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Effect of Exercise and Training on the Blood of Normal and Splenectomized Rats

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In 1929, Barcroft and Florey[5] observed that the exteriorized spleens of dogs contracted to about 65% of normal resting size following exercise. This constriction occurred in the denervated as well as the normal innervated spleen[4]. Electrical stimulation of the voluntary muscles of cats produced a similar contraction of the exposed spleen[11]. Previously, Cruikshank[12] demonstrated that the contracting spleen could expel two times its post-mortem weight of blood and that this blood contained twenty to forty per cent more hemoglobin than blood of the general circulation. These findings have led to the conclusion that contraction of the normal spleen during exercise produces a significant increase in the hemoglobin and erythrocytes per unit of blood in the general circulation. Broun's observation that the spleens of trained dogs were larger than those of untrained dogs further implicated it as an important hemoglobin and erythrocyte reservoir for exercise [10].However, Steinhaus found that the spleens of trained dogs were smaller than those of the untrained controls[40]. While some[6,22,28] maintain that the spleen is an important erythrocyte and hemoglobin reservoir for exercise, others[15,23,29] have questioned this concept. The importance of the rat spleen on these components of blood during exercise and training has not previously been studied. Reports on the effect of training on spleen size are also varied with increases, decreases, and no change, all having been reported[16,17,20,30,33].

Presently, little agreement exists as to the effect of training on erythrocyte count, hematocrit, hemoglobin concentration and red cell fragility. Investigators have found increases, decreases and no change following training of dogs, horses and man.* In this study the effects of exercise and training on hemoglobin, hematocrit and red cell fragility were evaluated in the normal and splenectomized rat.

* 1,13,18,19,25,27,31,32,34,35,37,42,43,44

Materials and Methods

Twenty-seven male, albino rats of the Sprague-Dawley strain, with initial body weights ranging from 260 to 270 grams, were used in the experimental procedures. They were housed individually in $7 \times 10 \times 7$-inch self-cleaning cages in an air-conditioned room with the ambient temperature maintained at 23° C. The animals were fed *ad libitum* a commercial pellet diet. Water was available at all times.

Half of the animals were splenectomized using an abdominal approach employing a midline incision of about one-half inch. The remaining rats were subjected to a similar surgical procedure, with spleens being left intact. Surgery was alternated between splenectomized and sham-operated animals. All surgery was performed with ether anesthesia and completed within a four-hour period. A previously conducted pilot study had shown that a two-week, post-operative period was sufficient for complete wound healing.

Following surgery, the animals were divided into four experimental subgroups of untrained normal, untrained splenectomized, trained normal and trained splenectomized. The training program consisted of swimming in water at 35° C. On the first day of the training period, the animals were individually introduced to swimming with a five-minute swim. Hematocrit and hemoglobin determinations were made immediately after this introductory period as the first swim period causes the animals to become somewhat excited or adrenalized, and such excitement has been purported to produce splenic contraction [3,23,28]. Daily exercise periods following this introductory swim were administered by placing several animals in a tank at once. This procedure was used to insure vigorous activity and to avoid the effortless floating which occurs when rats are allowed to swim alone. The first session of group swimming was for 35 minutes; this period was increased 5 minutes per day, finally reaching 60 minutes per day. The training period continued for 42 consecutive days.

Blood samples were obtained by nicking the tails with a scapel while the animals were under light ether anesthesia. Samples were taken prior to the surgical treatment and subsequently at one-week intervals. Weekly samples were taken following a 24-hour rest period. On the final day of the experiment, untrained animals were anesthetized and blood samples were taken from the bifurcation of the abdominal aorta and collected in heparinized tubes. Organs were quickly removed and weighed on a Roller-Smith torsion balance. At this time the incisions made during splenectomization were examined to insure that no surgical complications had occurred which could have effected the experimental results. Trained animals were subjected to a similar procedure following a 60-minute swim.

Red cell volumes were estimated from duplicate samples collected in heparinized 75 mm capillary tubes which were heatsealed and centrifuged at $1700 \times g$ for 25 minutes. Cell volumes were determined using an International Micro-Capillary Reader. No attempt was made to correct these readings for entrapped plasma as it has been shown that this error is less than the error of reading [3]. Hemoglobin concentrations were determined by the method described by SUNDERMANN [38]. These values are expressed as grams per 100 ml of blood. Red cell fragilities were determined by mixing 0.02 ml of blood in 5.0 ml of .55, .50, .45, and .40% solutions of sodium chloride. These preparations were allowed to stand at room temperature for two hours. Cell fragments and intact cells were then removed by centrifugation. The supernatant fluid was decanted and the hemoglobin released by the red cell hemolysis estimated as described above. Red cell fragilities are expressed as per cent of the total hemoglobin released in the various ionic solutions (Fragility $=$ $\frac{\text{Hemoglobin released} \times 100}{\text{Total hemoglobin}}$).

Results

Table 1 contains the results of the organ weight data. Comparisons between the untrained normal and splenectomized, and between the trained normal and splenectomized groups with a t test, revealed that splenectomization had no effect on organ weights. Thus, the effect of training was determined by comparing the combined trained and untrained groups with the t test. This revealed that on a absolute weight basis, the trained animals had significantly heavier adrenals and heart ventricles ($P < 0.01$), whereas their livers and spleens were lighter ($P < 0.01$).

Hemoglobin and hematocrit data are presented in Table 2. An analysis of variance was used to determine the effect of splenectomization on these components. In no instance were significant differences found between splenectomized and sham-operated untrained or between normal and splenectomized trained groups. The two untrained and trained

Table 1. *Final organ weights of normal and splenectomized rats trained by daily swimming for six weeks*

Group	No. of Animals	Adrenals mg	Heart Ventricles mg	Liver mg	Spleen mg
Untrained Splenectomized	8	36.90 ± 3.16[a]	1033.30 ± 28.43	14.24 ± 0.43	
Untrained Normal	5	39.60 ± 2.72	1056.80 ± 32.49	15.46 ± 1.16	804.48 ± 62.03
Trained Splenectomized	5	40.24 ± 3.38	1084.08 ± 50.13	12.96 ± 0.38	
Trained Normal	9	41.64 ± 1.36	1038.70 ± 20.37	12.51 ± 0.37	596.38 ± 27.79[d]
Combined Untrained[b]	13	37.94 ± 2.16	1042.34 ± 20.97	14.71 ± 0.52	
Combined Trained[c]	14	41.14 ± 1.42[d]	1054.91[d] ± 21.92	12.67 ± 0.37[d]	

[a] Value represents mean ± SEM. [b] Combination of both untrained groups. [c] Combination of both trained groups. [d] Trained vs. untrained $P < 0.01$.

Table 2. *Weekly hemoglobin and hematocrit of normal and splenectomized rats during a six-week training period*

Group	No. of Animals		Weeks 0[a]	1	2	3	4	5	6
Untrained Splenectomized	8	Hematocrit	48.13 ± 0.63[b]	49.75 ± 1.09	49.81 ± 0.77	48.60 ± 0.36	48.60 ± 0.61	47.11 ± 0.94	41.79 ± 1.26
		Hemoglobin	14.40 ± 0.27	15.58 ± 0.49	15.94 ± 0.19	15.01 ± 0.24	15.26 ± 0.30	13.90 ± 0.86	11.98 ± 0.57
Untrained Normal	5	Hematocrit	49.30 ± 0.26	49.90 ± 0.25	51.20 ± 0.60	50.10 ± 0.64	49.16 ± 0.85	48.60 ± 0.58	44.96 ± 0.57
		Hemoglobin	15.12 ± 0.20	16.14 ± 0.24	16.92 ± 0.33	15.96 ± 0.21	15.86 ± 0.43	15.20 ± 0.36	13.46 ± 0.32
Trained Splenectomized	5	Hematocrit	50.20 ± 0.68	52.62 ± 1.36	51.66 ± 1.03	48.60 ± 0.98	49.70 ± 0.70	47.70 ± 0.89	44.96 ± 0.83
		Hemoglobin	14.96 ± 0.25	16.70 ± 0.68	16.90 ± 0.49	15.70 ± 0.33	16.04 ± 0.37	15.12 ± 0.47	13.28 ± 0.31
Trained Normal	9	Hematocrit	49.60 ± 0.67	50.18 ± 1.05	51.17 ± 0.96	49.20 ± 0.42	48.74 ± 0.89	47.00 ± 0.77	44.90 ± 1.39
		Hemoglobin	14.26 ± 0.22	15.67 ± 0.51	16.66 ± 0.31	15.61 ± 0.06	15.52 ± 0.37	15.09 ± 0.30	13.11 ± 0.52
Combined Untrained[c]	13	Hematocrit	48.58[e] ± 0.42	49.81 ± 0.66	50.35 ± 0.55	49.14 ± 0.41	48.82 ± 0.48	47.75 ± 0.63	43.08[e] ± 0.93
		Hemoglobin	14.68 ± 0.20	15.79 ± 0.32	16.32 ± 0.21	15.38 ± 0.21	15.49 ± 0.25	14.39 ± 0.56	12.55 ± 0.42
Combined Trained[d]	14	Hematocrit	49.82 ± 0.48	51.05 ± 0.86	51.34 ± 0.70	48.99 ± 0.43	49.09 ± 0.62	47.25 ± 0.57	44.92 ± 0.86
		Hemoglobin	14.51 ± 0.13	16.04 ± 0.42	16.74 ± 0.26	15.64 ± 0.16	15.71 ± 0.27	15.10 ± 0.24	13.17 ± 0.34

[a] Sample taken the day the training program was started (see text). [b] Values represent means ± S.E.M. [c] Combination untrained animals. [d] Combination trained animals. [e] Trained vs. untrained $P < 0.01$.

groups were thus combined to determine the effects of training on the hemoglobin concentration and red cell volume of the blood. Significant weekly variations were found to exist within the untrained and trained groups. These weekly variations appear to be due to normal fluctuations as a *t* test between trained and untrained groups revealed that only the increase in hematocrit observed immediately following the first and last swim represented a statistically significant difference ($P < 0.01$) between the trained and untrained groups.

The results of a similar statistical analysis of the red cell fragility data appear in Table 3. Once again no statistically significant differences occurred as a result of splenectomization. Comparison of combined untrained with combined trained groups showed that the red cells of the trained animals were less resistant to osmotic pressure in the .50 and .45% NaCl solutions than cells from untrained animals. The lack of change at .40 and

Table 3. *Red cell fragility of normal and splenectomized rats after six weeks of training by swimming*

Group	No. of Animals	Ionic Strenght .55 %	.50 %	.45 %	.40 %
Untrained Splenectomized	8	8.49 ± 0.58[a]	17.56 ± 3.10	28.19 ± 4.03	65.03 ± 4.59
Untrained Normal	5	8.03 ± 1.04	18.21 ± 1.71	43.88 ± 3.96	76.88 ± 2.21
Trained Splenectomized	5	7.42 ± 0.56	21.73 ± 3.33	37.34 ± 1.67	71.92 ± 1.61
Trained Normal	9	8.53 ± 1.64	24.99 ± 2.34	47.07 ± 3.25	76.55 ± 2.16
Combined Untrained[b]	13	8.31 ± 1.12	17.81 ± 1.96	34.23 ± 3.41	69.59 ± 3.30
Combined Trained[c]	14	8.13 ± 1.06	23.83 ± 1.90[d]	43.59 ± 2.61[d]	71.78 ± 1.61

[a] Values represent mean ± SEM. [b] Combination of both untrained groups. [c] Combination of both trained groups. [d] Trained vs. untrained $P < 0.01$.

.55% solutions is probably a result of the low hemolysis at .55% and the high per cent at .40%.

Discussion

The organ weight changes found following training are in agreement with those previously reported*. The increase in adrenal weight and the heart ventricle hypertrophy indicated that the training program represented a significant physiological stress. Of particular interest is the finding that the spleens from the trained animals were significantly smaller ($P < 0.01$) than those from the untrained controls. This agrees with previously reported data for dogs[40], rats[17,20,24] and for rats fed a 1% cholesterol diet[20]. However, Broun[10] found that the spleens from trained dogs were larger than those of untrained controls. It could be postulated that the hour exercise prior to sacrifice resulted in an expulsion of storage blood from the spleens, thereby decreasing their weights. This seems unlikely as the reduction reported here is similar to that reported earlier[20] in which the animals were not exercised prior to sacrifice. In addition, all the rats were bled at the time of sacrifice and the spleens blotted to remove all excess blood prior to weighing.

It has been suggested, particularly in man, that a period of anemia follows splenectomization[45]. The weekly hematocrits indicated that this effect did not appear. The lack of any significant difference in either hemoglobin concentration or hematocrits between untrained groups (normal and splenectomized) and between trained groups indicated that the spleen was of little or no importance in the maintenance of these components of blood. Broun also found a temporary anemia in dogs at the start of training[7–10]. He attributed this to a differential between the destruction of fragile erythrocytes at the onset of training and the stimulation of the hemopoietic processes. This effect was not observed in this investigation with rats.

During the training period no significant differences in hematocrit or per cent hemoglobin occurred which could be attributed to the training program. While this agrees with several earlier reports, increases in erythrocytes**, hematocrit[32], hemoglobin[18,31,32], blood volume [27,31] and total body hemoglobin[27] have been reported. Altitude acclimatization has also been shown to produce polycthemia and to increase the per cent hemoglobin in man[28]. However, in rats, red cell counts were unchanged whereas per cent hemoglobin and blood volume were increased by altitude acclimatization[36,41]. These adaptations to altitude increase the capacity of the blood to transport oxygen to the tissue. The consistent heavy exercise of training in which large oxygen debts occur may

* 16,17,20,21,24,26,30,33,40,45

** 13,18,19,25,31,32,35,37,44

invoke similar changes. HOLMGREN et. al.[27] and KJELBERG et. al.[31] have reported increased blood volume and total body hemoglobin following training. These adaptive responses to altitude and training in which no increase in erythrocyte count occurs are interesting in that they increase the oxygen carrying capacity of the blood without increasing the viscosity as occurs with polycythemia. While blood volume and total hemoglobin were not measured, the lack of any positive change in hematocrit or per cent hemoglobin reported here may indicate that swimming did not adequately increase the metabolic rate of the rats. BAKER and HORVATH[2] demonstrated that the rats swimming individually increase their metabolic rate only three times that of resting. Group swimming is more strenuous than swimming rats individually, however, its effect on their metabolic rate has not been determined.

Exercise did not produce any changes in hematocrit or per cent hemoglobin between the normal and splenectomized rats. This indicates that if splenic contraction does occur during exercise, its contribution to the concentration of red cells is negligible. STEINHAUS[39] suggested that the contraction of the spleen is of little importance in man, but may be significant in smaller animals such as dogs and cats. This contention is supported by GUYTON[22] and is probably based on the changes in spleen size following exercise reported by BARCROFT and co-workers with dogs[4,5], COOK and ROSE with cats[11], and the observations of DILL et. al.[15] in which the increased red cell count of exercised splenectomized subjects was about the same as that for normal subjects (4%) and corresponded to the hemocentration following exercise[29]. The small difference between exercised and nonexercised animals is of the same magnitude reported by DILL et. al. and probably is due to hemocentration. The results reported here do not support the contention that spleen is more important to small animals during exercise than it is to man.

The decreased resistance of red cells from trained rats to osmotic pressure agrees with the findings of DAVIS[14] for man. However, THÖRNER has reported increased osmotic resistance in red cells of man and dogs following training[42,43]. From these findings and those of DAVIS it is evident that the structural integrity of the red cell is weakened by the regular exercise of training. The higher circulatory rate, increased temperature and increased compression of the cells by the muscles during exercise probably contribute to this weakening of the cell wall.

Summary and Conclusion

The immediate and chronic effects of exercise on hematocrit, hemoglobin and red cell fragility have been studied in splenectomized and sham-operated rats. The training program consisted of an initial 35 minute swim in water at 35° C. The duration of the swim was increased

5 minutes per day until reaching 60 minutes and continued for 42 days. Splenectomization had no effect on organ weights, hemoglobin content, hematocrit or red cell fragility. The adrenals and heart ventricles of the trained animals were heavier, whereas their livers and spleens were significantly smaller. Training had no effect on per cent hemoglobin or hematocrit, however, red cells from trained animals were less resistant to osmotic pressure. No differences in hemoglobin or hematocrits were found between splenectomized and sham-operated animals immediately after exercise. It is concluded that in the rat the spleen does not make a significant contribution to these components of blood during exercise.

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