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Glycogen Levels in Cardiac and Skeletal Muscle of Rats Recovering from Exercise

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by

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I wish to thank my advisor and teacher Dr. James L. Poland for being so interested in athletics and exercise and for helping me so much in carrying out this project.

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Introduction

In seeking answers to questions about how the human body functions under stressful situations, scientists, coaches, doctors, and athletes in general have all wondered about the role of exercise in the maintenance of good health. Good cardiovascular health, especially, is sought ardently, as it appears to be a major key to overall body fitness. Good cardiovascular health appears to go hand-in-hand with proper diet, proper rest, and proper exercise (1, 2).

The exercise phenomenon has attracted much attention and poses many questions. Just what is occurring when a person or an animal gets "in shape" by exercising. What bodily changes are initiated during exercises which tax the body's metabolic machinery?

To understand better the changes occurring with exercise, it would be of interest to examine and compare the metabolism of cardiac and skeletal muscles. Glycogen is considered a storage form of energy, as it is made up of glucose moieties, and it appears in all metabolizing mammalian cells (3). Yet because the level of glycogen remains at a fairly stable level in resting cells--reflecting a balance of synthesis and degradation of glycogen, any change in that level seen after a stressful situation, such as exercise, could be used as an indication of changes in cell metabolism associated with the stress (3). Thus, a change in the level of glycogen in either heart or skeletal muscle indicates a change in the utilization of energy-providing substrates, of which glycogen is one (4); an alteration in the concentration of the enzymes associated with glycogen synthesis or degradation (5, 6); or a change in some other regulatory mechanism associated with glycogenesis or glycogenolysis (7, 8).

To clarify those changes occurring in the glycogen metabolism of heart and skeletal muscle, the changes can be monitored by measuring differences in glycogen levels in rats after various regimens of exercise and can be related to the conditions under which they happened (4, 9).

Literature Review

Exercise can be considered the training of the body to do work which involves an enhanced state of metabolism of the body. It is usually felt by most physicians and coaches to be desirable and beneficial for good health. Astrand (2) says that the diet can make a big difference in maintaining good health and in performing work.

In measuring the amount of work done or metabolic changes observed, certain standard tests and assays have been established to measure the effects of exercise. Yet Cummings (10) in his editorial on physical fitness and cardiovascular health has stated the need for a more precise definition of exercise, its tests, and its various effects on the body.

In the process of standardization of exercise and its associated phenomena, a good place to start is with the major types of exercise employed by researchers. Cummings (10) and others (11, 12) speak of step tests, treadmill walking, marching (11), running, and ergometer or bicycle tests (12) in relation to human subjects, whereas many other authors have worked with rats (14-25), guinea pigs (6), and dogs (26) in running and swimming exercises. One reads the terms acute exercise and chronic exercise often in the literature and they mean respectively one spontaneous bout of exercise and an extended program of training by swimming or running. The conditions of the exercise do affect the animals' performances to some degree, so special prescribed sets of circumstances are usually established by an author before carrying out the research (14-25).

Swimming

When swimming an animal to exercise it, certain variables must be

considered. The sizes of the animal and the swimming area usually limit the research to either rats or guinea pigs, animals small enough to swim in a sink or large tub while large enough to be easily handled by the investigator. The time length of swimming varies greatly from author to author (14-16). The term seen frequently in the literature "swam to exhaustion" raises problems in the standardization of the exercise, for exhaustion comes to intact, control rats in some studies after one hour of swimming (4, 14), while in others the rats swim for two to three hours before becoming exhausted (15, 18). Exhaustion is, incidentally, measured by the inability of the rat to regain the surface of the water on his own power (22). Many authors (21, 22) also feel that the rats should be swum in groups of four or more in close enough quarters to cause interaction or activity between the rats, since they think that rats swum singly tend to passively float, rather than actively swim. Others say nothing about rats swimming in groups and imply that any swimming by the rat for long periods of time will cause the changes they are seeking (4, 17, 27).

The temperature of the water in which the rats are swimming makes a difference, too, authors feel. Some prefer 32-33°C for their experiments (15, 18), while others insist on 25-26°C (4, 20). The latter temperature is slightly cooler than a rat's body temperature and tends to dissipate some of the heat evolved from the working muscles and body. Cooler temperatures (less than 20°C) tend to affect the swimming ability of the rats and, consequently, the rats do not swim as long.

Running

Rats and guinea pigs are often used by investigators to run because of their convenient sizes. The times of the exercise depend usually on

the method of running employed and the goal of the program in mind. Either motorized treadmills equipped with electrical shock stimulators or rotating drums are used to exercise the animals, and scientists have found that to use the treadmill, a program of learning and training must be included to teach the rats to run. This fact precludes any "run to exhaustion" studies for non-trained animals on a treadmill. Although the rotating drum has been used to run untrained rats, once a rat tires, he will just roll for the remainder of the running time (5).

To skirt around the problem of setting up degrees of exercise using a treadmill, several authors simply increase the speed of the treadmill for sufficient times to exhaust the animals which had previously been taught to run (13). Then the speed and time length of running can be variables between groups of rats taught to run, rather than the ability of the rat to run on the treadmill. Most authors using running machines train their rats to run at speeds varying from one mph to two mph (4, 6, 7, 14), and use electrical shock stimulators to provide a noxious stimulus for the rat not running at the desired speed (6, 7).

Because of differences noticed in electron micrographs between the skeletal muscles of rats swum and rats run, some authors feel swimming is not as strenuous to the rat as running (13). This may be true in relation to some skeletal muscles, but the changes seen in heart tissue indicates that either exercise form can nearly equally affect cardiac muscle (13). Before choosing which exercise to use in an experiment, scientists should examine that exercise in light of the muscle actively used by the animal in the performance of the exercise and select their program with this in mind.

Glycogen as an indicator of exercise

To measure the effects of exercise or stress on either skeletal or cardiac muscle, the tissue glycogen levels are closely monitored. Glycogen is used as an indicator of the severity of an exercise since the amount becoming depleted from the muscles per exercise appears to be related to the amount of work performed by the heart or skeletal muscle, (9, 25, 28) or the degree of anoxia or hypoxia endured by the muscle tissues (29). To fully understand the effects of exercise, though, one must be knowledgable about the pathways of synthesis and degradation of glycogen in the fed, intact animal. This short overview of glycogen metabolism, as outlined by White, Handler, and Smith in their biochemistry text (3) and the Handbook of Physiology on Circulation, Section 2, Volume 1 (30), and further clarified by Green and Goldberger in 1961 (31) and the Reviews of Physiological Chemistry in 1969 (32), covers only the pertinent pathways which may be directly altered by exercise or starvation, two conditions of this experiment.

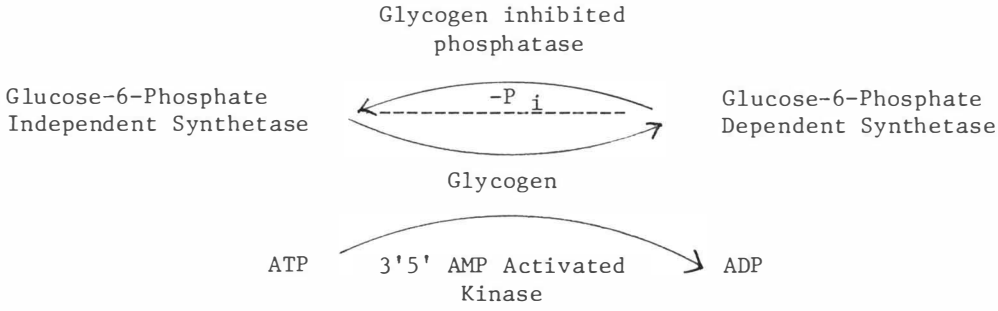
Glycogenesis, the synthesis of glycogen, involves the transfer of glucose-6-phosphate to glucose-1-phosphate by the action of the enzyme phosphoglucomutase; the enzymatic reaction of glucose-1-phosphate with uridinetriphosphate (UTP) to give UDP-glucose plus pyrophosphate; the addition of glucose from UDP-glucose to a glycogen primer in α1,4 glucosidic bonds with the help of the synthetase enzyme, called also UDP-glucose-glycogen transferase; and finally the transferring of some glucose moieties on the glycogen primer from the linear α1,4 sequence to α1,6 glucosidic bonds by the action of the branching enzyme, amylo (1:4--1:6) transglucosidase (3). The glycogen molecule may grow in molecular weight and size by adding on more glucose moieties and more branches.

Glycogenolysis, the breakdown of glycogen, involves the debranching of glucose moieties from the glycogen polymer by the debranching enzyme, amylo (1,6)--glucosidase, and the phosphorylation of those glucose subunits to glucose-1-phosphate by the action of the active phosphorylase "a" enzyme. Glucose-1-phosphate is converted back to glucose-6-phosphate by the same phosphoglucomutase enzyme as before, and the glucose-6-phosphate is then metabolized by the body.

Both glycogen synthetase and phosphorylase enzymes are influenced by other enzyme and substrate systems which can be regulated by levels of ATP, AMP, glycogen, epinephrine, glucose, or several other enzymes. The control of these two enzymes is important, since the relative rates of synthesis and degradation of glycogen depend on them. Hence, the measure of glycogen in the tissue at any one time reflects a balance of the two systems (3, 6).

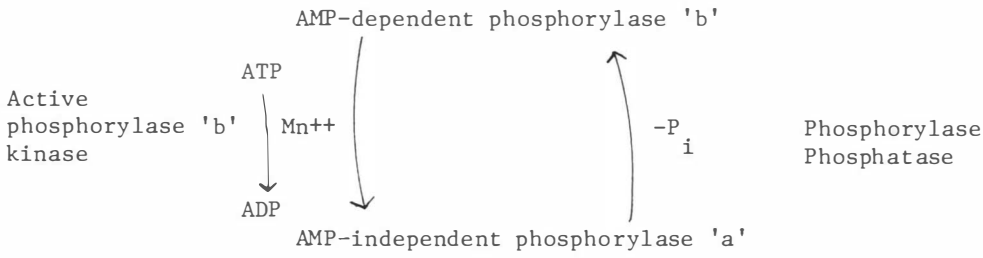
Mechanisms of glycogen synthetase and phosphorylase

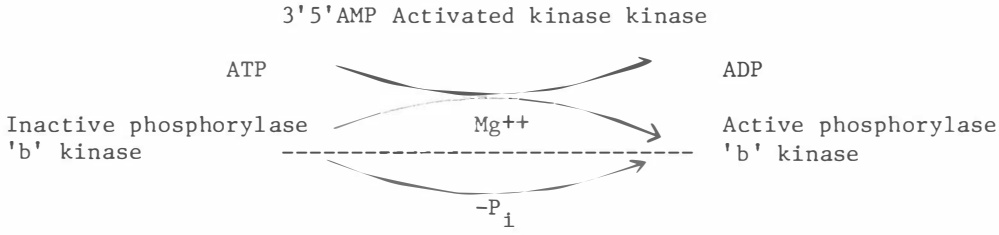
There are two forms of glycogen synthetase enzyme, a glucose-6-phosphate dependent form, the D one, and a glucose-6-phosphate independent form, the I enzyme. Depending on body conditions one or the other enzyme form will be dominant at any given time. The D enzyme is so dependent on the glucose-6-phosphate molecule that it is inactive without that substrate. The enzyme is inhibited by free UDP which accumulates when concentrations of ATP and glucose are low. The conversion of synthetase I to the D form occurs with the aid of 3', 5' AMP-activated glycogen synthetase kinase, an enzyme which is itself activated by low levels of 3', 5' cyclic AMP, while the reverse conversion from synthetase D to form I happens with the help of a glycogen synthetase phosphatase, an enzyme which is inhibited by increasing concentrations of glycogen.



"During sudden stress epinephrine effects the transformation of I to D synthetase so that glycogen formation may occur only if the glucose-6-phosphate concentration is high enough to assure formation of glucose-1-phosphate and to activate the synthetase. When heart or skeletal muscle glycogen is depleted, form I is dominant; as glycogen concentration increases, the relative fraction of the D form increases (3)."

"The phosphorylase enzyme has two forms also, an active 'a' form and an inactive 'b' form, with the enzyme phosphorylase 'b' kinase aiding the conversion from 'b' to 'a'. Phosphorylase phosphatase is the enzyme speeding along the transfer from form 'a' to 'b'. The phosphorylase 'b' kinase has two forms also--an active phosphorylated one and an inactive non-phosphorylated form. The conversion of the inactive to the active phosphorylase 'b' kinase is mediated by a 3'5' AMP activated kinase kinase enzyme. Epinephrine also can activate the kinase kinase enzyme to convert the inactive phosphorylase 'b' to the active 'a' form, so that glycogen will be broken down (3)."





To sum up the activity going on: White, Handler, and Smith say that resting muscle converts glucose to glycogen, and since the ATP concentration is high while the AMP concentration is low, glycogen breakdown is minimal. Contraction of the muscle, by using ATP and generating AMP, reverses these relationships. Available glucose-6-phosphate enters the glycolytic pathway, leading to ATP synthesis, and there is a demand for production from glycogen of more glucose-6-phosphate for this function. The increase in AMP concentration, decrease in ATP concentration, and decrease in glucose-6-phosphate concentration permit operation of phosphorylase 'b' by this method: a) epinephrine induces formation from ATP of 3'5' cyclic AMP, which activates phosphorylase kinase kinase, b) this, in turn, activates phosphorylase kinase and, hence, c) converts phosphorylase 'b' to 'a', thereby permitting maximal glycogen breakdown (3).

"Concurrently, the diminished glucose-6-phosphate concentration has minimized the activity of existing synthetase D, and form I is converted

to D by the 3'5' cyclic AMP-activated synthetase kinase. When contraction ceases, glucose from the blood is phosphorylated, glucose-6-phosphate concentrations increase, and synthetase D activity is initiated.

"The diminished glycogen concentration releases glycogen inhibited phosphatase, and as conversion of synthetase D to I occurs, glycogen synthesis accelerates. Phosphorylase phosphatase converts phosphorylase 'a' to 'b' and, as the ATP concentration is restored and the AMP concentration declines, glycogen breakdown declines markedly, permitting restoration of the glycogen stores (3)."

That liver glycogen content usually reflects the level of carbohydrates in the blood is well known, since the liver is found to have much higher glycogen levels after sugar feeding or infusion when the blood sugar levels are high than after having been fasted overnight, when the blood sugar levels are lower (33). While, on the other hand, the fact that muscle glycogen does not vary much with the changing levels of blood sugar has also been demonstrated (9). The glycogen level of the muscle tissue appears to be related either to the muscle's functioning state--whether it is at rest or contracting; to its rate of contraction, as with exercise states; or to the conditions of oxygen availability under which it is working--aerobic or hypoxic.

Hypoxia ? Exercise

Many authors have looked at the metabolic effects of hypoxia on cardiac and skeletal muscle glycogen levels (9, 25, 28, 29, 34, 35, 43). Evans in 1934 found that by lowering the oxygen tension in the air inspired by rats for three hour periods, he could observe significant decreases in cardiac and gastrocnemius muscles' glycogen content (9). Scheuer and Stezoski in 1970 (29) using a perfused heart preparation found that hearts

perfused for twenty minutes anoxically after fifteen minutes of aerobic perfusion displayed marked depletions in cardiac glycogen. In the most drastic of all the conditions used to study anoxia, Michal, et al. (34) sectioned the hearts of dead dogs immediately and at minute intervals after death, and glycogen depletion was monitored, along with other energy components, such as ATP, CP, ADP, DPN, TPN, and AMP. The decrease in DPN, which reflects a decrease in lactate in the muscle, was also seen as a response to hypoxia in heart muscle by Danforth and Bing (35).

From Danforth and Bing's studies on hypoxia (35) have come the ideas that heart muscle has less creatine phosphate (CP), an energy conserving compound which works directly with ATP, than does skeletal muscle, and that cardiac muscle is designed metabolically and anatomically for aerobic work. Myocardial muscle can tolerate little, and thusly builds up little, oxygen debt (35). Skeletal muscle, on the other hand, can build up an oxygen debt frequently without readily apparent harm to the muscle tissue (22).

Danforth and Bing in 1958 (35) tried to show a direct relationship between coronary blood flow and oxygen utilization by the heart, while Scheuer and Stezoski in 1970 (29) reported a similar relationship between coronary blood flow and anoxia in the tissue. Their experiments indicated such relationships did, in fact, exist.

Garbus, Highman and Altland in 1964 (43) after training rats by running found that hypoxia caused a release of the enzymes of glycolysis from tissue slices of heart and skeletal muscle of both trained and non-trained rats. The enzymes released were lactic dehydrogenase (LDH), aldolase (Ald), glutamic oxalacetic transaminase (GOT), and glutamic pyruvic transaminase (GPT), all enzymes found with studies of the Krebs

citric acid cycle and glycolysis. Similar reports of increased serum enzyme levels have come from authors working with exercised and trained animals and human subjects. Fowler, et al. (32) reported increased serum concentrations of the same enzymes of glycolysis plus malic dehydrogenase from exercising human subjects, while Altland and Highman (5) found in rats run for long times on a rotating drum increased concentrations of serum enzymes GOT, GPT, LDH, and alkaline phosphatase (AkP).

Significant drops in cardiac and skeletal muscle glycogen concentrations have also been reported for exercised animals as well as for animals forced to undergo periods of hypoxia. Blount and Meyer (25) found significant drops in cardiac glycogen after only fifteen minutes of swimming, while myocardial, liver, and skeletal muscle glycogen depletions in rats after running exercise were reported by Gollnick, et al. (7). Grasnin, et al. in 1958 (39) and Poland and Blount in 1966, 1967 and 1968 (14, 16, 27) among many others, have also reported glycogen level drops in heart muscle after exercise.

Gerald Evans in 1934 (9), however, observed no change in cardiac glycogen and lowering of gastrocnemius muscle glycogen after exercise, but his results are clouded by the effects of a twelve-hour fast which was part of his pre-exercise regimen. Gollnick, et al., (7) in 1970 also reported increased cardiac glycogen values after exercise, but those researchers waited to sacrifice the animals ten-to-twelve hours after the exercise. And Poland and Trauner in 1971 (4) showed that even though cardiac glycogen values are elevated significantly above non-swim controls six hours after exercise, up to one hour after the exercise the values were significantly lower.

From these papers cited and others reporting similar evidence, the

correlation between the effects of hypoxia and exercise on glycogen levels appears to be valid. Perhaps the shunting of blood from certain organs, such as the liver, during exercise lends credence to hypoxia in that organ (7), while local hypoxia sites within the well blood-perfused skeletal and cardiac muscles could exist during times of great activity, such as occurs during exercise. Gollnick and King in two papers (13, 22) have shown electron micrograph studies of skeletal and cardiac tissues after exercise. Each paper shows mitochondrial changes as evidence that there are some areas within the muscles which are more affected than others at the end of exercise. Their ideas for these differences in the muscle layers revolve around the postulation that some areas of tissue are better perfused than others and that local hypoxia sites are larger in areas furthest from the blood supply with its transient oxygen cargo. Thus, the areas showing the greatest change in structure at the end of the exercising period are the areas undergoing the greatest hypoxia during the exercise.

Cardiac metabolism, exercise, and hypoxia

In normal heart muscle carbohydrates, free fatty acids (FFA), and ketone bodies are utilized as food stuffs. Miller, et al., in 1971 (26) reported that perhaps greater than 65% of the sources of energy for the heart were from FFA, while the other 35% were from carbohydrates (30-35%) and ketone bodies (0-5%). The carbohydrates used may be in the form of glucose, glycogen, pyruvate or lactate, and under various conditions one of those is usually preferentially metabolized. Scheuer and Stezoski (29) reported that glycogen may be used preferentially to glucose during hypoxia, while Miller, et al., (26) said that lactate might be used more often than glucose during exercise if there was plenty of oxygen available.

The specific effect hypoxia has on cardiac glycogen metabolism has been postulated by Scheuer and Stezoski (29) to be a stimulation of phosphorylase activation and ensuing glycogenolysis. The activation of adenyl cyclase, the enzyme which mediates the breakdown of ATP into AMP, by catecholamines and the resultant conversion of phosphorylase 'b' to the active form 'a' is the mechanism for the initiation of glycogenolysis suggested by Scheuer and Stezoski. The greater availability as seen by higher relative concentrations of lactate over glucose in an exercising animal might explain the observed phenomenon of lactate being used preferentially to glucose in cardiac muscle, as described by Miller, et al. (26). Whatever the condition setting it up, the fact remains that cardiac muscle when adequately perfused with oxygenated blood can metabolize either glucose or lactate equally well.

That glycogen values in the heart are fairly stable during resting body conditions can be seen by comparing values obtained by Evans (3), Poland and Blount (13), Drasnin, et al. (39) and that muscle glycogen is depleted during hypoxia and exercise as an energy substrate can be observed, but that muscle glycogen is constantly being turned over metabolically under resting body conditions has not yet been shown. Some authors feel that the relative rates of glycogen synthesis and degradation are nearly equal for resting muscle levels and that only with special conditions, such as exercise, hypoxia, or fasting, does either process show dominance over the other; others feel that glycogen is a fixed, static molecule during resting body conditions which can be added to and taken from only during certain specific conditions such as fasting or exercise (40). Obviously only labelling studies and further research could settle that question conclusively.

Sacrificing technique

Several methods have been used to sacrifice rats in the process of collecting data on cardiac glycogen, although the results differ somewhat from technique to technique. Bloom in 1956 (28) reminded his readers that cardiac glycogenolysis did not occur with just hypoxia but did happen when the tissue was actively working under hypoxic conditions. Having this sort of reasoning in mind, Evans (9), Shelley, et al. (17), and Blount and Meyer (25), among many others (4, 21, 24, 41, 42), reported using anesthesia rather than decapitation as their method of sacrifice. They all found higher, less fluctuating values for cardiac glycogen when using the anesthesia method. Although other authors (7, 27) have used decapitation as a sacrifice method, they are allowing an extra variable condition, that of the heart working under hypoxic conditions for several seconds, to bias their data.

Effects of exercise

Before examining the metabolic effects of exercise in any more depth, one should look at the grosser effects of a week or more of training. Poland and Trauner (4) and Scheuer, et al. (21) found significant differences between the full body weights of rats exercised for periods longer than one week and those not exercised daily, while Altland and Highman (5) showed a difference, but not a significant difference, between the control and trained rats' body weights. Yet the latter authors mentioned used much older animals in their study, and older, heavier rats do not show the same rapid growth pattern as do younger, less heavy rats (5).

Evan though Gollnick, et al. (23) found increased heart weights with training, most authors report no increase in heart weights (4, 5, 21) but a significant increase in heart weight to full body weight ratios (5, 17,

21, 36, 39). A controversy arises when authors explain why their ratios are higher for trained than for control rats. Some say the rise in ratio value is due to the loss in body weight (4, 21), while others state that increased ratio is due to higher heart weights (17), while still others do not say anything except that exercise caused an increased ratio (5, 36, 39). Shelley, et al., Drasnin, et al., and Hearn and Wainio report that the hypertrophy of the heart present in exercised rats is indicated by the increased heart weight to body weight ratio, yet, in light of the studies of Poland and Trauner and Scheuer, et al. which showed significant differences in body weights between control and trained rats, the heart weight/body weight ratio may not be a very good indicator for hypertrophy in exercised hearts.

King and Gollnick in two recent papers (13, 22) have reported with the aid of electron micrographs gross structural changes in skeletal and cardiac muscle after exhaustive exercise in rats. In both tissues the sarcomeres were somewhat spread out by the tissue being distended or swollen after the exercise. The mitochondria, however, showed the biggest changes, as they were greatly enlarged, had lost many of their cristae, and, because of the swelling in the tissues, were more sparsely distributed than in the non-exercised tissue.

King and Gollnick (22) also showed the cardiac tissue two hours after the end of the exhausting exercise. The structures had recovered their normal appearance, although there was still some swelling of mitochondria. After twenty-four hours there appeared to be a greater number of cristae than normal in the still slightly swollen mitochondria. The authors agreed that hypoxia probably triggered the changes observed but they also suggested that, because the increase in cardiac work might not have allowed complete

oxidation time, the metabolic capacity of tissues had been impaired during the exercise and that this impairment might have been the rate limiting factor in the performance of heavy work.

Serum enzymes' change with exercise

Several authors including Altland, Highman, Garbus, Hearn, Wainio, and Gollnick have studied the effects of exercise and training on the serum levels of certain enzymes which are parts of the glycolysis process. The method of reasoning is that an increase in the serum content of enzymes not usually found in the serum in large amounts would indicate an increase in organ tissue levels of the enzymes and an increased breakdown of carbohydrates (11).

Altland and Highman (5) linked increased serum enzyme levels in rats to changes in body weight, tissue glycogen, and tissue pathology, after they had measured serum concentrations of GOT, GPT, LDH, and AkP, and blood urea nitrogen (BUN) levels at the completion of running periods of sixteen hours. The same authors (24) later noticed again large rises after exercise of GOT, Ald, and BUN and lesser rises in LDH and GPT. After four or more days of training, however, the rats being run showed smaller increases in the serum levels of enzymes, indicating, possibly, that adaptations to the training were occurring. Again still later in 1964, Garbus, Highman, and Altland (43) found increases with prolonged exercise of the serum enzymes of untrained rats only and not of trained ones. The levels of LDH, Ald, GOT, and GPT all increased in the rats running for the first time, while those enzymes were not found in increased levels in the serum of rats trained, which had run already at least four times. Those authors proposed that hypoxia at the local tissue sites was helping to cause permeability changes in cell membranes,

but they admitted factors other than local hypoxia sites might be affecting the release of the glycolytic enzymes into the serum after exercise. Altland et al. (36) in a second 1964 paper also stated that exercise training lessens both cellular permeability and the escape of certain cellular enzymes induced by exercise in untrained rats, implying that training for a time causes adaptations by the body to exercise work loads.

Fowler, et al. (38) and Halonen and Konttinen (11) worked with human subjects exercising and found increases in the serum enzymes. Halonen et al. found increases in LDH, MDH, and Ald, but none in GOT, in the serum of young male soldiers after a hike. They agreed that permeability changes were effected to cause the release of enzymes into the serum, and they postulated that cell damage might be involved also. Fowler, et al. observed increases in serum enzymes of untrained or non-athletic humans. Although they noticed increases in GOT, Ald, LDH, GPT, and MDH after exercise, they saw no significant differences in pre-exercise levels of serum enzymes between the trained and non-trained groups. These authors also mentioned the relationship of increased serum levels of enzymes to cell permeability changes after exercise.

Gollnick and Hearn (23) saw increased activities of LDH in heart but not skeletal muscle of exercised rats, while Hearn and Wainio (15, 18) found in two studies no change in activity of succinic dehydrogenase, a Krebs citric acid cycle enzyme, and increased activities of heart tissue aldolase, but no changes in gastrocnemius muscle aldolase activity. From the latter information cited (18) Hearn and Wainio suggested that anaerobic cycle enzymes might be rate limiting during exercise.

Glycogen changes with exercise

Although most studies on glycogen levels in muscle have involved a fast to enhance the recoverable amounts (9, 14, 39), Blount and Meyer in 1959 (25) and Poland and Trauner in 1971 (4) have reported studies on cardiac glycogen which contained data for exercised, non-fasted muscle's glycogen level. Blount and Meyer observed cardiac glycogen levels in rats sacrificed immediately after five, fifteen, or sixty minutes of swimming. The levels of glycogen depletion were as low after fifteen minutes of swimming as after one hour of swimming. Poland and Blount (16) found that glycogen depletion during exercise was dependent on the severity of the exercise performed.

Poland and Trauner (4) presented data for the recovery period of cardiac muscle after exercise--either swimming or running. They also found a significant decrease in glycogen levels in the heart immediately after exercise which was similar for one hour of swimming to the value obtained by Blount and Meyer in their study.

To establish the relationship between exercise, hypoxia, and glycogen depletion, one must consider the paper cited (28) in which Bloom said that not hypoxia alone, but cardiac work under hypoxic conditions, caused glycogenolysis. If an exercise is severe enough to cause a local hypoxic state in cardiac tissue, the condition of cardiac work done under an hypoxic environment is met, and glycogenolysis is expected. The depletion of cardiac glycogen in untrained rats during swimming should be the expected phenomenon to occur, and it does happen (4, 25).

ATP

During exercise just as during a period of hypoxia, ATP levels in

the affected tissues decrease. Just as Michal, et al. (34) observed that ATP disappears quicker than any of the energy supplying materials in hypoxic heart tissue, so can one observe a decrease in ATP levels during severe exercises as indicated by increases in ADP--and AMP--regulated enzyme reactions. The breakdown of glycogen in tissues is triggered by phosphorylase, which is dependent on AMP, a breakdown product of ATP. A fine balance of ATP--AMP is maintained in the cells as manifested by the accumulation of glycogen stores when AMP levels are below a threshold value and the depletion of glycogen whenever those AMP levels become somewhat higher. The glycogen lost during depletion is used, of course, to form more ATP's through the glycogenolysis scheme previously described.

Free fatty acids and catecholamines during exercise

Earlier in this paper in the section on cardiac metabolism, reference was made to the role of FFA (free fatty acids) as substrates supplying energy for cardiac work. The report made by Miller, et al. (26) indicated that FFA might provide 65% or more of the energy necessary for cardiac work in exercising animals and showed that 100% of the FFA taken up in the heart was oxidized. Gollnick (44) agreed with Miller, et al. that FFA were utilized in cardiac muscle during exercise, while the plasma and cardiac tissue levels of lactate and glucose were low, as the major energy source, and Gollnick reported that plasma levels of FFA remained elevated until increasing levels of either blood sugar or lactate depressed those FFA levels.

Upon observing the source of the FFA, Gollnick noted that FFA used by the hearts of untrained rats during exercise were mobilized from lipid stores by circulating catecholamines, while the FFA supplied in trained

rats were initiated by other mechanisms, such as increased sympathetic nervous system activity (44).

Astrand (2) and Hultman (12) both were impressed with the work energy supplied by a diet rich in fats and carbohydrates. Astrand found that a person could not endure hard work as long after a high protein diet, as he could after a high carbohydrate and fat meal. Obviously easier accessibility of the energy-providing substrates is the major feature of Dr. Astrand's special diet. Hultman in his study with human athletes exercising on an ergometer found that with lighter work loads FFA alone were metabolized, while with heavier work glycogen was also depleted from the skeletal muscle. This latter study by Hultman indicates that there may have been factors operating with heavy work that were not with lighter work. Such a notion is consistant with the hypoxia-due-to-exercise idea, as is the fact that the FFA were used before the glycogen reserves for increasing levels of work in exercising skeletal muscle.

Because the catecholamines are known to be mobilizers of lipid in raising serum FFA levels (44), researchers have traced their activity during exercise when greater quantities of FFA are being metabolized. DeSchryver, et al. (45) exercised rats by running them for ninety minutes three days a week for several weeks then waited for three days before sacrificing them. These authors observed a lower level of catecholamines in the hearts of the trained rats than the controls, although in acutely exercised rats sacrificed in the same manner the catecholamine levels were higher. The skeletal muscle levels remained the same with training.

These findings are consistant with Gollnick's data (44) described earlier which suggested two mechanisms were affecting rats during exercise. Since DeSchryver's three day wait after exercise should be long enough to

effect a return to resting levels, the serum catecholamine level being low might indicate some other process for increasing the catecholamines during exercise--such as an increased sympathetic nervous system activity, or suggest that increased FFA mobilization during exercise was not dependent solely on prior increased catecholamine levels.

Chin and Evonuk (46) noticed in rats sacrificed immediately after an exhausting swim, which followed a six-week training period, that the epinephrine levels were decreased while norepinephrine and total catecholamine levels were increased. This is somewhat consistent with DeSchryver's results as both groups of scientists noticed increases in total catecholamine levels following exhaustive exercise. It should now be apparent that at least two separate catecholamine states exist after exercise in rats--one after chronic training and another after exhaustive, acute exercise, yet the overall body metabolism effects of each of these separate conditions as related to the catecholamine levels are not readily definable at this time.

Fasting

Evans in 1934 (9) conducted a study to find a standard technique for determining in the albino rat cardiac glycogen values which could be seen regularly with small standard deviations. Among the many relationships he observed was the fact that hearts taken from unfasted rats contained only 69% as much glycogen as hearts from rats after a 24 hour fast. Yet Evans still used fasted rats in all his studies, since he could, after all, get higher glycogen values and, although he did not discuss in his paper the effects of a fast, all his data noted contained that bias. He had trouble explaining the effects observed after exercise and after epinephrine (adrenalin) injection since the effects of both of these stimuli were

masked by the fast.

Shelley, et al. in 1943 (17) also studied rat heart glycogen after exercise, and they too fasted the rats 24 hours before sacrifice. They, like Poland and Blount in 1968 (14), noticed after exercise that the cardiac glycogen values were even greater than in a non-exercised fasted rat group. That cardiac glycogen values are increased after a fast has been demonstrated and that enhanced glycogenesis occurs in fasted rats after exercise has also now been observed (9, 14, 17).

Russell in several papers and with various co-authors looked at the problem of observing what was happening during a fast to cardiac glycogen levels. Russell and Wilhelmi in 1950 (47) in working with hypophysectomized rats after 24 hour fasts showed that tissue glycogen in the gastrocnemius muscle decreased unless growth hormone was added. With the injection of growth hormone, glycogen levels stayed near normal. In 1951 Illingworth and Russell (41) postulated that growth hormone and some cortical adrenal hormones were acting synergistically during a fast, as the growth hormone maintained fasting carbohydrate levels or inhibited further use of glycogen, while the cortical hormones effected use of carbohydrate fragments for fat formation.

Adrouny and Russell in 1956 (20) looked at fasting times in relation to the greatest value of cardiac glycogen and noticed that after 48 hours of fasting the cardiac glycogen levels were at their peak value. They also dried the rat hearts at 110°C to a constant weight and found that the heart tissue contained the same water content--from 75.7 to 77.6% water. Upon the rats being fed either carbohydrate or protein at the end of a 48 hour fast, the cardiac glycogen values dropped, while upon each rat receiving a fatty meal at the end of the fast, the cardiac glycogen levels remained

high at the same level. This observation may indicate that rat hearts during a fast are metabolizing FFA as substrates and that glycogen is being spared by the metabolizing heart during the fast.

In another 1956 paper (33), Russell and Bloom found a similar relationship for rats' cardiac glycogen values as before; the fasted rats' values being higher than the non-fasted rats'. Yet in hypophysectomized fasted rats, there was no change in cardiac glycogen until growth hormone was injected into the animals. These researchers found no increase in skeletal muscle glycogen during a fast, though, even after the injection of growth hormone, and saw nothing but a loss in liver glycogen with the fast. Insulin had little effect on the cardiac glycogen during the fast while it did help in the deposition of glucose into skeletal muscle glycogen.

In one of the last papers published before she died, Russell with McKee in 1968 (48) looked at the effect of acute hypophysectomy on FFA mobilization and cardiac glycogen in fasted rats. With hypophysectomy the rats' cardiac glycogen levels were reduced by one half from the control levels. Upon the injection of growth hormone, these glycogen values returned to normal for both the 24 and 48 hour fasted rats. The researchers found with hypophysectomized rats no mobilization of FFA during a fast, but after the addition of growth hormone the levels of FFA were near normal. With these pieces of information in mind, these authors postulated that growth hormone might affect FFA mobilization which in turn might affect carbohydrate and nitrogen metabolism. They implied a relationship between the adrenals and the pituitary gland with increased glycogenesis observed after a fast and said that ACTH and growth hormone affected the adrenals to help mobilize FFA.

Schimmel and Knobil (49) in working with liver slices in vitro concluded that plasma FFA levels do not play a major role in the control of gluconeogenesis but they observed that FFA did effect a large increase in glycogen in liver slices from fasted animals. Thus even though FFA levels might not be a controlling factor in glycogenesis, those FFA were important in fasted animals for use as substrates, if nothing else.

Control of glycogen during a fast

Wermers, et al. (50) in working with rat diaphragm muscles in vitro decided that the greatest degree of net glycogen synthesis occurs in the resting state with both fasted and non-fasted rat preparations and that the progressive decline in glycogen synthesis observed with increasing contraction rates might be explained by a proportionately greater dominance in activity of the phosphorylase system over the synthetase system. Presumably the progressive increase in glycogen values can be explained by the converse of Wermers' idea--that the synthetase system activity becomes dominant to the phosphorylase one.

Adrouny in 1969 (8) sums up the differences between skeletal and cardiac muscle glycogen metabolism during a fast and in so doing describes the biochemical changes affecting both the phosphorylase and the synthetase systems. A 48 hour fast was used in this study since earlier work by Adrouny and Russell (20) had indicated that the plateau of cardiac glycogen levels is attained at the end of this interval of fasting. The effects of fasting are listed below with an explanation supplied by Adrouny:

1) No effects on activities of 'a' or 'a' + 'b' heart phosphorylases, but significant decreases in both 'a' and 'a' + 'b' activities of skeletal phosphorylases. a) Since AMP levels in the heart are approximately twice

those in the gastrocnemius, the nucleotide may be more effective as a controlling factor in the case of the heart. b) The fall in cardiac AMP concentrations in the fasted state may be looked upon as a factor in favor of cardiac glycogen accumulation, since it indicates a decreased activation of phosphorylase 'b' and a diminished counteraction to ATP inhibition of the enzyme. c) The increase of cardiac glucose-6-phosphate levels induced by fasting also works toward accumulation of glycogen levels by inhibiting the AMP activation of phosphorylase 'b'. d) The total phosphorylase activity in the heart is approximately one-half of that seen in the gastrocnemius.

2) The total (I+D) activity of the heart synthetase is unaffected, the I form activity is significantly lowered, and the D form activity is significantly higher. The observed increase in D activity in the fasted state allows for higher rates of glycogen synthesis. It appears, therefore, that variations in glucose-6-phosphate concentrations and the conversion of the I form of synthetase to the D form play important intermediary roles in the different effects of fasting on glycogen values in skeletal and cardiac muscles.

3) The increase in UDPG concentration in heart and concurrent decreases in skeletal muscle are not statistically significant. Increased UDPG-glycogen transglucosylase activity in the fasted heart maintains the levels of glycogen at a fairly steady level despite the increased availability of glucose-6-phosphate as a precursor.

4) The heart glucose-6-phosphate content increases by 80% while the gastrocnemius content shows no change. Glucose-6-phosphate is a key metabolite in the control of muscle glycogen levels, since it serves a a precursor of glycogen and as a regulator of phosphorylase and transglucosylase

activities. There is a parallel between glycogen and glucose-6-phosphate levels during a fast.

5) There is a significant increase in cardiac citrate levels. Citrate along with ATP and AMP is an inhibitor of the enzyme phosphofructokinase. A decrease in AMP with a fast may promote glycogen build up by low AMP inhibition of that enzyme mentioned above. The inhibition of phosphofructokinase with the elevation of citrate leads to higher levels of glucose-6-phosphate and hence to glycogen. The parallel mentioned above between glycogen and glucose-6-phosphate levels can be extended now to include citrate levels also.

Adrouny also said that FFA cause elevations in glycogen and citrate and that the action of growth hormone, which influences cardiac glycogen, may well be increased mobilization of fats and ketone bodies. He showed further, however, that increases in muscle citrate levels would follow growth hormone treatment in hypophysectomized rats but not in intact rats. Although puzzling, this last statement is corroborated by Trenkle (51), who found decreases in plasma growth hormone in fasted, intact rats along with increases in pituitary level growth hormone. The answer to why in intact, fasted rats the growth hormone levels remain low probably will be discovered later in studies searching for other hormones from the hypophysis--besides the growth hormone--which can either effect increases in muscle citrate or work synergistically with growth hormone to the same function. Certain adrenal corticoids are probably involved since several researchers have already postulated the synergism between those cortical hormones and growth hormone in fasting rats (44).

Exercise and fasting effects observed

That fasting causes an increase in cardiac glycogenesis has been shown before (8) and that fasting after a prolonged program of exercise causes an enhancement of the glycogenesis seen has also been demonstrated (9), yet in

1971 Poland and Trauner (4) observed that when untrained rats were acutely exercised by swimming and then fasted, their cardiac glycogen values were similar to those rats which were fasted after their last bout of exercise in a prolonged training schedule of running. This study suggested that perhaps the enhanced glycogenesis seen after exercise and a fast might be due to the last bout of exercise and not the prolonged training period, as supposed before.

Poland and Trauner also noticed in non-fasted rats a characteristic pattern of glycogen recovery by the heart muscle after exercise, in which the cardiac glycogen levels were depleted significantly immediately after the exercise and then in gaining back the glycogen lost those tissue levels rose above the control, pre-exercise levels. The supercompensation noticed was determined later to last as long as 24 hours after completion of exercise (unpublished data from Dr. Poland's laboratory).

Among the questions yet to be answered is whether the extent of utilization and speed of recovery of cardiac glycogen are similar for trained and untrained rats after a single bout of exercise. The next step would be to observe the differences--if any--between animals, trained and not trained, fasted and not fasted, after a single bout of exercise. This research project currently being presented undertakes to fill in that gap in the information spectrum of exercise and its many effects.

Procedure - Methods and Materials

Male Wistar strain albino rats weighing between 150 and 250 grams when purchased were used throughout these experiments. After arrival all rats were caged individually and were permitted to adjust to the new environment for one week. The rats were fed Wayne Laboratory chow and watered ad libitum. Those rats chosen at random to be conditioned by running on the treadmill were called the trained group; those which did not run and stayed in cages for the training period were called sedentary or untrained rats.

Trained groups of rats were taught to run on a motor-driven treadmill, purchased from the Quinton Equipment Company. The treadmill held ten running compartments enabling ten rats to run at a time. The posterior part of each compartment side was clear plexiglass while the anterior part was painted black. The front area then was darker than the rest and may have helped the rats to run near the front of the compartment. To insure adequate ventilation for breathing and heat elimination, while keeping each rat in one compartment, the top of each compartment was made of uniformly- and widely-spaced thin wire strips. A grid arrangement at the back of the compartment was set up to shock rats if they did not run at the speed the treadmill was moving. Whenever a rat backed into two adjacent wire prongs projecting from the back of the compartment, a small amp, high voltage shock was administered. The voltage used was approximately 350 volts with a maximal current of 20 milliamperes to minimize tissue damage. The electrical shock was supplied by a stimulator from the Quinton Equipment Company.

The exercised rats were trained to run on the treadmill by initially running it at slower speeds and then gradually speeding up the treadmill

until a speed of one MPH was reached. One MPH was the speed at which the rats were exercised daily. The training process involved increasing the time length of the runs a few minutes per day until the rats could run for one hour at one MPH twice a day, with a four hour rest period between exercising sessions. The overall training regimen called for the rats to run for one hour twice a day for the last two weeks of a four week training period.

At the end of four weeks of running, part of the trained rats along with sedentary rats underwent a single bout of exercise during which they were forced to swim for one hour in a large sink filled with water at 25°C ± 1C° (25). Since there were usually four to six rats swimming at the same time in a fairly confined area, there was observed little passive floating and much interaction between the rats (21).

Some rats were sacrificed either before or at 0, 1, 2, 4, or 8 hours after the swim and others were fasted for 48 hours with the fast beginning either before or at 0, 1, 2, 4, or 8 hours after the swimming. The non-fasted rats prior to sacrifice and the fasted rats prior to the beginning of their fast had access to food and water. Fasting rats received water only.

All rats were sacrificed by injecting approximately 0.5 cc nembutal (60 mg/cc) intraperitoneally (IP) and quickly excising the heart as soon as the rat was asleep (approx. 3-5 minutes). The hearts were excised by opening the thorax, exposing the heart, and with a single cut freeing it away from the body (3, 42). These hearts were trimmed of vessels and atria, blotted, and then placed into preweighed tubes of 30% KOH (16, 27). The time between freeing the heart from its blood supply and placing it into the KOH was less than 10 seconds (3). The weight of the tissue sample

was determined from the difference in the weights of the tube before and after the muscle was added.

The analysis of the rat cardiac glycogen was done by the anthrone method first set up by Pfluger in 1905 (53), then modified by Good, et al., in 1933 (54), and finally worked out by Seifter, et al., in 1950 (55). After the hearts were placed into small tubes containing 2 ml. of a 30% KOH and 0.5% Na_2SO_4 solution, the muscle tissue was digested by heating for ten to twenty minutes at 100°C in aluminum blocks mounted on a hot plate until the "mixture formed a homogeneous fluid" (54). Two ml. of 95% ethanol were added to each digestion mixture to precipitate the glycogen and, after stirring thoroughly, the tubes were heated to boiling in a water bath (54). Although the boiling step hastened the precipitation of glycogen, a twenty-four hour cooling period was allowed to assure the total settlement out of solution.

Centrifugation for fifteen minutes at three thousand rpm's helped gather the glycogen into a bolus, which was not broken up when the supernatant was carefully poured off (55).

The glycogen pellets or residues were next dissolved in ten ml. distilled water and mixed thoroughly by inverting the tubes several times. A 0.4 ml. aliquot was transferred from each glycogen solution to a larger test tube diluted up to five ml. with distilled water. After the sample solutions were placed in an ice water bath in anticipation of the addition of anthrone solution, standard sugar solution (dextrose in water-100 mg/100 ml. water) and blank (five ml. distilled water) solution tubes were readied. The anthrone solution was dispensed to the sample tubes by a large autopipette, designed to deliver ten ml. each time, such that there was no danger in working with the highly caustic material.

While the sample tubes remained in the ice water bath, they received ten ml. of anthrone solution each. The mixtures were thoroughly stirred manually by a glass rod. From the ice water bath, all tubes were moved to a boiling water bath for ten minutes during which the glycogen-anthrone color reaction occurred (56, 57). Eight to ten minutes were recommended as the maximum color change time (55). Subsequently, returning the tubes to the cold water bath arrested the color reaction and kept the color stable for several hours (55).

Color densities of the samples and standard solutions were measured with a Beckman Model B Spectrophotometer set at 620 mu and zeroed by the blank solution. The color differences were read in optical density units. A sample calculation for the milligrams glycogen per 100 mg. wet tissue (cardiac muscle) is:

1. $\frac{50}{1.11} \times \frac{\text{OD sample}}{\text{OD standard}}$ = micrograms glycogen,

2. heart weight in mg. x 0.04 = mg. heart tissue,

3. $\frac{\text{ugms. glycogen}}{\text{mg. heart tissue}} \times 100$ = mg.% glycogen (55).

The factor 50 in the first calculation represents the standard sugar solution's concentration, and the factor 1.11 is the number relationship determined by Morris between the concentration of glycogen and its equivalent concentration of glucose (57). The OD sample represents the optical density of the sample tube, and the OD standard stands for the optical density of the standard sugar solution. The heart weight in calculation two is the measured weight recorded for each heart tissue after extraction from the animal, while the 0.04 is the dilution factor for each glycogen sample analyzed (55).

Means and standard errors of the mean were found for all sets of data

on a Wang programmed calculator (Model 362) and using the same machine Student "t" tests were run on all data involved in comparisons of mean values.

Programs were also set up to study the effects of acute and chronic exercise on skeletal muscle glycogen. The gastrocnemius muscle was chosen for study because of its apparent use by the rat in both running and swimming and because of the relative ease in excising the muscle quickly (9).

The chronic exercise program involved a similar schedule as described previously for treadmill running. In this program, however, all the rats were fasted for 48 hours beginning immediately after the last bout of running.

The acute exercise program used no training on its subjects. The non-trained, sedentary rats were simply swum for one hour and either sacrificed at 0, 1, or 6 hours after the swim or fasted at 0, 1, or 6 hours after the exercise. The fast lasted 48 hours (20), following which the rats were sacrificed in the manner previously described, and the gastrocnemius muscle excised and placed into the 30% KOH-filled tube. To excise the leg skeletal muscle, the hind limb's posterior skin and fascia was cut to an area proximal to the knee, the gastrocnemius was separated from surrounding tissue by a dull probe, and the muscle was excised--distal end first (42). Excision of the muscle from the rat's leg took only about 30 seconds from the time the heart was removed. Muscle tissue from either leg was used in the study to rule out any non-random selection of samples. The muscle glycogen was then extracted and analyzed as previously described.

Results

In Table 1 is printed all the data collected for the fasted rats, both trained and sedentary animals, involved in the cardiac muscle study, while in Table 3A are listed and in figure 1 are pictured the mean glycogen concentrations for each group of fasted rats. There were no significant differences at any one point between the glycogen levels of trained and sedentary rats of the fasted groups, although the fasted sedentary rats, whose fasts started immediately or at one or two hours after exercise, contained significantly higher cardiac glycogen levels than the fasted, non-swim rats ($P<.01$). The difference between the fasted, trained, non-swim control rats' values and the values for the fasted, trained rats whose fast was begun one hour after swimming was not quite significant with the value being $\simeq.10$.

In Table 2 all the data collected for the non-fasted rats, both trained and sedentary, of the myocardial muscle study is listed, while in Table 3B are printed the means of the glycogen concentrations for each group of non-fasted rats. Figure 2 also shows those mean glycogen values for the non-fasted rats.

The cardiac glycogen values of non-fasted rats dropped significantly from the control, non-swim rats' levels in rats sacrificed immediately ($P<.01$ for trained and sedentary rats) and one hour after the swim ($P<.01$ for trained and sedentary rats). Two hours after exercise the levels were only slightly elevated (no significance), while four hours ($P<.01$ for trained rats; $P<.05$ for sedentary rats) and eight hours ($P<.05$ for trained and sedentary rats) after exercise the levels were significantly raised, showing the supercompensation noted earlier in this report. At

Figure 1. Cardiac glycogen in rats sacrificed following a 48 hour fast which was begun either before (NS) or at 0, 1, 2, 4, or 8 hours following one hour of swimming. The numbers above each bar stand for the number of rats in that group, while the lines at the top of each bar represent the standard error of the mean for that group.

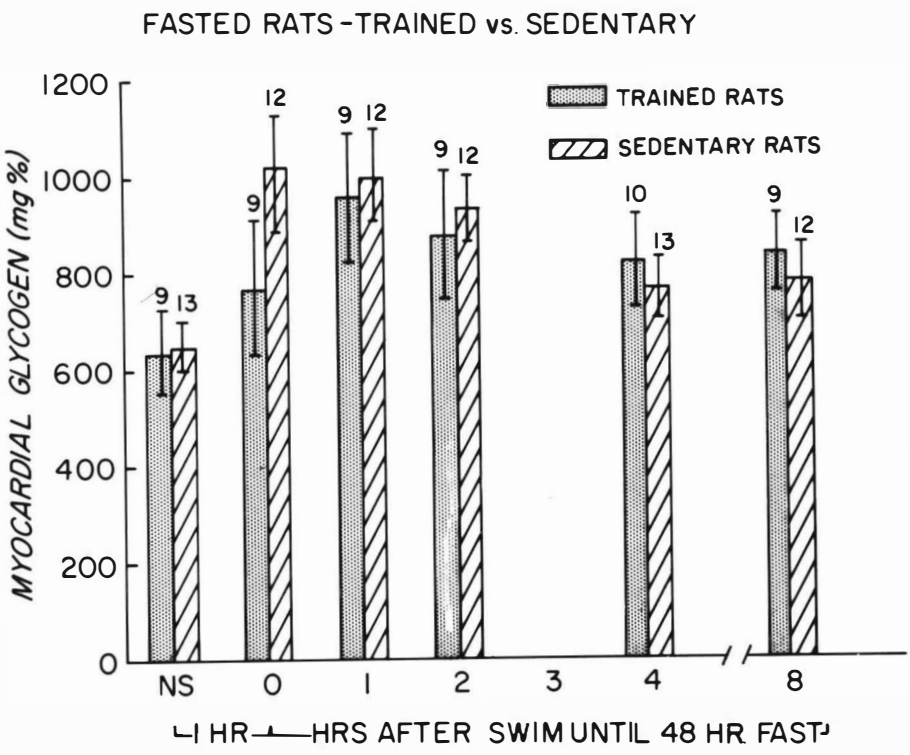


Figure 2. Cardiac glycogen in non-fasted rats sacrificed before (NS) or at 0, 1, 2, 4, or 8 hours after one hour of swimming. The numbers above each bar are the number of rats in that group, while the line at the top of each bar represents the standard error of the mean for that group.

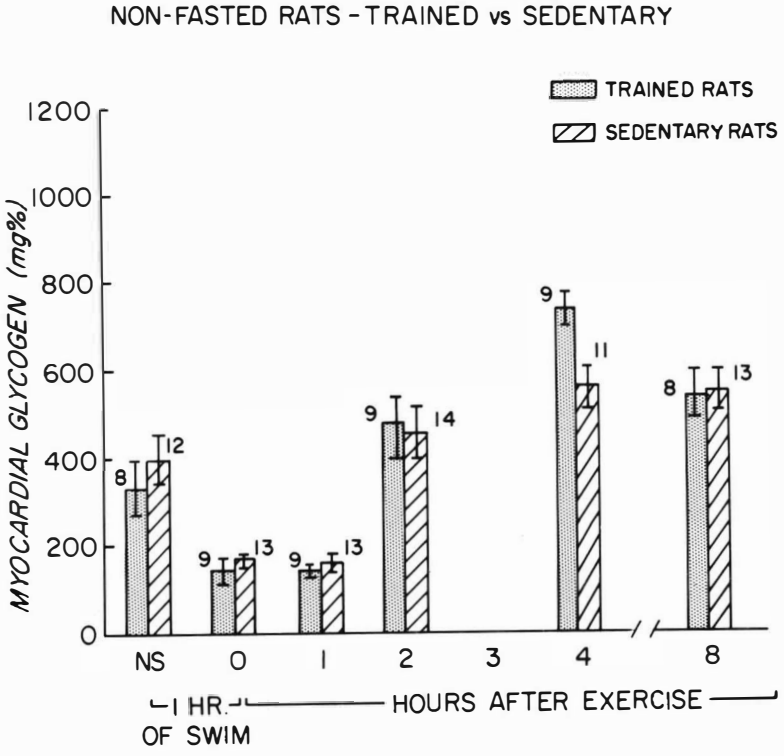


Table 3A. Cardiac glycogen and heart weight-body weight ratios for trained and sedentary rats fasted for 48 hours with the fast beginning before (NS) or at 0, 1, 2, 4, or 8 hours after one hour of swimming. Mean values ± standard errors of the means are given.

	Hours after swim					
	NS	0	1	2	4	8
Glycogen (mg%)						
Trained	646±92	775±138	966±126	885±130	831±102	846±78
Sedentary	655±52	1022±113	1005±113	938±74	777±59	789±80
Ratio HW/BW x 10^3						
Trained	2.7±.33	3.1±.09	3.1±.10	2.9±.04	3.0±.07	3.1±.06
Sedentary	2.8±.07*	2.8±.06	2.9±.07	2.9±.08	2.8±.07	2.7±.04**

Table 3B. Cardiac glycogen and heart weight-body weight ratios for trained and sedentary, non-fasted rats sacrificed before (NS) or 0, 1, 2, 4, or 8 hours after one hour of swimming. Mean values ± standard errors of the means are given.

	Hours after swim					
	NS	0	1	2	4	8
Glycogen (mg%)						
Trained	337±58	145±30	147±14	484±81	743±40	542±57
Sedentary	397±60	170±14	162±21	457±62	563±51*	552±38
Ratio HW/BW x 10^3						
Trained	2.8±.11	2.9±.10	2.7±.08	3.0±.08	2.7±.07	2.8±.06
Sedentary	2.6±.05	2.5±.09*	2.5±.05*	2.5±.04*	2.5±.06*	2.4±.20*

* $P<.05$
** $P<.01$

only four hours after swimming were the non-fasted, trained and non-fasted, sedentary rats' cardiac glycogen levels significantly different from each other ($P<.05$).

As an indication of the severity of the training program on the trained rats, a weight chart was kept and weight differences between the sedentary and trained rats were observed. The trained rats gained significantly less weight than the control, sedentary rats ($P<.01$) as is shown in figure 3, while the heart weights varied little between the two groups (Tables 1 and 2). Table 4 indicates the differences between the heart weight to body weight ratios in the fasted and non-fasted groups. This ratio, used as a sign of cardiac hypertrophy by some researchers (9, 17, 39), was shown to be significantly higher for sedentary rats 0, 1, 2, 4, and 8 hours after exercise than for trained rats ($P<.05$). No such significant difference was found in the ratios of the non-fasted rats before training. The differences may be attributed, however, not necessarily to cardiac hypertrophy, but to the smaller increases in body weight of the trained to the sedentary rats.

In the skeletal muscle study the rats chronically trained and fasted after the last bout of running had gastrocnemii which were found to contain glycogen at levels not significantly different from the control, non-trained rats (Table 5) (figure 4). The acutely exercised rats involved in only a single bout of swimming showed in non-fasted animals a significant drop in the tissue glycogen immediately ($P<.05$) and one hour after exercise ($P<.01$) with a return to normal by six hours after the swim (Table 6)(figure 5), while rats acutely exercised and begun on a fast at 0, 1, and 6 hours after the swim showed no significant difference at any point from the control values of skeletal muscle glycogen (Table 7)(figure 5). Also there were

Figure 3. Body weights of trained and sedentary rats involved in the cardiac muscle study. Standard errors of the means are indicated by the lines at the top of each bar.

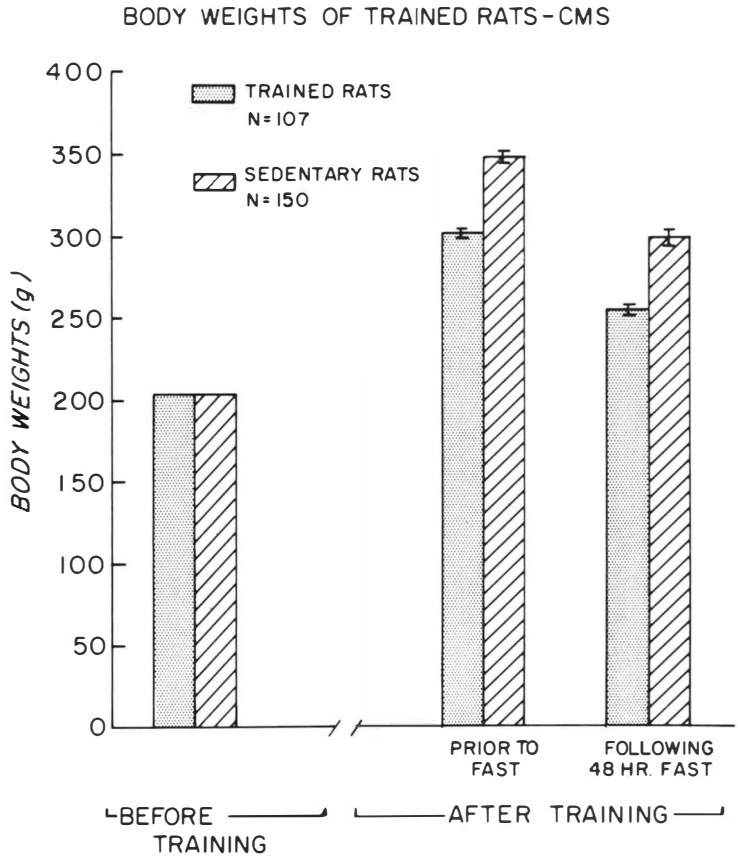


Table 4. Body weights and heart weight-body weight ratios for trained and sedentary rats of the cardiac muscle study. Mean values ± standard errors are given, as well as the number of rats in each group.

	Body Weights (g)			HW/BW
	Before Training Program	After Training Program		
		Before 48 hour fast	After 48 hour fast	Non-swim Non-fasted
Sedentary	204±2	349±4	299±4	2.6±.05
Trained	203±2	303±3 *	255±3 *	2.8±.11

* P<.01

Figure 4. Gastrocnemius glycogen levels in trained and control rats following a 48 hour fast with the trained rats fasted immediately after the last bout of exercise in the training program. The numbers above each bar are the number of rats in that group, and the standard errors of the means are represented by the lines at the top of each bar.

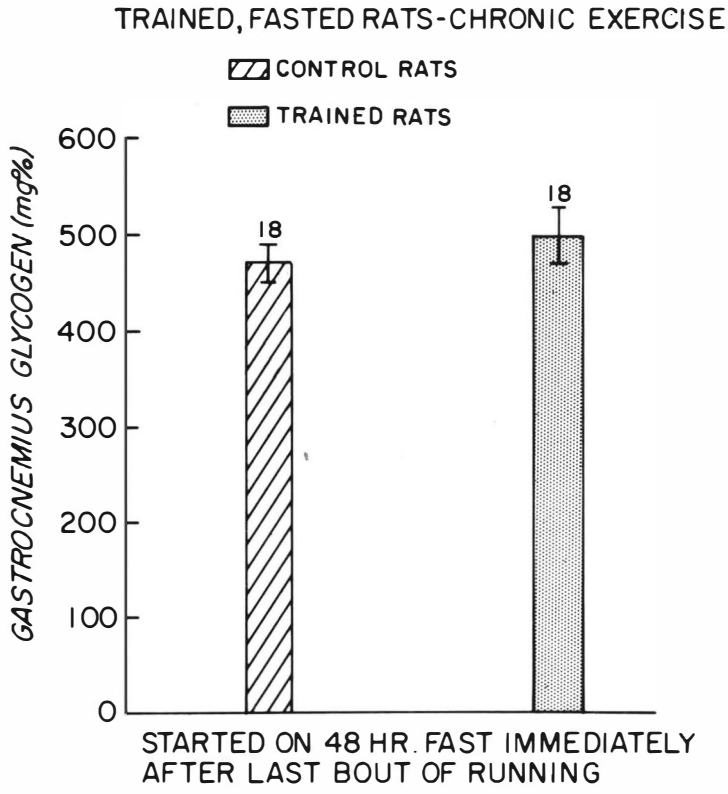
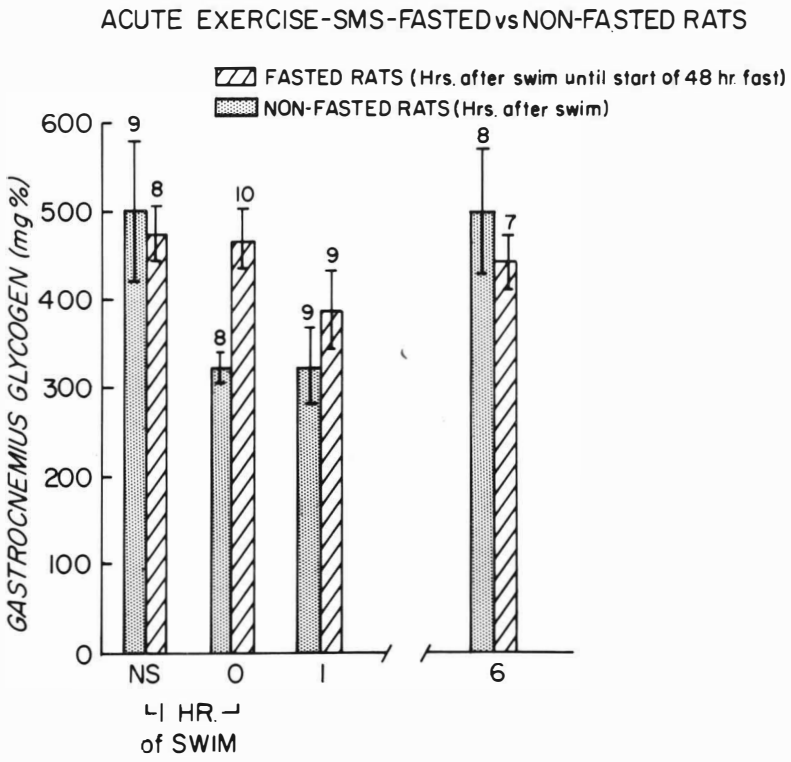


Figure 5. Gastrocnemius glycogen in untrained rats sacrificed before (NS) or at 0, 1, or 6 hours after one hour of swimming (Non-fasted rats) and in rats sacrificed following a 48 hour fast which was begun before (NS) or at 0, 1, or 6 hours after one hour of swimming (fasted rats). The number above each bar is the number of rats in that group, and the standard errors of the means are represented by the lines at the top of each bar.



no significant differences between glycogen values at any point, except the 0 hour point ($P<.01$), in a comparison between rats fasted and non-fasted after acute exercise (figure 5), showing that there was, in other words, no increase in glycogenesis during a fast in the skeletal muscle, as was observed in cardiac tissue.

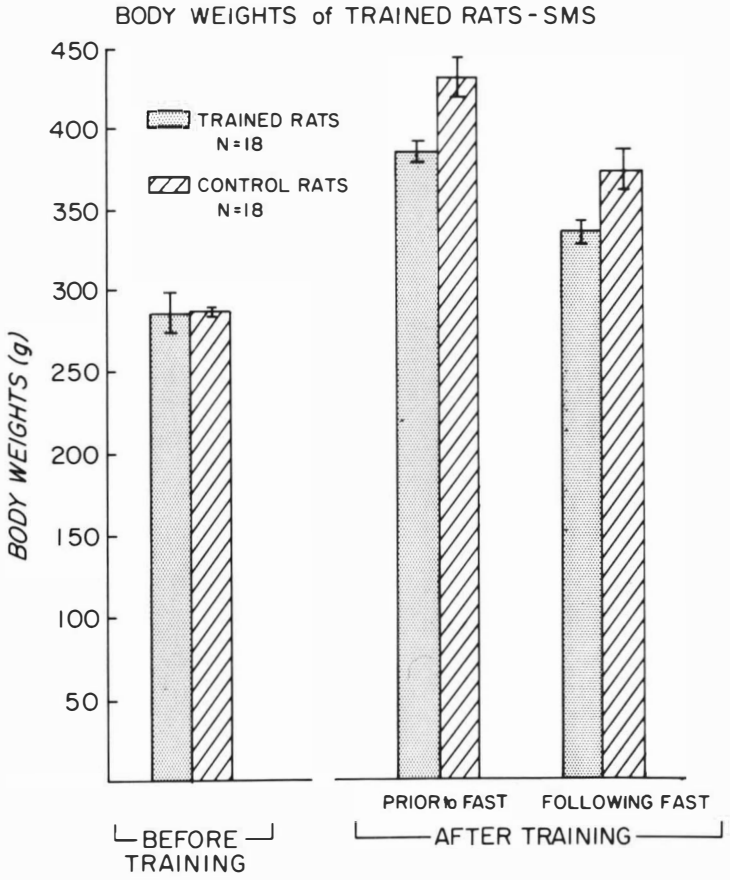
The body weights of control and trained rats were recorded and are summarized in Table 8 and figure 6, as with the other trained group of rats mentioned earlier, the trained rats gained less body weight than the sedentary rats did. There were no significant differences in the muscle weights or muscle weight to body weight ratios in these chronically trained rats whose gastrocnemii glycogen values were observed (Table 8).

Table 8. Body weights, muscle weights, and muscle weight-body weight ratios for control and trained rats before training and after training. Post-training values include those prior to and following a 48 hour fast. Mean values ± standard errors of the mean are given.

	Body Weights (g)				
	Before Training	After Training		Muscle (mg) Weight	MW/BW x 10^3
		Before fast	After fast		
Control	285±2	431±12	375±12	2337±71	6.2±1
Trained	282±12	382±7*	337±7*	2153±58	6.4±1

*P<.01

Figure 6. Body weights of control and trained rats used in the skeletal muscle study before and after the training period. Post-training values include those prior to and following a 48 hour fast.



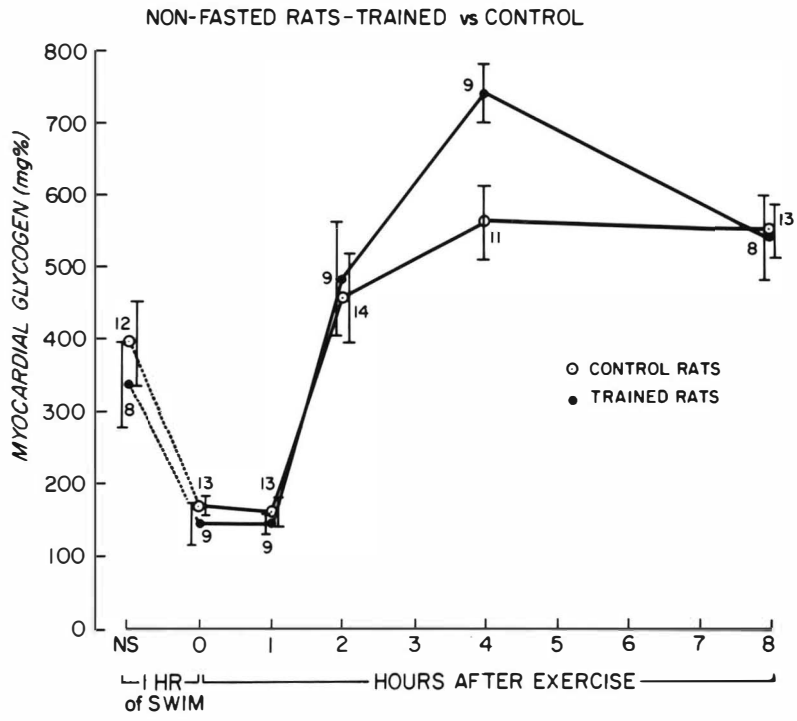
Discussion

Non-fasted rats after a single common bout of exercise showed a cardiac glycogen recovery pattern which was similar for both trained and sedentary rats (figure 7). A depletion of cardiac glycogen after one hour of swimming was seen, as expected from the results of Poland and Trauner (4) and Blount and Meyer (25), with the lowest levels approaching the values obtained by Evans (9) in his work with hypoxic rat hearts. After remaining low for at least an hour after the time of swimming, the cardiac glycogen values were observed to rise to approximately control levels at the two hour post-exercise time and to levels markedly above control at the four hour point. At eight hours post-swim the cardiac glycogen values were lower than the four hour mark but significantly higher than the control levels. Poland and Trauner (4) also saw this characteristic pattern in acutely exercised rats and have subsequently collected yet unpublished data showing the complete recovery time to be close to 24 hours post-exercise.

The only significant difference between the trained and sedentary rats' cardiac glycogen recovery patterns came at the peak four hour point ($P<.05$). The importance of that difference is not readily seen from this sort of data, although it might be that the trained rats, having been exercising and recovering for four weeks, made some special adaptation to exercise which enhanced the glycogenesis during recovery.

As far as postulating just what is happening such that the pattern described is observed, one must say that several related phenomena are probably occurring. Scheuer and Stezoski (29) state that glycogen is used preferentially to glucose during periods of hypoxia and that hypoxia

Figure 7. Myocardial glycogen in non-fasted rats of trained and control populations before (NS) and at various times after one hour of swimming. The number beside each point is the number of rats associated with that point and the vertical line represents the standard error of the mean.



might be a stimulus for phosphorylase activation with its accompanying glycogenolysis. These ideas probably account for the depletion of glycogen during heavy exercise, since the heart in an animal actively exercising is working hard and probably is working under hypoxic conditions (61).

The overshoot or supercompensation of cardiac glycogen after exercise has no explanation just yet, although once again several related factors may tie in. Wermers, et al. (50) found that the greatest degree of net glycogen synthesis occurs in muscle in the resting state, which for myocardial tissue is at resting body conditions. Lamb, et al. (6) found increased glycogen synthetase activities after exercise in both trained and sedentary animals in cardiac and skeletal muscles.

Probably the relative degrees of synthetase and phosphorylase activities are controlled by several factors after exercise which may be working either synergistically or antagonistically. Scheuer, et al. (29) postulated that activation of the enzyme adenyl cyclase by catecholamines and the resultant conversion of phosphorylase to its active form is one mechanism for the initiation of glycogenolysis in cardiac muscle. He also showed that by reducing the myocardial norepinephrine up to 80% by the drug reserpine, he could observe a raised cardiac glycogen level and in reserpinized hearts a greater glycolytic reserve than in controls, more consumption of energy, and more generation of ATP by anaerobic pathways. In short Scheuer noticed that the tissue norepinephrine level affected the cardiac glycogen level inversely.

Bloom and Russell (52), however, found in nonexercised rats an increase of cardiac glycogen at two and four hours after injection of epinephrine and norepinephrine, the two major catecholamines. Chin and

Evonuk (46) after seeing increases in both norepinephrine and total catecholamine levels along with a decrease in epinephrine after a six week training program decided that with exercise the sympathetic nervous system is putting out more tissue catecholamines, mostly norepinephrine, while the adrenals, the source of most plasma catecholamines, are releasing less. This interaction between the local sympathetic nervous system outpourings of norepinephrine and the plasma levels of catecholamines released by the adrenals must make a difference in heart tissue glycogen after exercise, yet the specific actions in every situation are not clear.

Much attention has been paid to levels of catecholamines in the tissue and plasma since they are supposed to help mobilize FFA, which are in turn metabolized by the heart during exercise. Under certain conditions the FFA are metabolized almost exclusively by the heart as energy sources, while glycogen is being stored up or replenished. Perhaps the overshoot of glycogen in non-fasted rats' myocardial muscle after exercise is just a reflection of the FFA metabolism scheme remaining turned on.

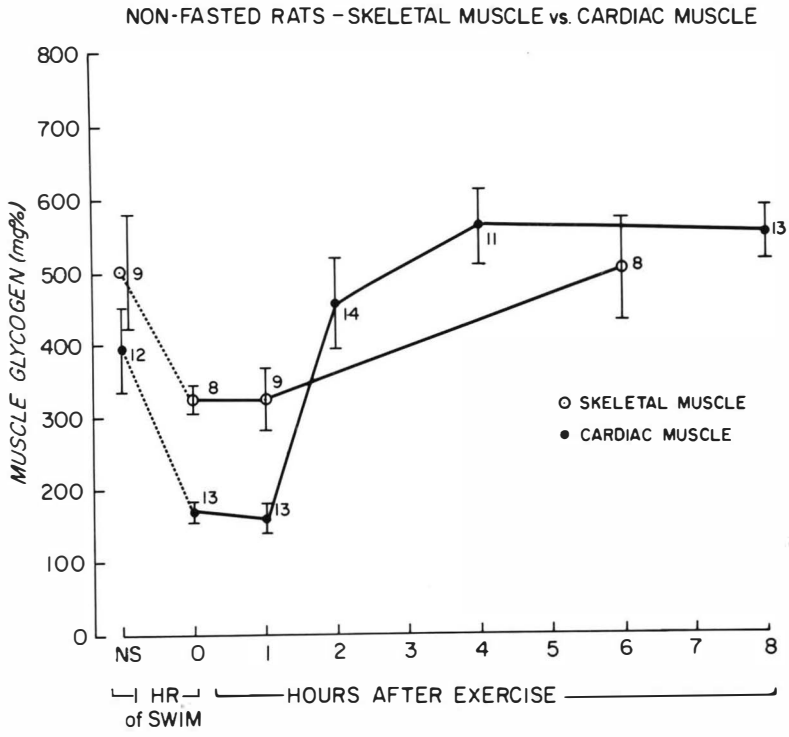
Gollnick (44) demonstrated that FFA mobilization in untrained rats during exercise is controlled almost exclusively by circulating catecholamines, while in trained rats, other mechanisms, such as increased sympathetic nervous system activity, appear to control this function. He showed later (9) that lipid mobilization may be controlled during exercise by two systems; one an adrenergic system, the sympathetic nervous system, and the other a non-adrenergic system, the one involving non-adrenergic hormones with adipokinetic or FFA mobilizing activity. Those non-adrenergic hormones are ACTH, GH, TSH, adrenal glucocorticoids, glucagon, and several other pituitary polypeptides. Gollnick, et al. (9) discovered that dual system when they found that FFA mobilization and increased catecholamine circulation

occurred during exercise even without direct mediation of the sympathetic nervous system, which was blocked by hexamethonium, or with the β-blocking agent propanolol being administered. Russell implied a similar dual system of FFA regulation in her work with McKee (48), when she stated that the adrenals and pituitary hormones worked synergistically to help mobilize FFA and increase glycogenesis during a fast.

One more approach to the problem of postulating what is happening to the heart muscle after exercise is to view the electron micrographs of tissue taken before and after exercise. King and Gollnick (22) exercised rats acutely and chronically and then made sections of those rats' heart muscles. They observed disruption of the cardiac mitochondria and sarcoplasmic reticulum in rats sacrificed immediately after exercising, while after waiting two hours they found the tissue to be mostly repaired. Yet even after waiting 24 hours after the exhausting exercise, they still saw slight swelling of the mitochondria. From these observations can be implied that the aerobic activities of the cells were lessened by the decreased ability of the mitochondria to operate its electron transport system and that, because of the rapid repair time (less than two hours), the cell would be fully functional metabolically soon after exercise when the repair of the mitochondria was complete. It can be noted here that two hours after the completion of exercise was also the time it took for the exercised rats' cardiac glycogen levels to rise near normal again.

Skeletal muscle glycogen as measured from the gastrocnemius muscle of non-fasted albino rats showed a similar pattern of depletion after one acute bout of exercise as did the heart tissue glycogen (figure 8). The levels of glycogen observed immediately and one hour after the swim were significantly lower than control, non-swim levels ($P<.05$)($P<.01$), while the six

Figure 8. Glycogen levels in cardiac and skeletal muscles of untrained rats before (NS) and at various times after one hour of swimming. The number beside each point is the number of rats associated with that point, and the vertical line represents the standard error of the mean.



hour post-swim value was back to normal. The depletion of glycogen observed immediately after exercise with the skeletal muscle, like that seen in heart tissue, is probably explained best by the hypoxia theory, which states that hypoxia is a stimulus to glycogenolysis.

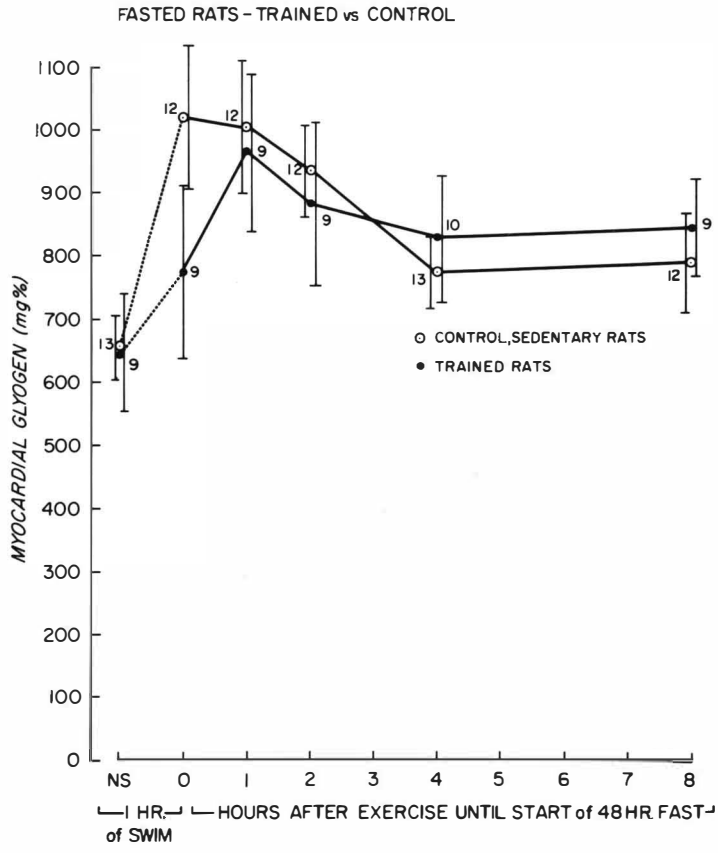
Local tissue hypoxia sites occur in hard working skeletal muscles and trigger the use of glycogen as an energy substrate. The gradual recovery of glycogen from low to normal values is at present best explained by the fact that glycogen synthesis seems to occur most readily in resting muscle than in contracting muscle (50). Low ATP levels and higher AMP concentrations trigger the synthetase enzyme to activity and stem the activation of the phosphorylase system (5).

One difference has been noted thus far between the skeletal and cardiac muscles of exercised non-fasted rats and that is the supercompensation or overshoot of glycogen seen in cardiac muscle two hours or more after exercise (figure 8). The skeletal muscle glycogen observed did not rebound from its low value after exercise to a level any higher than the control, non-swim level. That difference probably would be explained by whatever explains the overshoot in cardiac glycogen, and that has not yet been worked out.

Fasting

No appreciable differences were noted at any point between the trained and sedentary rats fasted for 48 hours with the fast beginning before or at various times after a single common bout of exercise (figure 9). The glycogenesis noticed during a fast is enhanced when the fast is begun shortly after exercise but the glycogenesis itself may be related more to the fast than the exercise, since non-swim rats display a similar pattern of glycogenesis.

Figure 9. Myocardial glycogen in fasted rats of trained and control populations with the fast initiated before (NS) or at various times after one hour of swimming. The number beside each point is the number of rats associated with that point, and the vertical line represents the standard error of the mean.



Because of the greater accessibility of cardiac glycogen after a fast, researchers have for years fasted their animals either before or after exercise in studies done to measure the effects of exercise (9, 14, 16, 17, 27). Yet the levels of glycogen in rat hearts after both fast and exercise are different from those levels in non-fasted exercised rats (4). The effect of the fast in exercised animals, no matter whether the exercise is acute (with non-trained rats) or chronic (with trained rats), is the same: enhanced cardiac glycogenesis seen in rats started on a fast immediately after an exercise (figure 9). The glycogenesis was noted in this study for all fasted rats, since both groups of non-swim fasted rats (trained and sedentary) had cardiac glycogen levels significantly higher than the non-fasted control rats' values ($P<.05$) (figure 10). The glycogenesis in fasting rats after an exercise is considered enhanced, since the increase in glycogen seen when the non-trained rats were started on a 48 hour fast one hour after exercise is significantly greater than the increase noted for non-swim rats either trained or non-trained, which were fasted also for 48 hours ($P<.01$) (figure 10).

In rats acutely and chronically exercised, skeletal muscle glycogen levels were also observed after a fast (figure 11). The gastrocnemii of rats trained for one month by running on a treadmill showed no appreciable change in muscle glycogen with a fast after the last session of running. Neither was there any appreciable change noted when a fast was begun immediately, one, or six hours after a single acute bout of swimming in non-trained or sedentary rats. By remaining close to the normal levels of the non-fasted control rats yet still agreeing with results gained by Russell and Bloom (33), the skeletal muscle glycogen levels showed none

Figure 10. Myocardial glycogen in trained and sedentary rats. The lower two lines are for non-fasted rats sacrificed before (NS) or at various times after one hour of swimming. The top two lines are for rats fasted for 48 hours with the fast beginning before (NS) or at various times after one hour of swimming. The number beside each point is the number of rats associated with that point, and the vertical line represents the standard error of the mean.

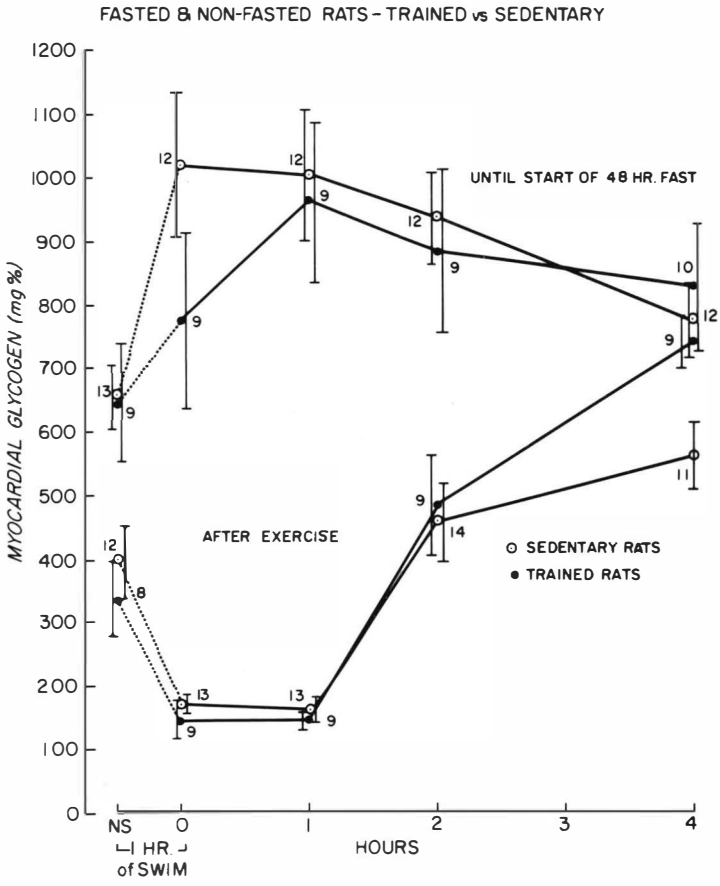
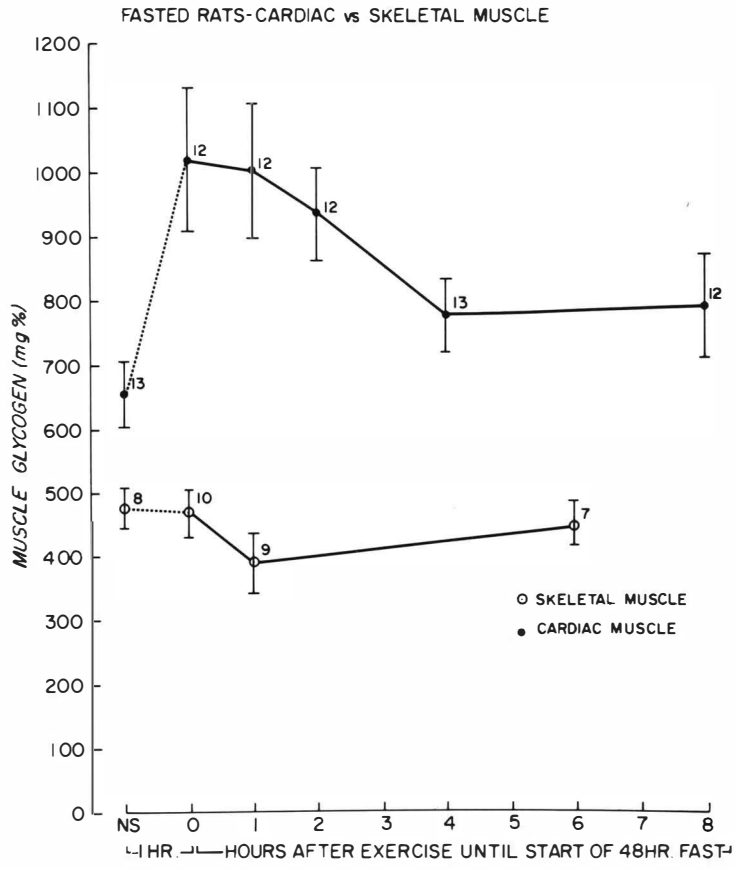


Figure 11. Glycogen in cardiac and skeletal muscles of rats fasted for 48 hours with the fast beginning before (NS) or at various times after one hour of swimming. The number of rats associated with each point is given, and the standard error of the mean is represented by the vertical lines.



of the enhanced glycogenesis that the cardiac muscle had. The great observable difference then between the cardiac and skeletal muscle glycogen values during a fast is simply the increased glycogenesis in cardiac muscle (figure 11).

During a fast several phenomena must be occurring to allow the storage of glycogen in cardiac tissue in both trained and non-trained animals. FFA appear to be the main sources of energy for the fasting heart (58), although several authors have stated a case for the expanded use of ketone bodies during the starvation time (59). Russell and Adrouny (20) demonstrated that FFA were integrally involved in the sparing of cardiac glycogen during a fast by monitoring the glycogen levels in the hearts of animals coming off a fast and being fed either carbohydrate, fat, or protein diets. The rats fed fatty foods continued to have hearts loaded with glycogen, while the rats eating proteins or carbohydrates had hearts which dropped in glycogen content shortly after the meal.

After having carried out many research projects using fasted rats, Russell (48) postulated that growth hormone is probably working with some other hormones, both adrenal and pituitary in origin, to regular FFA mobilization and glycogenesis. This idea was corroborated by Schimmel and Knobil (49) who observed with FFA infusion in liver slices from fasted rats no glycogenesis, yet who saw later with liver slices from fasted rats a large increase in tissue glycogen. Obviously some factors are at work not only mobilizing the FFA but also utilizing it to spare glycogen breakdown in fasting animals.

Adrouny sums up some of these factors in a more specific way in the paper described earlier in this report (8). His conclusions about the basic differences in cardiac and skeletal muscle during a fast relate the

differential changes in metabolites, especially AMP and citrate, which in turn affect the activities of phosphorylase, phosphofructokinase, and transglucosylase, the latter via variations in glucose-6-phosphate levels; as well as describe the differences in pattern of I and D interconversions in cardiac versus skeletal muscle transglucosylase, the branching enzyme. Those differences discussed still lead to the idea of carbohydrate, or glycogen, sparing in the heart and the lack of any such mechanism in skeletal muscle.

Summary

The results of this project indicate that cardiac glycogen values do not vary significantly in most instances between trained and sedentary rats before or after they all have engaged in a single common bout of exercise. There was one significant difference noticed, however, between the cardiac glycogen values for non-fasted rats--both trained and sedentary--which were swum for one hour and that was found at the four hour post-swim point ($P<.05$). There were no differences noticed between the values for trained and sedentary rats fasted before or after the single swimming session. This lack of difference between the cardiac glycogen values for rats trained and for rats not trained before the one swimming session implies that the changes observed in cardiac glycogen after exercise are due more to the last bout of exercise than to any training program.

In seeking similarities and differences between the cardiac and skeletal muscle glycogen loads, gastrocnemius glycogen values were found for acutely exercised rats, fasted and non-fasted, as well as for chronically trained rats fasted immediately after their last running session. The fasted rats' glycogen values were not significantly different at any point from either of the control levels of rats--fasted or non-fasted, indicating no enhancement of glycogenesis during a fast as is characteristic of cardiac glycogen levels. The non-fasted rats' skeletal muscle glycogen, however, was depleted significantly during acute exercise in the same manner as cardiac glycogen and rose back only to control values after six hours post-swim. The lack of an overcompensation of skeletal muscle glycogen as seen in cardiac muscle after exercise marked the second observed difference in cardiac and skeletal muscle glycogen levels.

Table 1. Data for cardiac muscle study involving trained and sedentary rats fasted 48 hours without swimming or fasted at 0, 1, 2, 4, or 8 hours after one hour of swimming.

Rats fasted without swimming

	Rat No.	Date Sacrificed	Body Weights[1] (grams)			HW[2] (mg)	HW/BW[3] x 10^3	Glycogen (mg%)
Trained	24	12-70	199	273	236	649	2.8	28
	13	12-70	193	290	240	750	3.1	436
	24	2-71	181	314	271	912	3.4	787
	17	2-71	187	300	263	850	3.2	817
	39	3-71	198	300	258	650	2.5	748
	17	4-71	218	295	252	852	3.4	581
	43	4-71	232	345	291	897	3.1	536
	23	5-71	226	363	311	874	2.8	863
	48	5-71	215	296	296	824	3.3	1018
	$\bar{X}$		203	324	269	859	2.7	646
	SE		6	10	8	94	.33	92
	N=9							
Sedentary	51	12-70	180	329	272	843	3.1	646
	7	12-70	189	376	317	834	2.6	140
	48	2-71	237	394	333	895	2.7	557
	8	2-71	186	312	259	713	2.8	670
	76	3-71	186	282	240	720	3.0	690
	56	3-71	208	341	300	855	2.8	709
	14	3-71	191	328	286	747	2.6	708
	27	4-71	192	336	282	961	3.4	819
	55	4-71	179	265	227	664	2.9	513
	73	4-71	233	401	348	802	2.3	589
	54	5-71	214	439	376	978	2.6	854
	69	5-71	261	457	397	1019	2.6	929
	68	5-71	236	350	297	794	2.7	696
	$\bar{X}$		209	344	303	833	2.8	655
	SE		7	17	13	29	.07	52
	N=13							

[1]Body weights--before training, after training, after fast.
[2]Heart weight.
[3]Heart weight to body weight ratio.

Table 1 (continued)

Rats fasted for 48 hours beginning 0 hours after one hour of swimming

	Rat No.	Date Sacrificed	Body Weights (grams)			HW (mg)	HW/BW x 10^3	Glycogen (mg%)
Trained	32	12-70	162	219	160	527	3.3	80
	1	2-71	193	317	266	799	3.0	1230
	44	3-71	186	269	233	893	3.8	410
	48	3-71	201	296	257	740	2.9	945
	64	3-71	190	259	241	696	2.9	1257
	24	4-71	216	324	265	854	3.2	841
	34	4-71	235	241	182	576	3.2	227
	9	5-71	252	342	289	816	2.8	1169
	11	5-71	234	308	256	786	3.1	816
	$\bar{X}$		208	286	239	743	3.1	775
	SE		9	13	13	39	.09	138
	N=9							
Sedentary	29	12-70	190	309	252	733	2.9	459
	48	12-70	209	364	307	840	2.7	700
	40	2-71	210	368	235	729	2.4	1290
	9	3-71	158	276	217	637	2.9	479
	23	3-71	193	217	274	855	3.1	1072
	29	3-71	182	329	277	723	2.6	989
	33	3-71	156	271	230	695	3.0	776
	12	4-71	226	324	271	830	3.1	656
	35	4-71	251	395	344	916	2.7	1705
	39	4-71	225	358	313	917	2.9	1330
	12	5-71	219	415	351	910	2.6	1439
	67	5-71	249	384	317	875	2.8	1367
	$\bar{X}$		206	334	282	805	2.8	1022
	SE		9	16	12	27	.06	113
	N=12							

Table 1 (continued)

Rats fasted for 48 hours beginning one hour after one hour of swimming

	Rat No.	Date Sacrificed	Body Weights (grams)			HW (mg)	HW/BW x 10^3	Glycogen (mg%)
Trained	17	12-70	191	309	247	827	3.3	452
	2	12-70	204	306	249	885	3.6	929
	10	2-71	172	293	256	716	2.8	911
	19	3-71	164	217	175	575	3.3	1779
	69	3-71	187	300	258	679	2.6	789
	16	4-71	231	262	211	732	3.5	567
	2	5-71	234	326	271	807	3.0	822
	31	5-71	205	339	280	843	3.0	1158
	36	5-71	216	300	248	736	3.0	1289
	$\bar{X}$		200	295	244	756	3.1	966
	SE		8	11	10	30	.10	126
	N=9							
Sedentary	45	12-70	194	354	298	090	3.1	1152
	14	12-70	183	329	294	782	2.7	443
	35	2-71	165	310	267	787	3.0	1220
	26	2-71	154	314	276	707	2.6	1335
	5	3-71	169	311	261	841	3.2	811
	18	3-71	185	301	252	795	3.2	848
	47	3-71	197	298	260	796	3.1	971
	61	3-71	211	396	350	1012	2.9	199
	29	1-71	219	384	332	936	2.8	1501
	56	4-71	222	384	309	958	3.1	1291
	17	5-71	242	392	347	832	2.4	1153
	58	5-71	231	441	375	1006	2.7	1132
	$\bar{X}$		198	351	301	888	2.9	1005
	SE		8	13	11	54	.07	104
	N=12							

Table 1 (continued)

Rats fasted for 48 hours beginning two hours after one hour of swimming

	Rat No.	Date Sacrificed	Body Weights (grams)			HW (mg)	HW/BW x 10^3	Glycogen (mg%)
Trained	30	12-70	209	341	276	781	2.8	1261
	22	12-70	169	315	244	714	2.9	296
	25	2-71	192	285	238	667	2.8	665
	34	3-71	199	302	266	777	2.9	1033
	58	3-71	160	288	248	752	3.0	1078
	75	3-71	159	262	222	699	3.1	762
	54	4-71	238	289	228	645	2.8	258
	18	5-71	229	337	283	838	3.0	1322
	37	5-71	183	339	276	878	3.2	1289
	$\bar{X}$		193	306	253	750	2.9	885
	SE		9	9	7	24	.04	130
	N=9							
Sedentary	54	12-70	205	367	306	1042	3.4	729
	44	12-70	160	308	260	794	3.1	333
	31	2-71	177	277	230	640	2.8	1122
	39	2-71	179	330	284	833	2.9	1189
	16	3-71	182	333	287	787	2.7	1393
	53	3-71	193	369	319	928	2.9	786
	37	4-71	230	409	342	983	2.9	936
	72	4-71	228	418	353	987	2.8	1040
	71	4-71	223	362	302	912	3.0	1070
	38	5-71	231	419	357	960	2.7	987
	61	5-71	232	380	323	775	2.4	865
	72	5-71	226	273	232	780	3.4	806
	$\bar{X}$		206	354	300	868	2.9	938
	SE		7	14	12	33	.08	74
	N=12							

Table 1 (continued)

Rats fasted for 48 hours beginning four hours after one hour of swimming

	Rat No.	Date Sacrificed	Body Weights (grams)			HW (mg)	HW/BW x 10^3	Glycogen (mg%)
Trained	42	12-70	202	308	244	834	3.4	138
	5	2-71	179	289	247	690	2.8	1191
	24	3-71	192	301	260	769	3.0	1178
	36	3-71	174	266	225	656	2.9	1026
	77	3-71	184	299	254	672	2.6	718
	9	4-71	214	292	235	800	3.4	323
	15	4-71	241	347	297	893	3.0	763
	43	5-71	225	305	258	784	3.0	885
	65	5-71	264	343	284	863	3.0	962
	6	5-71	256	339	278	802	2.9	1076
	$\bar{X}$		213	309	258	776	3.0	831
	SE		10	8	7	24	.07	102
	N=10							
Sedentary	28	12-70	192	269	220	758	3.4	295
	16	2-71	190	367	316	968	3.1	752
	33	2-71	215	383	338	935	2.8	883
	12	3-71	189	335	297	805	2.7	796
	40	3-71	166	321	275	814	3.0	826
	49	3-71	197	324	272	734	2.7	1067
	73	3-71	166	275	235	659	2.8	946
	47	4-71	234	370	311	904	2.9	714
	74	4-71	240	433	373	923	2.5	572
	65	4-71	239	413	353	871	2.5	439
	14	5-71	263	430	373	927	2.5	936
	33	5-71	213	355	301	813	2.7	953
	15	5-71	238	364	308	850	2.8	918
	$\bar{X}$		210	357	373	843	2.8	777
	SE		8	14	58	24	.17	59
	N=13							

Table 1 (continued)

Rats fasted for 48 hours beginning eight hours after one hour of swimming

	Rat No.	Date Sacrificed	Body Weights (grams)			HW (mg)	HW/BW x 10^3	Glycogen (mg%)
Trained	39	12-70	174	312	255	910	2.9	309
	41	2-71	174	277	235	810	3.5	941
	9	2-71	185	287	235	676	2.9	919
	21	3-71	191	277	247	749	3.0	995
	32	3-71	168	269	227	696	3.1	871
	10	4-71	239	359	305	990	3.2	1069
	36	4-71	230	340	285	852	3.0	571
	41	5-71	226	336	288	829	2.9	1032
	51	5-71	217	299	252	744	3.0	907
	$\bar{X}$		200	306	259	806	3.1	846
	SE		9	10	9	32	.06	78
	N=9							
Sedentary	52	12-70	181	332	279	778	2.8	293
	43	2-71	171	355	301	799	2.7	934
	50	2-71	194	322	272	720	2.6	970
	54	3-71	145	270	231	692	3.0	350
	38	3-71	190	360	314	857	2.7	759
	1	3-71	191	357	312	853	2.7	789
	51	4-71	234	381	327	865	2.6	575
	69	4-71	240	381	328	850	2.6	627
	1	5-71	229	380	326	834	2.6	964
	19	5-71	200	323	272	688	2.5	1140
	56	5-71	210	387	329	927	2.8	1242
	71	5-71	244	356	305	817	2.7	827
	$\bar{X}$		202	350	300	807	2.7	789
	SE		8	9	8	21	.04	80
	N=12							

Table 2. Data for cardiac muscle study involving non-fasted trained and sedentary rats sacrificed before (non-swim rats) or 0, 1, 2, 4, or 8 hours after one hour of swimming.

Non-swim rats

	Rat No.	Date Sacrificed	Body Weights[1] (grams)		HW[2] (mg)	HW/BW[3] x 10^3	Glycogen (mg%)
Trained	27	12-70	180	233	787	3.4	318
	31	12-70	186	286	793	2.8	200
	37	2-71	179	275	828	3.0	443
	78	3-71	195	293	827	2.8	218
	38	4-71	213	308	740	2.4	54
	5	4-71	232	365	888	2.4	416
	16	5-71	227	349	904	2.6	446
	46	5-71	232	343	888	2.6	603
	$\bar{X}$		206	307	832	2.8	337
	SE		8	15	19	.11	58
	N=8						
Sedentary	6	12-70	199	348	853	2.5	266
	10	12-70	196	320	843	2.6	75
	6	2-71	166	328	808	2.5	368
	2	2-71	160	276	704	2.8	219
	7	3-71	171	292	742	2.5	366
	13	3-71	157	279	708	2.5	500
	26	4-71	248	389	936	2.4	412
	67	4-71	223	344	867	2.5	352
	42	4-71	207	258	809	3.1	384
	20	5-71	204	373	982	2.6	418
	27	5-71	236	365	945	2.6	992
	66	5-71	240	411	989	2.4	413
	$\bar{X}$		201	332	849	2.6	397
	SE		9	13	28	.05	60
	N=12						

[1]Body weights--before and after training.
[2]Heart weight.
[3]Heart weight to body weight ratio.

Table 2 (continued)

Rats sacrificed 0 hours after one hour of swimming

	Rat No.	Date Sacrificed	Body Weights (grams)		HW (mg)	HW/BW x 10^3	Glycogen (mg%)
Trained	20	12-70	153	257	815	3.2	259
	46	12-70	224	314	872	2.8	188
	29	2-71	194	280	994	3.6	76
	41	3-71	182	316	844	2.7	23
	74	3-71	185	257	736	2.9	246
	19	4-71	231	349	913	2.6	25
	50	4-71	226	284	810	2.9	70
	25	5-71	191	297	815	2.7	194
	53	5-71	216	318	827	2.6	223
	$\bar{X}$		200	297	847	2.9	145
	SE		8	10	23	.10	30
	N=9						
Sedentary	25	12-70	183	319	933	2.9	112
	43	12-70	191	370	821	2.2	187
	45	2-71	187	354	745	2.1	150
	14	2-71	211	435	1171	2.7	170
	72	3-71	179	224	586	2.6	186
	50	3-71	206	358	964	2.7	162
	15	3-71	184	338	697	2.1	181
	23	4-71	237	377	1214	3.2	43
	52	4-71	206	333	871	2.6	202
	62	4-71	185	361	889	2.5	281
	63	5-71	240	341	796	2.3	181
	22	5-71	235	430	997	2.3	174
	64	5-71	232	363	859	2.4	181
	$\bar{X}$		206	354	888	2.5	170
	SE		6	14	47	.09	14
	N=13						

Table 2 (continued)

Rats sacrificed one hour after one hour of swimming

	Rat No.	Date Sacrificed	Body Weights (grams)		HW (mg)	HW/BW x 10^3	Glycogen (mg%)
Trained	3	12-70	214	286	947	3.3	78
	38	2-71	194	320	836	2.6	194
	22	3-71	177	265	756	2.9	166
	70	3-71	189	305	895	2.9	181
	71	3-71	172	245	781	2.8	160
	41	4-71	239	292	816	2.8	98
	18	4-71	234	312	819	2.6	189
	32	5-71	203	326	928	2.8	155
	45	5-71	208	296	721	2.4	98
	$\bar{X}$		203	294	822	2.7	147
	SE		7	8	29	.08	14
	N=9						
Sedentary	15	12-70	209	363	911	2.5	107
	41	12-70	184	334	778	2.3	56
	19	2-71	180	356	920	2.6	81
	46	2-71	180	332	961	2.9	150
	27	3-71	176	323	784	2.4	236
	42	3-71	206	327	829	2.5	239
	59	3-71	163	215	525	2.4	132
	32	4-71	240	394	1016	2.6	222
	44	4-71	236	416	1157	2.8	47
	57	4-71	240	424	1103	2.6	158
	8	5-71	231	368	896	2.4	219
	24	5-71	194	377	861	2.3	296
	30	5-71	224	371	908	2.4	166
	$\bar{X}$		205	354	896	2.5	162
	SE		7	14	42	.05	21
	N=13						

Table 2 (continued)

Rats sacrificed two hours after one hour of swimming

	Rat No.	Date Sacrificed	Body Weights (grams)		HW (mg)	HW/BW x 10^3	Glycogen (mg%)
Trained	50	12-70	161	291	865	3.0	227
	40	12-70	171	268	756	2.8	135
	42	2-71	178	250	887	3.5	366
	63	3-71	203	331	956	2.9	578
	68	3-71	180	281	794	2.8	890
	46	4-71	246	317	955	3.0	400
	28	4-71	247	335	1121	3.3	514
	40	5-71	227	276	792	2.9	537
	50	5-71	217	294	816	2.8	814
	$\bar{X}$		203	294	882	3.0	484
	SE		10	9	36	.08	81
	N=9						
Sedentary	18	12-70	199	404	994	2.5	207
	23	12-70	203	373	957	2.6	56
	49	2-71	187	369	922	2.5	461
	4	2-71	194	367	951	2.6	438
	17	3-71	172	324	895	2.7	731
	28	3-71	197	332	844	2.5	649
	37	3-71	196	297	801	2.7	750
	43	3-71	168	310	756	2.4	379
	31	4-71	229	432	1021	2.4	396
	70	4-71	218	329	847	2.6	263
	60	4-71	193	356	984	2.8	88
	5	5-71	249	386	905	2.3	749
	70	5-71	229	400	986	2.5	663
	21	5-71	229	420	936	2.2	565
	$\bar{X}$		205	364	914	2.5	457
	SE		6	11	20	.04	62
	N=14						

Table 2 (continued)

Rats sacrificed four hours after one hour of swimming

	Rat No.	Date Sacrificed	Body Weights (grams)		HW (mg)	HW/BW x 10^3	Glycogen (mg%)
Trained	11	2-71	197	283	849	3.0	638
	46	3-71	176	277	722	2.6	859
	65	3-71	189	271	716	2.6	692
	11	4-71	233	312	857	2.7	773
	30	4-71	245	338	1097	3.2	627
	8	4-71	217	316	856	2.7	646
	75	4-71	208	285	725	2.5	694
	4	5-71	230	353	956	2.7	730
	49	5-71	227	335	900	2.7	1025
	$\bar{X}$		214	308	853	2.7	743
	SE		7	9	39	.07	40
	N=9						
Sedentary	53	12-70	197	328	863	2.6	632
	18	2-71	197	331	815	2.5	680
	36	2-71	178	357	1037	2.9	424
	8	3-71	205	323	762	2.4	283
	10	3-71	194	314	870	2.8	296
	11	3-71	177	342	924	2.7	414
	49	4-71	245	345	819	2.4	783
	63	4-71	253	409	951	2.3	594
	77	4-71	218	367	999	2.7	706
	26	5-71	209	362	784	2.2	677
	62	5-71	240	394	991	2.5	709
	$\bar{X}$		210	352	892	2.5	563
	SE		7	9	27	.06	51
	N=11						

Table 2 (continued)

Rats sacrificed eight hours after one hour of swimming

	Rat No.	Date Sacrificed	Body Weights (grams)		HW (mg)	HW/BW x 10^3	Glycogen (mg%)
Trained	1	12-70	178	284	754	2.7	542
	30	2-71	166	281	758	2.7	750
	22	2-71	152	293	802	2.7	585
	60	3-71	197	269	754	2.8	674
	57	3-71	191	321	940	2.9	173
	22	4-71	215	311	820	2.6	566
	21	4-71	234	328	1018	3.1	458
	39	5-71	206	365	935	2.6	587
	$\bar{X}$		192	307	848	2.8	542
	SE		9	10	34	.06	57
	N=8						
Sedentary	49	12-70	192	327	810	2.5	511
	20	2-71	180	254	758	3.0	691
	51	2-71	126	284	750	2.6	678
	2	3-71	164	264	700	2.7	541
	25	3-71	179	282	759	2.7	195
	30	3-71	163	301	753	2.5	489
	31	3-71	193	344	985	2.9	593
	78	4-71	238	368	979	2.7	423
	61	4-71	167	301	722	2.4	498
	20	4-71	235	452	1085	2.4	530
	28	5-71	210	360	841	2.3	613
	10	5-71	237	398	890	2.2	682
	29	5-71	229	433	1005	2.3	735
	$\bar{X}$		193	336	849	2.4	552
	SE		9	17	34	.20	38
	N=13						

Table 5. Data for the skeletal muscle study involving trained and control rats following a 48 hour fast.

	Rat No.	Date Sacrificed	Body Weights[1] (grams)			HW[2] (mg)	HW/BW[3] x 10^3	Glycogen (mg%)
Control	2	7-70	340	517	457	2888	6.4	349
	4	7-70	258	335	298	2021	6.8	443
	6	7-70	306	433	390	2293	5.9	474
	8	7-70	304	452	400	2776	7.0	395
	12	7-70	315	450	407	2068	5.1	422
	14	7-70	307	442	393	2397	6.1	545
	16	7-70	318	462	411	2501	6.1	481
	18	7-70	354	532	459	2731	6.0	526
	20	7-70	240	343	287	1835	6.4	465
	23	7-70	309	458	400	2395	6.0	375
	62	7-70	255	429	374	2187	5.8	549
	64	7-70	253	382	329	2070	6.3	518
	66	7-70	241	366	353	2456	7.0	357
	68	7-70	257	419	378	2321	6.2	600
	70	7-70	263	436	378	2418	6.4	342
	74	7-70	269	420	374	2095	5.6	625
	76	7-70	269	481	417	2596	6.3	588
	78	7-70	237	398	345	2056	6.0	451
	$\bar{X}$		285	431	375	2337	6.2	472
	SE		2	12	12	71	.10	21
	N=18							
Trained	3	7-70	292	350	313	2075	6.7	565
	7	7-70	315	382	340	2139	6.3	588
	9	7-70	338	430	380	2482	6.6	184
	13	7-70	308	370	329	2229	6.8	386
	15	7-70	341	395	353	2275	6.5	552
	17	7-70	340	440	396	2459	6.2	540
	19	7-70	330	396	353	2207	6.3	537
	22	7-70	332	356	314	1902	6.1	704
	61	7-70	265	343	296	1908	6.5	633
	63	7-70	140	317	271	1677	6.2	449
	65	7-70	260	378	319	2093	6.6	445
	67	7-70	266	398	356	2408	6.8	328
	69	7-70	268	361	316	2169	6.9	644
	71	7-70	274	428	379	2395	6.4	464
	73	7-70	268	406	362	2368	6.6	437
	75	7-70	246	358	307	2043	6.7	423
	77	7-70	223	402	354	2325	6.6	499
	79	7-70	269	368	325	1598	5.0	667
	$\bar{X}$		282	382	337	2153	6.4	502
	SE		12	7	7	58	.10	30
	N=18							

[1]Body weights--before training, after training, after fasting.
[2]Muscle weight.
[3]Muscle weight to body weight ratio.

Table 6. Skeletal muscle data of non-fasted rats sacrificed before (control) or 0, 1, or 6 hours after one hour of swimming.

	Rat No.	Date Sacrificed	BW (g)	MW (mg)	MW/BW $\times 10^3$	Glycogen (mg%)
Control	61	7-14	267	1366	5.2	625
	73	7-14	203	1047	5.2	999
	42	7-9	252	1505	6.0	525
	38	7-9	250	1346	5.4	539
	78	7-20	339	1862	5.5	446
	79	7-20	326	1785	5.5	72
	87	7-20	363	1897	5.3	412
	91	7-22	327	1704	5.3	347
	92	7-22	320	1533	4.8	554
	$\bar{X}$		294	1561	5.4	502
	SE		17	88	.10	78
	N=9					
0 hours	67	7-14	294	1501	5.1	421
	40	7-9	275	1836	6.7	296
	35	7-9	263	1510	5.8	346
	77	7-20	329	1770	5.4	317
	83	7-20	315	1640	5.2	223
	84	7-20	338	1830	5.5	327
	88	7-22	377	1849	4.9	322
	89	7-22	367	1641	4.5	348
	$\bar{X}$		320	1697	5.4	325 *
	SE		14	48	.20	19
	N=8					

*P<.05 when compared with controls.

Table 6 (continued)

	Rat No.	Date Sacrificed	BW (g)	MW (mg)	MW/BW^3 x 10	Glycogen (mg%)
1 hour	54	7-14	254	1356	5.4	426
	70	7-14	275	1538	5.6	434
	36	7-9	268	1287	4.8	377
	41	7-9	243	1225	5.1	450
	74	7-20	324	1731	5.4	374
	75	7-20	315	1812	5.8	165
	85	7-20	335	1858	5.6	66
	86	7-20	329	1830	5.6	358
	90	7-20	338	1910	5.7	286
	$\bar{X}$		298	1616	5.4	326 *
	SE		12	84	.10	42
	N=9					
6 hours	51	7-14	278	1560	5.7	550
	64	7-14	272	1468	5.4	698
	66	7-14	269	1466	5.5	660
	34	7-9	240	1444	6.1	587
	39	7-9	251	1363	5.5	667
	71	7-20	310	1821	5.9	127
	72	7-20	323	1782	5.6	227
	76	7-20	320	1730	5.4	515
	$\bar{X}$		283	1579	5.6	504
	SE		10	58	.10	71
	N=8					

*P<.01 when compared with controls.

Table 7. Skeletal muscle data of rats sacrificed following a 48 hour fast which was begun before (controls) or 0, 1, or 6 hours after one hour of swimming.

	Rat No.	Date Sacrificed	BW (g)	MW (mg)	MW/BW x 10^3	Glycogen (mg%)
Control	82	7-20	281	1867	6.6	266
	33	7-17	199	1207	6.1	511
	26	7-17	185	1053	5.7	531
	25	7-17	217	1129	5.2	476
	63	7-14	244	1471	6.0	540
	62	7-14	268	1668	6.2	408
	50	7-14	236	1367	5.8	501
	49	7-14	249	1280	5.1	579
	$\bar{X}$		235	1380	5.8	477
	SE		11	92	.20	33
	N=8					
0 hours	47	7-14	236	1261	5.4	547
	48	7-14	211	1298	6.2	426
	58	7-14	222	1328	6.0	292
	59	7-14	250	1677	6.7	492
	60	7-14	215	1351	6.3	560
	68	7-14	244	1574	6.5	393
	69	7-14	259	1643	6.4	320
	22	7-17	240	1382	5.8	539
	29	7-17	174	1049	6.1	695
	30	7-17	195	1185	6.1	438
	$\bar{X}$		225	1375	6.2	470
	SE		8	61	.10	37
	N=10					

Table 7 (continued)

	Rat No.	Date Sacrificed	BW (g)	MW (mg)	MW/BW x 10^3	Glycogen (mg%)
1 hour	45	7-14	219	1335	6.1	457
	46	7-14	210	1164	5.5	480
	55	7-14	239	1411	5.9	401
	56	7-14	219	1333	6.1	642
	24	7-17	229	1297	5.7	299
	31	7-17	178	1050	5.9	85
	32	7-17	177	1049	5.9	420
	80	7-20	255	1544	6.1	345
	81	7-20	256	1607	6.3	381
	$\bar{X}$		220	1310	5.9	390
	SE		8	62	.10	47
	N=9					
6 hours	43	7-14	241	1416	5.9	535
	44	7-14	231	1299	5.7	299
	52	7-14	224	1135	5.1	516
	53	7-14	241	1371	5.7	369
	23	7-17	263	1556	6.0	414
	27	7-17	187	1135	6.1	495
	28	7-17	193	1198	6.2	489
	$\bar{X}$		226	1301	5.8	445
	SE		10	55	.10	31
	N=7					

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