Glycogen Depletion in Rat Ventricles During Graded Exercise.* (30830)

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It has been shown that the glycogenolysis observed in the rat heart during brief exercise (up to 15 minutes) is dependent upon the duration of the exercise and that after this initial period the glycogen levels remain relatively stable during further exertion(2). The present study relates myocardial glycogenolysis to the severity of exercise in this initial dynamic period.

Materials and methods. Male Sprague-Dawley rats weighing between 175 and 350 g were used in the experimental procedures. All rats underwent a 24-hour fast prior to being sacrificed but were allowed free access to water at all times. The separation of trichloroacetic acid (TCA) and residual glycogen fractions was carried out by the procedure developed by Bloom *et al*(1). Both fractions were extracted from the same tissue and analyzed by the anthrone method of Seifter *et al*(4) using a Beckman model B spectrophotometer at a wavelength of 620 mμ. Glycogen values are reported as mean values in milligrams of glycogen per 100 g of wet tissue with their standard errors. The Student's "t" test was used with confidence limits set at $P<0.05$.

Two basic forms of exercise were used: (a) swimming for 5 or 15 minutes in water at 25 ± 0.5°C and (b) running on a treadmill for 15 minutes. In (a) additional rats were forced to swim 15 minutes with weights added to their tails amounting to 3% of their fasted body weight and in another group for 5 minutes with a 6% load. In (b) rats were forced to run at 2 speeds (0.57 and 1.08 miles per hour). All rats were exercised singly with no previous training periods, and the cardiac glycogen concentrations were compared with unexercised rats.

After the desired period of exercise each rat was beheaded and the ventricles rapidly isolated. The right ventricle was immediately frozen between blocks of dry ice. The remaining tissue, the left ventricular wall and interventricular septum, was weighed to the nearest 0.2 mg and transferred to a Ten-Broeck tissue grinder. Removal of the heart, trimming and weighing the left ventricle required approximately 2.5 minutes from time of decapitation. The frozen right ventricle was likewise weighed and transferred to a separate tissue grinder. Unpublished work (Blount) indicates that the pattern of glycogen depletion is the same whether the tissue is frozen before being weighed or allowed to remain nonfrozen while being weighed prior to homogenization.

Results. Five minutes of unburdened swimming was sufficient to produce a significant decrease in myocardial glycogen (Fig. 1 (A) and 1 (B)). This decrease reflected essentially a decline in the TCA fraction, from 280 ± 14 to 209 ± 9 mg% ($P<0.005$) in the left ventricle and from 255 ± 12 to 210 ± 9 mg% ($P<0.024$) in the right ventricle. The residual fraction displayed no significant changes. The rats with the 6% load showed no significant difference from the non-weighted rats swimming for the same time.

Fifteen minutes of unburdened swimming produced no greater glycogenolysis than did swimming for 5 minutes. Greater glycogenolysis was found, however, in the left ventricular TCA fraction of rats forced to swim for 15 minutes with a 3% load when compared with unburdened rats swimming for 5 to 15 minutes ($P<0.015$ and $P<0.04$ respectively). The right ventricular TCA glycogen level for unburdened rats swimming for 15 minutes was high (254 ± 15 mg%) and did not differ from the resting value. However, the 15-minute TCA glycogen concentration was considerably greater than the 5-minute swimming value ($P<0.04$) for unburdened rats.

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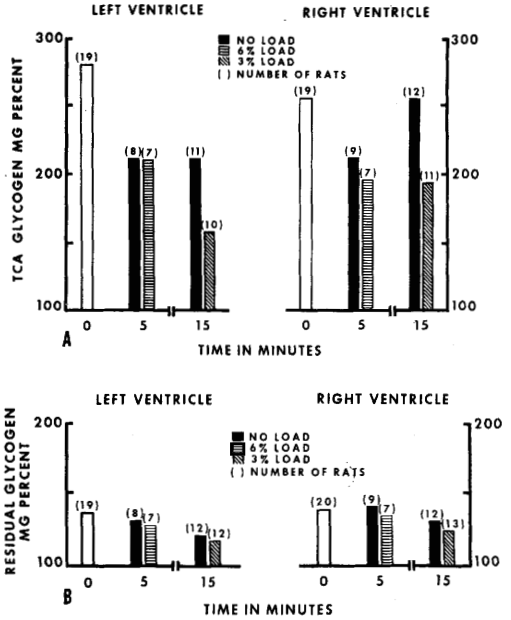

FIG. 1. (A) TCA glycogen fractions of left and right ventricles after various periods of swimming exercise with different burdens. (B) Residual glycogen fractions of left and right ventricles after various periods of swimming exercise with different burdens.

The pattern of glycogenolysis for the right ventricle in animals swimming for 15 minutes with a 3% load was different from the left ventricular pattern. This 15-minute value (194 ± 9 mg%) while lower than the elevated 15-minute value ($P<0.004$) for non-weighted rats was no different from the two glycogen concentrations found in either of the groups swimming for the 5-minute period. The residual glycogen for both ventricles was essentially unaffected by any of the experimental procedures.

In rats forced to run at a slow speed (0.57 mile/hour) on a treadmill the TCA glycogen fraction was reduced from 297 ± 8 to 228 ± 10 mg% ($P<0.001$) (Fig. 2) in the left ventricle and in the right ventricle from 258 ± 11 to 231 ± 14 mg% (not statistically significant). Increasing the speed to 1.08 miles/hour produced even greater glycogenolysis with the TCA fraction being lowered to 185 ± 8 mg% in the left ventricle and to 188 ± 7 mg% in the right ventricle. Both of these values were significantly lower than the values of the corresponding fractions in the slow runners. Less difference was noted in the left ventricular residual glycogen levels between the fast running rats (132 ± 5 mg%) and the rested value (152 ± 7 mg%) ($0.052>P>0.043$) (borderline significance) and between fast runners and the slow runners (145 ± 3 mg%) ($0.045>P>0.037$). No differences were noted in the residual fractions in the right ventricle.

Discussion. The 6% weight represented an almost overwhelming load for the swimming animals, and they were unable to swim much longer than 5 minutes. However, no greater glycogenolysis occurred here than with the unburdened rats. When a lesser weight, 3%, was attached to the animals they were able to swim for a longer time and the left ventricular TCA glycogen level was depleted further than that produced by swimming without weights. Therefore the burden the animal had to carry as well as the duration of the swimming exercise proved to be important considerations.

An interventricular difference was noted in both swimming and running experiments. Left ventricular TCA glycogen of the 3% loaded, 15-minute swimmers was lower than that of the 5-minute swimmers. Fifteen minutes of swimming with a 3% load was not sufficient to lower the TCA fraction below that of the 5-minute swimmers in the right ventricle. In the treadmill trials fast and slow speeds lowered left ventricular TCA glycogen while in the right ventricle only the fast speed affected the carbohydrate reserve.

Wilber has suggested that during physical exercise the heart is maintained in an hypoxic environment(5). Results by Kirk and Honig

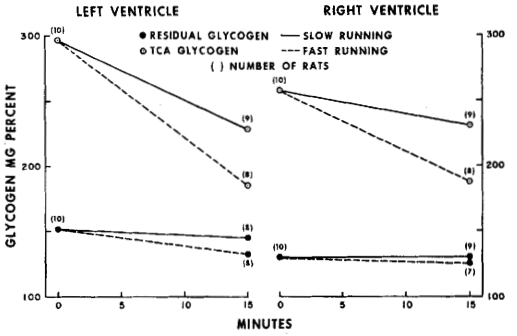

FIG. 2. Glycogen fractions of left and right ventricles after running at slow and fast speeds on a treadmill.

(3) suggest that since tissue oxygen tension is less in the subendocardium of the dog than in the subepicardial layer, greater oxygen lack may be encountered in the thicker left ventricle than in the right ventricle. They suggested that under conditions of exercise the inner regions may meet part of their energy requirements by anaerobic mechanisms. In the present experiment the greater workloads encountered by the left ventricle possibly increased the anaerobic demands of this tissue and therefore produced the interventricular differences seen in Fig. 1 and 2.

Summary. Graded cardiac work loads were imposed on fasted rats by varying the severity of exercise in acute experiments. Trichloroacetic acid soluble (TCA) and residual glycogen fractions were determined in both ventricles. Glycogen depletion varied with the severity of the exercise and was produced almost exclusively in the TCA fraction. Left ventricular TCA glycogen was affected more than that of the right ventricle.

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Multiple Virus Infection of Monkey Kidney Cells in Culture.* (30831)

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Infection of a single animal cell with 2 viruses has been studied previously(1,2,3,4,5), often with inclusions localized in different intracellular sites. For example, a single cell infected with both herpes simplex and vaccinia viruses showed herpes intranuclear inclusions and vaccinia intracytoplasmic inclusions. This report describes a double infection with SV40 and measles virus occurring in the same nucleus of single cells. In addition, these doubly infected cells were highly susceptible to superinfection with poliovirus.

Materials and methods. Virus strains. SV40 and measles virus were isolated from cell cultures prepared from the kidneys of a sick green monkey. SV5 was isolated from rhesus monkey kidney cultures during a simian virus surveillance study(6). Poliovirus type 1, LSC strain, was used in the interference experiments.

Tissue cultures. Primary rhesus or green monkey kidney tissues were trypsinized in the usual manner(7). Roller tubes or Leighton tubes containing 11 × 22 mm coverslips each were seeded with 1-2 ml of a cell suspension containing 3×10^5 cells per ml. These cultures were incubated at 37°C in a stationary position and complete cell monolayers were obtained 6-7 days after seeding.

Staining cultures. Infected cultures on coverslips were washed in phosphate buffered saline and fixed in Zenker's acetic acid solution for hematoxylin-eosin (H&E) staining. For the Feulgen reaction, or for acridine orange fluorescent preparations, Carnoy's fixative fluid was employed. The acridine orange fluorescent preparation described previously by Gluck and Kulovich(8) was used with some modification. After rapid hydration in 80, 70, and 50% alcohols, the coverslips with infected cells were rinsed in 0.002 M $MgSO_4$ solution. Then they were stained for 3 minutes in 0.01% acridine orange in 0.067 M Na_2HPO_4 and KH_2PO_4 buffer of pH 8.0.

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