volume IX, May 1961}\]
"Sedentary" guinea pigs in this experiment does not disclose what might happen at higher work loads. That intercoronary anastomoses of the type seen with equal frequency in both "trained" and "untrained" guinea pigs may have physiological significance is suggested by the retrograde filling of a ligated vessel described herein. In a discussion of the "pathology of inactivity" in a recent paper, Morris suggested the possibility that less effective production of coronary collaterals may be one of the biological consequences of sedentary living.

There is no need to belabor the possible public health importance of the necessity to gain further insight into some of the questions raised in this discussion. The purpose of the experiments reported herein is to raise the following questions: (a) Are there structural variations in the coronary arterial tree of man that are of great importance in establishing susceptibility or resistance to coronary occlusion? (b) Can the structure of the coronary arterial tree of man be modified by exercise in such a way as to protect against this disease?

Summary

Vinyl acetate corrosion casts were prepared of the coronary arterial trees of chronically exercised rats and of suitable control animals. In four experiments, there was a statistically significant increase in the mean coronary-cast weight of the exercised animals over that of controls. In two experiments, the coronary-cast/heart-weight ratios were significantly increased.

In three-week-old ducklings rendered anemic by an iron-deficient diet, corrosion casts of the coronary vessels showed marked increases in cast weight, increase in size of homologous vessels, and generally more luxuriant arborization of the tree. No differences were found when coronary casts of thyroxin-injected or of pentaerythritol tetranitrate-injected rats were compared with those of control animals. Right-to-left-side coronary anastomoses were demonstrated in 75 per cent of guinea-pig hearts studied by the corrosion-cast technique. In five experiments, retrograde filling of a ligated anterior descending branch of the left coronary artery was demonstrated when the vinyl acetate solution was injected into the aorta.

Acknowledgment

The pentaerythritol tetranitrate (Peritrate) was obtained from the Warner-Chilcott Co., through the courtesy of Mr. Fred Houghton.

References


BOOK REVIEW


This book reviews some of the literature on the clinical symptoms and electrocardiographic signs of electrolytic disturbances. The first part deals with effects of changes in the potassium concentration, mainly the relationships between the serum potassium level and the electrocardiographic abnormalities. The view is upheld that the ratio of intracellular to extracellular potassium is the main factor in determining the electrocardiographic pattern, a rise in this ratio causing the hypokalemic electrocardiogram and vice versa. The problem is oversimplified by employment of neuromuscular excitability as the index of intracellular potassium level. Electrocardiograms of patients with various metabolic disorders are examined and usually interpreted in favor of the concept mentioned above. The second part of the book presents data on electrocardiographic changes due to disturbances in metabolism of calcium, magnesium, rubidium, cesium, lithium, barium, boron, strontium, and cobalt.
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Circ Res. 1961;9:576-584
doi: 10.1161/01.RES.9.3.576

Circulation Research is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
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Print ISSN: 0009-7330. Online ISSN: 1524-4571

The online version of this article, along with updated information and services, is located on the World Wide Web at:
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