Effect of Swimming Exercise on Serum Glutamic-Oxalacetic Transaminase and Hematocrit of Rats.* (30708)

JERRY B. CRITZ
(With technical assistance of Robert Yee and Edward Ehrichs)
Department of Physiology and Pharmacology, University of South Dakota School of Medicine, Vermillion

LaDue, Wroblewski and Karmen demonstrated that serum glutamic-oxalacetic transaminase (SGOT) varied from one individual to another when they found normal SGOT levels ranged from 10 to 40 units(1). However, other investigators reported the SGOT activity was relatively constant in the same individual under normal conditions(2,3). Many reports now indicate that SGOT may vary in the same individual under special circumstances, such as physical exercise. Conflicting reports have appeared in the literature concerning the effects of exercise on SGOT. Apparently SGOT may decrease(4-7), increase(8-16) or remain unchanged(17, 18) in response to exercise. It is necessary to resolve these differences since the SGOT test is widely used to aid in the diagnosis of myocardial infarction.

Several attempts have been made to explain these controversial results. These include: (a) different types of exercise; (b) different durations of exercise; (c) different time intervals for obtaining samples after exercise; (d) different methods used to determine GOT activity in the serum.

Nerdrum and Berg(11) have pointed out that Laets(6), Critz and Merrick(4,5) and Nerdrum and Nordøy(7), all using spectrophotometric methods, have reported decreases in SGOT titers following exercises of a specific type or duration.

Nerdrum and Berg(11) have demonstrated an increase in ketone bodies after exercise. Ketone bodies are known to affect the SGOT values obtained by use of the Reitman and Frankel colorimetric method. In earlier work from this laboratory(4,5) a modified version (19) of the Tonhazy, White and Umbreit (20) method for tissue GOT was utilized. In this modification a serum sample was inactivated and then carried through the assay procedure. Thus, any color developed was due to non-transaminase material and should be subtracted from the color developed in the assay procedure by the same serum. In the raw data from these experiments we have noted that under resting conditions there is little, if any, chromogenic material but after exercise there is a large amount of color present, probably ketone bodies. The amount of color present is such that if the values found in the sample blanks had not been subtracted from the assay samples we would have found an increased SGOT level following exercise.

In view of the possible errors inherent in such a method we were pleased with the introduction of a new method for determination of SGOT in 1962(21). This method employs a diazonium salt which is specific for oxalacetic acid(22). The availability of this new method, which is not influenced by other keto acids in the reaction mixture, led us to examine this problem more rigorously. In particular we were concerned with the effects of the duration of a specific type of exercise (swimming) on SGOT levels.

Materials and methods. Adult, female albino rats of the Sprague-Dawley strain, weighing an average of 186 g, were housed in air-conditioned animal quarters and were fed standard Purina Laboratory Chow and given water *ad libitum*.

These animals were divided randomly into groups for either control, exercise (swimming) or splenectomy experiments. The hematocrit was determined before and after exercise in normal and splenectomized rats to investigate the effects of fluid exchanges on SGOT levels during and immediately after exercise. Blood was obtained for the hematocrit studies from the tail. Splenectomized animals were allowed 10-14 days for recovery from surgery before they were utilized in the

* Supported in part by Grant AM 06154, USPHS, Nat. Inst. Health and South Dakota Heart Assn.

TABLE I. Hematocrit and Serum Glutamic-Oxalacetic Transaminase Activity in Normal and Splenectomized Rats.

Exp series	SGOT in units/liter (normal animals)	Hematocrit (%) (normal animals)	Hematocrit (%) (splenectomized animals)
Control	79.0 ± 2.18† (26)*	43.4 ± .025 (80)	42.4 ± .30 (81)
Swimming, 1 min	63.6 ± 5.40‡ (13)	47.1 ± 1.03‡ (11)	46.3 ± .73‡ (12)
" 5 "	111.0 ± 5.40‡ (17)	46.3 ± .90‡ (11)	47.6 ± .93‡ (14)
" 10 "	80.5 ± 2.64 (24)	45.5 ± .54‡ (25)	47.0 ± .81‡ (15)
" 15 "	75.8 ± 3.28 (14)	—	—
" 30 "	92.7 ± 4.63‡ (19)	45.0 ± .79‡ (9)	42.7 ± .81 (11)
" 60 "	101.1 ± 5.88‡ (21)	46.1 ± .63‡ (9)	46.5 ± .62‡ (11)
" 120 "	103.3 ± 4.50‡ (15)	45.2 ± .85‡ (14)	44.9 ± .82‡ (10)

* No. of animals. † S.E. of mean. ‡ $p < 0.05$ compared to control.

swimming experiments. The animals swam for either 1, 5, 10, 15, 30, 60 or 120 minutes.

Serum for the SGOT determination was obtained by a direct heart stab on unanesthetized, restrained rats immediately after exercise. The serum was separated and stored in cold until analyzed. Analysis was usually performed within 2 days after the serum was collected. The enzyme is reportedly quite stable when refrigerated for this period(1,23, 24). Hemolyzed samples were discarded.

A modification of the Furuno and Sheena (25) method was utilized for determination of SGOT. The modification eleminated ethylenediaminetetraacetic acid (EDTA) and polyvinylpyrrolidone (PVP) from the color developer solution. The diazo salt (6-benzamido-4-methoxy-m-toluidine diazonium chloride) reacts only with oxalacetic acid and apparently owes its specificity to interaction with the β-carbon rather than the keto group of oxalacetic acid as does 2, 4-dinitrophenylhydrazine(22). SGOT activity was expressed in terms of units/liter.

Statistical comparison of experimental mean values to control mean values was made by Student's "t" test of significance. Chauvenet's Criterion(26) for the rejection of suspected data was applied to all series of experiments. The null hypothesis was rejected at the 5% level.

Results. The effect of variable swimming time on SGOT activity and hematocrit is presented in Table I. SGOT activity was decreased significantly ($p = 0.011$) by swimming for 1 minute, while swimming for 5 minutes resulted in a highly significant rise in SGOT activity ($p = <0.001$). It can be seen that swimming for either 10 or 15 minutes resulted in SGOT levels near the resting (control) level. However, swimming for 30, 60, or 120 minutes resulted in a progressive increase in SGOT titers.

The hematocrits of the intact rats were the same as those of splenectomized rats with the following exception: at a swimming duration of 30 minutes in the splenectomized group the hematocrit was at the control level. No explanation is offered for the control hematocrit observed in this group. Hematocrit data was not obtained for the 15-minute swimming group, however, in previously published work on intact rats swimming for 15 minutes we found the hematocrit to be increased to 47.5% from a resting (control) value of 42.0%(5). This is a somewhat larger increase than that found in this study at either the 10- or 30-minute swimming time.

Discussion. The decrease in SGOT activity observed after 1 minute of swimming and the large increase in SGOT activity found after 5 minutes of swimming are difficult to ex-

plain. Fluid shifts could have been responsible for the early, rapid changes in SGOT activity. Sréter and Friedman(27,28) observed a decrease in hematocrit (51.5% to 50.2%) in rats running on a treadmill for 24 minutes at 20 m/min. The hematocrit remained low for 40 minutes. However, we found in rats, as Auvergnat found in man (29), that swimming for either 1 minute or 5 minutes caused a hemoconcentration. The spleen and other red cell reservoirs could have been masking fluid shifts by forcing red cell rich blood into the circulation during the first few seconds of exercise. To investigate this possibility a group of rats were splenectomized and their hematocrits determined before and after various swimming times. We found no significant difference in hematocrit response to exercise between splenectomized and intact rats. The decrease in SGOT activity reported by several investigators appears to be related to the intensity and duration of the exercise load(7,11). The time the sample is obtained after exercise may also be important(11). We are unable to explain completely the rapid fluctuations in SGOT activity occurring during the first 5 minutes of swimming exercise.

After 10 and 15 minutes of swimming SGOT activity returns to normal levels. With the longer swimming times there is a gradual and significant elevation in SGOT titer. The rise in transaminase activity in the longer swimming groups is probably related to hypoxia(30-33) developing in the active muscles. The hypoxic condition could result in increased permeability of the cell membrane and thus allow leakage of the GOT enzyme into the serum. Highman *et al*(30,34) have suggested that the hypoxic condition acts indirectly by causing release of catechol amines, which in turn are responsible for the increased cell permeability. However, the work of Gray and Beetham(35) suggests the large dose of catechol amines used to demonstrate this was far in excess of the physiological release of catechol amines during exercise.

This laboratory has demonstrated elevated GOT activity in heart, skeletal muscle and liver from exercised rats(5). Such an increase would result in a larger tissue/serum ratio for GOT and would favor diffusion of GOT from such tissues into the serum, and thus might explain, at least in part, the elevated SGOT activity associated with the longer duration swimming exercise.

The function of GOT in serum is unknown. It may have no function in serum, but may be present as a result of slow leakage from normal heart, skeletal muscle and hepatic tissue. Likewise, the function of GOT in heart or skeletal muscle remains obscure but it appears to be closely related to the contractile process(36). The increased GOT in heart and skeletal muscle associated with exercise may represent an attempt by the organism to convert an increased amount of amino acids to keto acids. As pointed out by Cohen(37), the rapid rate of transamination (GOT), and its independence of aerobic conditions, indicate its importance in making available such substances as α-ketoglutaric acid or oxalacetic acid for muscle metabolism. The appropriate keto acids could enter the Krebs cycle and result in an elevated production of ATP which would aid the exercising animal in meeting the increased work load.

Summary. The hematocrit and SGOT activity were determined in intact and splenectomized rats before and after swimming exercises of varying durations. The hematocrit was elevated in both the intact and splenectomized rats following a swimming exercise of any duration. SGOT activity was decreased by the very short duration swim (1 minute), but was elevated after swimming 5 minutes. A 10 or 15 minutes swim produced no significant change in the SGOT activity but swimming for 30, 60 or 120 minutes caused a progressive rise in such activity.

1. LaDue, J., Wroblewski, F., Karmen, A., Science, 1954, v120, 497.

2. Goble, A., O'Brien, E., Lancet, 1958, v2, 873.

3. Sampson, J., Prog. Cardiovasc. Dis., 1958, v1, 187.

4. Critz, J., Merrick, A., Proc. Soc. Exp. Biol. and Med., 1962, v109, 608.

5. ———, ibid., 1964, v115, 11.

6. Laets, G., Proc. IV Intern. Congr. Clin. Chem., E. & S. Livingstone, Ltd., Edinburgh & London, 1961, 172.

7. Nerdrum, H., Nordøy, S., Scandinav. J. Clin. & Lab. Invest., 1964, v16, 617.
8. Schlang, H., Am. J. Med. Sci., 1961, v242, 338.
9. Vacca, C., Lombardi, S., Mangoni, C., Scala, L., Vacca, L., Boll. Soc. Ital. Biol. Sper., 1962, v38, 1585.
10. Remmers, A., Kaljot, M., J.A.M.A., 1963, v185, 968.
11. Nerdrum, H., Berg, K., Scandinav. J. Clin. & Lab. Invest., 1964, v16, 624.
12. Cornelius, C., Burnham, L., Hill, H., J. Am. Vet. Med. Assn., 1963, v142, 639.
13. Altland, P., Highman, B., Am. J. Physiol., 1961, v201, 393.
14. Highman, B., Altland, P., ibid., 1963, v205, 162.
15. Garbas, J., Highman, B., Altland, P., ibid., 1964, v207, 467.
16. Franza, C., Palmieri, A., Arch. Obstet. Ginecol., 1964, v69, 270.
17. Halonen, P., Konttinen, A., Nature, 1962, v193, 942.
18. Swaiman, K., Awad, E., Neurology, 1964, v14, 977.
19. Helmsen, R., MS Dissertation, Univ. of Missouri, 1959.
20. Tonhazy, N., White, N., Umbreit, W., Arch. Biochem., 1950, v28, 36.
21. Babson, A., Schapiro, P., Williams, P., Phillips, G., Clin. Chim. Acta, 1962, v7, 199.
22. Schaffert, R., Kingsley, G., Getchell, G., Clin. Chem., 1964, v10, 519.
23. Karmen, A., Wroblewski, F., LaDue, J., J. Clin. Invest., 1955, v34, 126.
24. Eckstein, B., Lavi, M., Acta Endocrinol., 1964, v46, 440.
25. Furuno, M., Sheena, A., Clin. Chem., 1965, v11, 23.
26. Jarrett, A., AEC, Oak Ridge, Tenn., 1946, 300-A9417.
27. Sréter, F., Friedman, S., Canad. J. Biochem. Physiol., 1958, v36, 333.
28. ———, ibid., 1958, v36, 1193.
29. Auvergnat, R., C. Rend. Soc. Biol., 1958, v152, 176.
30. Highman, B., Altland, P., Am. J. Physiol., 1960, v199, 981.
31. Asvall, J., Scandinav. J. Clin. Lab. Invest., 1960, v12, 239.
32. Highman, B., Altland, P., Am. J. Physiol., 1961, v201, 603.
33. Altland, P., Highman, B., Garbus, J., Aerospace Med., 1964, v35, 1034.
34. Highman, B., Maling, H., Thompson, E., Am. J. Physiol., 1959, v196, 436.
35. Gray, I., Beetham, W., Proc. Soc. Exp. Biol. and Med., 1957, v96, 636.
36. Ponomareva, T., Drel, K., Biokhimiya, 1964, v29, 185. CA, 1964, v61, 3487.
37. Cohen, P., J. Biol. Chem., 1940, v136, 585.

Received September 20, 1965. P.S.E.B.M., 1966, v121.

Effect of Stress on Blood Levels of Guanine Nucleotides.* (30709)

VINCENT W. HOLLIS, JR.† AND ESTELLE R. RAMEY
(Introduced by L. S. Lilienfield)
Department of Physiology and Biophysics, Georgetown University Medical Center, Washington, D.C.

Previous work from this laboratory showed that pretreatment of stressed intact rats with guanine nucleotides abolished some of the characteristic metabolic alterations of stress (1,2). In addition, the survival time of adrenalectomized rats subjected to tourniquet application was significantly prolonged by guanylic acid pretreatment(2). These experiments suggested a physiological role for the guanine nucleotides in the stress response. The present study was undertaken to determine whether an applied stress alters the metabolic handling of the guanine nucleotides themselves in normal and adrenalectomized rats. As a first approach to this problem we measured the rate of disappearance of C^{14} labelled guanylic acid from the blood of tourniquet stressed and unstressed intact and adrenalectomized animals.

Materials and methods. Male albino rats of the Sprague-Dawley strain, weighing 200 g ± 10 g were used in these studies. The rats were maintained on Purina rat chow and

* Supported in part by U.S.P.H.S. Grant AM-04714.

† Predoctoral Fellow, U.S.P.H.S.