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## Transaminase Changes in Rats After Exercise.* (28815)

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Evidence has been accumulating since 1956 suggesting a relationship between the glutamic-oxalacetic transaminase (GOT) level and muscular or metabolic activity.

Vagotomy of the pigeon, presumably allowing an imbalance of activity in favor of the sympathetic nervous system, was found to result in increased GOT in skeletal, myocardial and hepatic tissue(1). Barbieri(2) measured the GOT content in the 4 chambers of the heart *(Bos taurus)* and reported the transaminase content of the left ventricle was highest with decreasing amounts present in the right ventricle, left auricle and right auricle. Administration of 2,4-dinitrophenol, a metabolic stimulant, resulted in an elevated GOT concentration in skeletal muscle and hepatic tissue(3). Serum glutamic-oxalacetic transaminase (SGOT) was determined in men before and after 3 three-minute rounds of boxing(4). A significant rise in SGOT was observed immediately after this type of exercise. Altland and Highman(5) forced rats to walk for 16 hours and found that this work load resulted in a significant increase in SGOT. Recent research indicated that untrained men, exposed to the Harvard Step Test, had a significant decrease in SGOT(6). Well-trained athletes, on the other hand, did not deplete the serum enzyme level following the same work load. The athletes, however, did have lower SGOT titers after time trials in their particular track events.

On the basis of the above evidence, experiments were designed to minimize tissue damage and to test further the exercise theories.

*Materials and methods.* Adult female rats of the Sprague-Dawley strain, weighing between 160 and 234 g, were fed standard Purina Laboratory Chow and given water *ad libitum.*

The exercise consisted of 2 series of swimming animals and 2 series subjected to a treadmill run. The rats swam in a glass tank (approximately 23°C) for either 15 or 60 minutes (or to exhaustion; the animal was considered exhausted if it remained below the surface for 60 seconds). The rats ran on the treadmill at a speed of 34 meters per minute. The first series ran 7 minutes, the second group ran 15 minutes. Immediately after the exercise the rat was killed by decapitation. A control animal was killed with each experimental animal. The first few drops of blood were collected in a waxed dish for determination of the hematocrit (International Hemacrit Centrifuge). The remainder of the blood was captured in a centrifuge tube. The clot was allowed to retract, then centrifuged at

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TABLE I. Mean Body Weight, Hematocrit, Adrenal Ascorbic Acid and Glutamic-Oxalacetic Transaminase Activity in Rats.

| Exp series | Wt (g) | Hematocrit (%) | GOT as $Q_T^{10}$ Heart | GOT as $Q_T^{10}$ Muscle | GOT as $Q_T^{10}$ Liver | Serum ($\mu$g/ml) | Adrenal ascorbic acid (mg %) |
|---|---|---|---|---|---|---|---|
| Control | 192 (39)* | 42.0 ± .51† (39) | 648 ± 8.36 (39) | 352 ±12.2 (38) | 255 ± 6.62 (28) | 113 ± 4.28 (34) | 360 ± 8.9 (6) |
| Treadmill, 7 min | 196 (11) | 39.4 ±1.15 (11) | 591 ±25.1 (10) | 301 ±23.7 (11) | — | 113 ± 6.94 (8) | 295 ± 17.9‡ (8) |
| Treadmill, 15 min | 206 (10) | 42.5 ± .64 (10) | 705 ±18.9‡ (10) | 369 ±15.5 (10) | 297 ±10.3 ‡ (10) | 79.9 ± 6.04‡ (10) | 279 ± 22.6‡ (7) |
| Swimming, 15 min | 194 (10) | 47.5 ± .62‡ (10) | 668 ±31.1 (10) | 419 ±20.7‡ (10) | 261 ± 8.62 (10) | 94.7 ± 7.83‡ (10) | 280 ± 10.6‡ (8) |
| Swimming, 60 min | 197 (10) | 45.2 ± .98‡ (10) | 742 ±34.2‡ (9) | 482 ±12.2‡ (10) | 312 ±13.8 ‡ (10) | 70.3 ± 4.03‡ (10) | 240 ± 4.2‡ (7) |

* No. of animals. † S.E. of mean. ‡ P = <0.02.

1500 RPM, the serum separated and stored in the refrigerated state. Analysis was carried out within 4 days of the time the serum was collected. Reports indicated that the stability of the serum enzyme under refrigeration was well beyond this period(7). Hemolyzed samples were discarded. Serum transaminase activity was expressed as micrograms of pyruvate per milliliter of serum. These values, if multiplied by the factor 1.2 (8), are equivalent to the spectrophotometric units originally used in the method proposed by LaDue, Wroblewski and Karmen(7).

The tissues analyzed for GOT content were the left ventricle of the heart, the right gastrocnemius and a sample from the central lobe of the liver. They were removed as quickly as possible to beakers of ice-cold, 0.1 M phosphate buffer (pH = 7.4) and homogenized in preparation for enzymatic assay. Glutamic-oxalacetic transaminase was determined by a modification of the Tonhazy, White and Umbreit method(9). The modification allowed both serum and tissue transaminase to be measured by the same method. The modification is outlined in detail elsewhere(6). Tissue GOT activity was stated in terms of the $Q_T^{10}$ notation(10).

The adrenal glands were removed as rapidly as possible, cleaned of adherent connective tissue and weighed on a torsion balance. Adrenal ascorbic acid was determined by the method of Briggs and Munson(11). Adrenal ascorbic acid was expressed as mg/100 g of wet adrenal tissue.

Experimental mean values were compared statistically to control mean values by use of Student's "t" test of significance. Chauvenet's criterion(12) for rejection of suspected data was applied to all series of experiments. The null hypothesis was rejected at the 2% level.

*Results.* The effects of running and swimming on the hematocrit, SGOT, tissue GOT and adrenal ascorbic acid are presented in Table I.

The hematocrit did not change after running but was increased by swimming 15 minutes or 60 minutes. The running and swimming data suggest that transaminase activity was related to the duration of the work effort. Rats running for 7 minutes exhibited no change in transaminase content in the tissues investigated nor was there any alteration in SGOT level. Animals swimming for 15 minutes had an elevation in skeletal muscle GOT while serum transaminase was depleted significantly. Heart and liver GOT of animals running for 15 minutes was increased and the GOT was elevated in all 3 tissues in rats swimming for 60 minutes. Serum glutamic-oxalacetic transaminase was depleted in both groups; however, the more significant SGOT loss occurred in the swimming rats.

Adrenal ascorbic acid was depleted in all of the experimental groups. The extent of depletion, however, was least in animals running for 7 minutes, the greatest in animals swimming for 60 minutes. Running or swimming for 15 minutes produced identical de-

pletion of ascorbic acid from the adrenal gland.

*Discussion.* Hematocrit results similar to those observed in the present series of experiments have been reported. Man, running at top speed on a treadmill for 90 seconds, lost 470 ml of plasma, presumably accounting for the concomitant 12% hematocrit increase(13). Auvergant reported that running elevated the hematocrit in man from a level of 43.1% to 50.2% and inferred that a plasma loss and an influx of red cells from various reservoirs were responsible for the increased hematocrit(14). It is generally agreed that with a fairly severe muscular effort the hematocrit will be elevated. The response probably is related to loss of plasma.

The duration of work appears to be a more important factor in regulating GOT levels than the rate of doing work. Moderate to severe exercise or psychological stress, sufficient to involve the hormones of the adrenal cortex, may be the mechanism responsible for the transaminase changes noted in this experiment. Adrenalectomized rats had lower GOT in heart, skeletal muscle and brain(15). Treatment of these animals with cortisone restored transaminase activity to normal levels in heart and brain, but increased the activity in muscle(16). Similar results were observed in the heart and kidney of the mouse (17). In the present investigation, short periods of exercise resulted in a significant depletion of adrenal ascorbic acid, while more prolonged exercise provoked an even greater depletion of adrenal ascorbic acid. These data suggest an interdependency between amount of GOT in a tissue and level of adrenal cortical hormones.

An animal not previously trained to a treadmill run or a swim appears to accept either stimulus as a stress and increase adrenal cortex activity. Untrained rats deplete serum transaminase and accumulate tissue transaminase as a result of the stress of moderate to severe exercise. Although a casual inspection might appear to suggest a dynamic equilibrium between SGOT and tissue GOT, our calculations did not indicate that such a relationship existed. The animals in the swimming experiments exhibited an elevated hematocrit. It was suggested above that hemoconcentration occurred, probably due to a loss of plasma. Despite this fact the SGOT level decreased. It is possible that these values could be corrected for the hemoconcentration effect and might reveal that a still greater decrease in SGOT occurred.

GOT converts aspartic acid to oxalacetic acid. The latter would then be available to the citric acid cycle and offer a potential source of energy to the animal. Accumulation of GOT by active tissue in response to an unusual work load may be of benefit to the animal.

*Summary.* The hematocrit, adrenal ascorbic acid, and glutamic-oxalacetic transaminase (GOT) of myocardial, skeletal and hepatic tissue and of serum (SGOT) were determined in normal and exercised (treadmill run or a free swim) adult female rats. The hematocrit was not changed in the running animals but was increased in the swimming rats. Adrenal ascorbic acid was decreased by exercise. In general, GOT increased while SGOT was depleted following exercise. These changes were more pronounced as the work load increased. The role of the adrenal cortex and its possible interrelationship with transaminase movements, as influenced by exercise and stress, was discussed.

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## Effects of Electrolytes Upon Adipose Tissue Membrane Electrical Potentials.* (28816)

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Transmembrane or resting membrane electrical potentials (REP) have been demonstrated in rat epididymal adipose tissue(1,2). A dose-response relationship has been established between increase of adipose tissue REP and insulin concentrations of 1-1000 micro-units/ml. The REP response to insulin diminishes in proportion to increase of rat weight(2). More recent studies have shown that insulin antibody blocks the insulin effects.

The investigation reported here reveals significant diminution of adipose tissue REP associated with increase of $K^+$ in the medium. This effect was not demonstrated when adipose tissue from older, larger animals was employed.

*Methods.* The apparatus and techniques for recording REP's and the design of the experiments have been described(1,2). Segments of male Wistar rat epididymal adipose tissue were penetrated with glass microelectrodes and the DC potentials recorded on motion picture film. Evidence that these were true electrical potentials has been presented (1,2). In many experiments, it was possible to employ a single glass micro-electrode for a single complete experiment or for a number of experiments, in some cases extending over several days' duration.

Normal male Wistar rats, in the fed state, weighing 160-390 g were employed. The usual control medium was Krebs-Ringer bicarbonate solution containing (mM) $Na^+ = 140$, $K^+ = 5$, $Ca^{++} = 2.5$, $Mg^{++} = 1$, $Cl^- = 125$, $H_2PO_4^- = 1$, $HCO^- = 25$ and $SO_4^= = 1$. Variations of potassium and sodium ion were obtained with media described by Hodgkin and Horowisz(3), the composition being (mM), $Ca^{++} = 8$, $HPO_4^= = 1.08$, $H_2PO_4^- = 0.43$, $SO_4^= = 48$, Sucrose $= 113$, $Cl^- = 0$. One solution, designated for convenience "high $K^+$", contained 80 mM of $K^+$ and 3 mM of $Na^+$. A second solution, designated "high $Na^+$", contained 83 mM of $Na^+$, and no $K^+$. Relative tonicity and ionic strength were approximately equivalent to the Krebs-Ringer control buffer, so that successive determinations of REP with control, "high $K^+$", and "high $Na^+$" solutions were usually characterized by a stable base-line. If major base-line alterations occurred, REP values were discarded. As previously described, the experimental and control REP's were determined on the same tissue segment. Usually, 3- to 5-minute periods of recording REP's were

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