The effect of muscular exercise on blood coagulation has been the subject of several investigations in both man and laboratory animals.\textsuperscript{1-7} Coagulation time has been measured by various methods of whole blood clotting time, one-stage prothrombin time,\textsuperscript{4} and more recently by thromboelastography.\textsuperscript{5} In general, results have indicated that clotting is accelerated immediately after muscular exertion. However, if blood is rendered hypercoagulable by exercise, it would be expected that intravascular clotting would be more frequent among those engaged in heavy physical labor or in athletics. This has not been found to be the case.\textsuperscript{8} In fact, some investigations have shown that delayed coagulation time is one of the chronic effects of habitually high levels of physical activity.\textsuperscript{6,7} In view of the possible role of exertion and hypercoagulability as etiological factors in coronary thrombosis, it seemed worthwhile to investigate further the relationship between blood coagulation and exercise, employing clotting tests of more recent origin than previous studies, and using a group of normal human subjects rather than animals or hospital patients as the more extensive of the prior investigations have done.\textsuperscript{3,4} In this way, it was hoped that more widely applicable conclusions could be drawn from the data.

\textbf{Methods}

The 32 subjects were male college students ranging from 17 to 37 years of age, from 67 to 74 inches in height, and from 150 to 185 pounds in weight. Twenty-five were moderately active, and seven were members of varsity football, track, swimming, or baseball teams. None of the subjects gave evidence of apprehension of the blood sampling or of the strenuous exercise. They were questioned before each testing period with regard to unusual emotional incidents, fatigue, alcoholic intake, or smoking in the 24 hours preceding the test.

Blood samples were drawn from the median cubital vein with clean, dry, siliconized syringes and 20-gauge needles. The veins were distended by applying a pneumatic cuff around the upper arm so that the venous return was obstructed but the arterial blood flow was not entirely cut off. Ethyl chloride was sprayed on the site of the sample before the puncture was made in order to reduce any possible effects of pain or discomfort. Only those samples drawn rapidly after a clean puncture were used for coagulation measurements.

Measurements were made of whole blood clotting time, thrombin generation rate, and thromboplastin generation rate on blood samples drawn before, immediately after, and 15 and 30 minutes after exercise. Preliminary experiments on two subjects had shown that blood samples taken two, four, six, or eight hours after vigorous exercise did not differ from the control sample taken before exercise.

A modified Lee-White technique was used to measure whole blood clotting time. One ml. of freshly drawn blood was placed in each of two newly siliconized 12 X 50 mm. serological tubes, and the tubes were incubated at 37.5 C. Starting five minutes after the tubes had been in the waterbath, the first tube was tilted at one-minute intervals until a firm clot was formed. The second tube was then tilted at 30-second intervals until clotting occurred. A stop watch was started the instant the needle was withdrawn from the subjects arm and stopped when the clot formed in the second test tube.

Three different technicians participated in this phase of the study. In order to avoid any possible bias incurred by differences in technique, only one technician performed the tests on a subject for any sequence of before and after exercise clotting times on a given day.

The rate of thrombin generation was measured by centrifuging oxalated blood samples (1 part 0.1M sodium oxalate to 9 parts blood) for 2 minutes at 1,000 r.p.m. The supernatant plasma was removed, reconstituted with M/40 CaCl\textsubscript{2}, and placed in a waterbath at 37.5 C, and the tube was tilted every 15 seconds until a clot formed. The clot was immediately expressed, and the remaining plasma was incubated for one minute after which a 0.025-ml. aliquot of the plasma was added to an equal amount of bovine fibrinogen solution.
Exhaustive run for measuring one-stage prothrombin time, which was recorded. This procedure was repeated at one-minute intervals for eight minutes. The apparatus used in this technique was adapted from a method for measuring one-stage prothrombin time, which has been described elsewhere.9

The thromboplastin generation rate was measured in serum from blood samples placed in unsiliconized, centrifuge tubes, stirred gently with an unsiliconized glass rod until a clot formed, and then incubated for two hours at 37.5 C. After incubation, the serum was diluted 1 to 9 with 0.85 per cent NaCl, and 1/10 ml. of diluted serum was added to equal amounts of reconstituted solutions of antihemophiliac globulin, platelet suspension (Warner-Chilcott thromboplastin generation test reagents), and M/40 CaCl₂. This mixture was incubated at 37.5 C. After one minute of incubation, a 0.025-ml aliquot of the incubation mixture was added to equal amounts of a platelet-free plasma substrate and M/40 CaCl₂, and the time required for clot formation was recorded. This procedure was repeated after three minutes of incubation and every minute thereafter for eight minutes. In order to include moderate, prolonged, and intensive exertion, the following four exercises were employed: (1) a 15-minute walk on a motor-driven treadmill at 2.5 m.p.h.; (2) a 2-hour walk on a motor-driven treadmill at 2.5 m.p.h.; (3) a run to near exhaustion on the treadmill at 6.25 m.p.h.; (4) a stepping exercise on a 12-inch bench, 30 steps per minute for 15 minutes. In the treadmill run, the subjects ran until they were unwilling to continue. The degree of exhaustion depended on the determination of the various subjects. Pulse rates taken immediately after exercise ranged from 140 to 204 beats per minute, with a mean of 170.3. The average running time was 28.7 minutes for the 12 subjects used in this phase of the investigation. Pulse rates taken immediately after the stepping exercise ranged from 100 to 164 beats per minute, with a mean of 139.4. Eosinophil counts made on 4 subjects, four to seven hours after the step test, showed 29 to 82 per cent decreases from resting values.

Whole blood clotting time was measured after all exercise; however, the thrombin generation rate and the thromboplastin generation rate were measured only after the stepping exercise. The study was divided into three phases: the first dealing with whole blood clotting time, the second with thrombin generation rate, and the third with thromboplastin generation rate.

In the first phase, 12 subjects completed each of the three treadmill exercises and the stepping exercises. At least a week elapsed between exercise bouts for any given subject. Blood samples were taken before, within 1 minute after exercise, 15 and 30 minutes after exercise, except after the stepping exercise, in which case the 15-minute sample was dispensed with. The data were treated with a two-way analysis of variance.

In measuring the thromboplastin generation rate, blood samples were obtained from 14 subjects, before, within 1 minute after, and 30 minutes after the stepping exercise. Time of maximum thromboplastin generation and the clotting time recorded at the time of maximum thromboplastin generation were compared among the three samples taken from each subject. The Friedman two-way analysis of variance, a nonparametric statistical method, was used to compare the times of maximum thromboplastin generation before and after exercise, since the times were recorded at minute intervals and thus did not meet the assumptions required for conventional analysis of variance.10

The clotting times at maximum thromboplastin generation were treated with a conventional two-way analysis of variance. Thrombin generation rate was measured in 16 subjects before exercise, within 1 minute after, and 30 minutes after exercise. The time of maximum thrombin generation and the clotting time at the time of maximum thrombin generation were compared in the same manner as with the thromboplastin generation test results.

Results

The results of the measurement of whole blood clotting time are presented in table 1. Analysis of variance indicated that there were no statistically significant differences among the mean clotting times of blood samples before and after exercise. There are significant differences among the clotting times of various subjects, and this effect is biased by the different technicians who measured clotting time in the different subjects. How-

<table>
<thead>
<tr>
<th>Exercise</th>
<th>Before exercise (min.)</th>
<th>1 minute after exercise (min.)</th>
<th>16 minutes after exercise (min.)</th>
<th>30 minutes after exercise (min.)</th>
</tr>
</thead>
<tbody>
<tr>
<td>15-minute walk</td>
<td>16.5</td>
<td>16.9</td>
<td>17.0</td>
<td>17.2</td>
</tr>
<tr>
<td>2-hour walk</td>
<td>18.3</td>
<td>18.8</td>
<td>18.7</td>
<td>18.8</td>
</tr>
<tr>
<td>Exhaustive run</td>
<td>17.3</td>
<td>17.3</td>
<td>17.5</td>
<td>17.4</td>
</tr>
<tr>
<td>Stepping exercise</td>
<td>17.7</td>
<td>17.0</td>
<td>17.1</td>
<td>17.1</td>
</tr>
</tbody>
</table>

*Means of 12 subjects. Standard deviation equals 1.1 minute. No differences between mean clotting times before and after exercise are statistically significant.
EXERCISE AND BLOOD COAGULATION

TABLE 2

<table>
<thead>
<tr>
<th>Time of blood sampling</th>
<th>Before exercise</th>
<th>1 minute after exercise</th>
<th>30 minutes after exercise</th>
</tr>
</thead>
<tbody>
<tr>
<td>Time of maximum thromboplastin generation (min.)</td>
<td>Mean 5.2</td>
<td>4.9</td>
<td>4.6</td>
</tr>
<tr>
<td></td>
<td>Range 3-7</td>
<td>2-7</td>
<td>3-6</td>
</tr>
<tr>
<td>Mean clotting times at maximum thromboplastin generation (sec.)</td>
<td>Mean 12.5</td>
<td>12.5</td>
<td>12.0</td>
</tr>
<tr>
<td></td>
<td>Range 9.5-15.7</td>
<td>8.5-16.2</td>
<td>10.6-15.8</td>
</tr>
</tbody>
</table>

*Fourteen subjects. Differences in mean times of maximum thromboplastin generation and mean clotting times are not statistically significant.

TABLE 3

<table>
<thead>
<tr>
<th>Time of blood sampling</th>
<th>Before exercise</th>
<th>1 minute after exercise</th>
<th>30 minutes after exercise</th>
</tr>
</thead>
<tbody>
<tr>
<td>Time of maximum generation (min.)</td>
<td>Mean 4.2</td>
<td>4.2</td>
<td>4.2</td>
</tr>
<tr>
<td></td>
<td>Range 3-6</td>
<td>3-6</td>
<td>3-5</td>
</tr>
<tr>
<td>10 subjects with no acceleration of thrombin generation</td>
<td>Clotting time at maximum thromboplastin generation (sec.)</td>
<td>Mean 10.7</td>
<td>11.3</td>
</tr>
<tr>
<td></td>
<td>Range 9.0-12.8</td>
<td>9.0-15.1</td>
<td>10.1-15.8</td>
</tr>
<tr>
<td>6 subjects with accelerated thrombin generation</td>
<td>Time of maximum generation (min.)</td>
<td>Mean 5.2</td>
<td>4.0†</td>
</tr>
<tr>
<td></td>
<td>Range 4-7</td>
<td>3-5</td>
<td>4-5</td>
</tr>
<tr>
<td>Clotting time at maximum thromboplastin generation (sec.)</td>
<td>Mean 10.7</td>
<td>9.5</td>
<td>11.1</td>
</tr>
<tr>
<td></td>
<td>Range 8.9-11.8</td>
<td>9.0-12.8</td>
<td>8.9-13.8</td>
</tr>
</tbody>
</table>

*Sixteen subjects. †Significantly shorter than before exercise thrombin generation time (P < 0.05).

However, this bias is not present in the comparison of clotting times before and after exercise, as in every case this was measured by only one technician on any given subject.

Table 2 gives the results of the thromboplastin generation test. As in the case with whole blood clotting, there are no significant differences among the blood samples taken before and after exercise.

The results of the thrombin generation test after exercise are somewhat different. In 10 subjects, there was no change in the time of maximum thrombin generation. However, in 6 subjects, there was an acceleration of one to two minutes in the time of maximum thrombin generation (table 3). The two groups of subjects were treated statistically as separate groups, since replicate tests showed the difference in thrombin generation after exercise to be consistent, and control tests of sequential blood sampling at the same time intervals, but without the exercise on...
three of the six subjects, failed to show any acceleration of thrombin generation. Clotting times at peak thrombin generation did not show significant variation after exercise in either group.

Discussion

The data obtained in this study do not support the widely held view that blood is rendered hypercoagulable by exercise. Although in 6 out of 16 subjects the thrombin generation rate was increased after exercise, there were no detectable effects of exertion on either the whole blood clotting time or the seric thromboplastin generation test.

This investigation employed subjects who differed from those used in the recent researches of Vuori and Schneider, whose subjects were hospital patients afflicted with nervous disorders. It is possible that this difference in the type of subject was responsible for the difference in the results.

Likewise, in comparing the results of this experiment with that of Hartman, one must consider the possible effect of emotion, which seems to be unavoidable in animals which are forced to exercise by running on a treadmill or by swimming.

It is interesting to note that four of the six subjects whose thrombin generation rate increased showed no change in the whole blood clotting time. The other two were not among those whose whole blood clotting time was measured. This inconsistent result may be explained by the well-known fact that the initiation of whole blood clotting requires only minute amounts of thrombin and is autocatalytic in its progress. In addition, there are many extrinsic factors which affect the clotting time of whole blood; hence it is probable that this test is not sensitive enough to detect such small changes as might have been present in the whole blood clotting time of these four subjects after exercise.

The negative findings in the thromboplastin generation test may be due to the choice of the seric method, which was initially made due to the larger number of recognized clotting factors present in the serum. Research reported since the completion of this phase of the investigation indicates that a modification of the plasmic variation of the thromboplastin generation test may be capable of detecting hypercoagulability. Another possible explanation lies in the sensitivity of the thrombin generation test to platelet concentration. Although the thromboplastin generation test is relatively insensitive to platelet concentration if a critical level is present, the thrombin generation test is extremely sensitive to platelet concentration. It has been shown that both the concentration and adhesiveness of platelets is increased by exercise.

This study indicates that coagulation changes following exercise are a matter of biochemical individuality. Furthermore, it does not support the hypothesis that accelerated blood coagulation is a stress phenomena, since exercise intensities severe enough to elicit eosinopenia failed to affect blood coagulation in most subjects.

Summary

Whole blood clotting time was measured before and after four different intensities and durations of exercise, using 12 male college students as subjects. The exercises consisted of a 15-minute walk on a motor-driven treadmill at 2.5 m.p.h., a 2-hour walk on the treadmill at 2.5 m.p.h., a run to near exhaustion on the treadmill at 6.25 m.p.h., and a stepping exercise on a 12-inch bench, 30 steps per minute for 15 minutes. Thromboplastin generation rate was measured before and after the stepping exercise on blood samples drawn from 14 subjects, and thrombin generation rate was similarly measured on samples drawn from 16 subjects.

No statistically significant variation was found in whole blood clotting time of samples drawn before the exercise, 1 minute after the exercise, or 15 and 30 minutes after. Neither was there any significant change in the thromboplastin generation rate of samples drawn before, 1 minute after, and 30 minutes after exercise. Ten of the 16 subjects showed no change in thrombin generation rate after exercise. However, six subjects experi-
enced an acceleration of thrombin generation from a mean time of maximum thrombin concentration at 5.2 minutes before exercise to 4.0 minutes one minute after exercise. This difference was statistically significant.

From these data it was concluded that, in general, exercise was not followed by hypercoagulability. However, in some subjects, thrombin generation rate was increased due to undetermined factors.

References
Effect of Exercise on Blood Coagulation
Clifford E. Keeney and David W. Laramie

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