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THE EFFECT OF GLUCOSE SUPPLEMENT ON GLYCOGEN STORAGE, UTILIZATION, AND PERFORMANCE CAPACITY DURING HIGH INTENSITY WORK

by

FRANK D. REARDON

A THESIS

SUBMITTED TO THE FACULTY OF GRADUATE STUDIES AND RESEARCH

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FACULTY OF GRADUATE STUDIES AND RESEARCH

The undersigned certify that they have read, and recommend to the Faculty of Graduate Studies and Research, for acceptance, a thesis entitled "The Effect of Glucose Supplement on Glycogen Storage, Utilization, and Performance Capacity During High Intensity Work", submitted by Frank D. Reardon in partial fulfilment of the requirements for the degree of Doctor of Philosophy in Physical Education.

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ABSTRACT

The effect of glucose supplements (2 mls of 50% glucose) given at different times before the performance of high intensity short term work, was evaluated using performance capacity measures and isotopically labelled glucose. Eighty-one male Wistar rats were oriented to treadmill sprint running (85 m/minute, 30% grade, 15 second run, 20 second rest, 10 repetitions) for three weeks prior to the experiment. The animals were then randomly assigned to various treatments of glucose supplement, exercise, no glucose and no exercise over a period of 2 days. The final performance consisted of running at the criterion rate until exhaustion. Performance was gauged by the actual running time on the treadmill.

It was found that carbohydrate was utilized anaerobically to a large extent in this type of work. The sources of this carbohydrate proved to be primarily the blood glucose pool and the muscle glycogen store in these rats. The liver glycogen levels did not decrease significantly during exercise. Blood lactate concentration rose significantly to a maximum mean value of 207 mg% in a group that was given glucose and exercise on both treatment days. The percent of the total lactate in the blood derived from the glucose supplement was appreciable in exercised animals with mean values in some cases as high as 61%.

Performance of this type of the day 2 sprint work was found to improve significantly only when the rats received the supplements prior to exercise on both days.

The orientation program to sprint running on the treadmill did not alter the fiber type composition of the soleus and plantaris muscles although the muscle glycogen concentration was found to be lower than accepted resting values.

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TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

INTRODUCTION

During the past decade, through the contributions of various lines of research, an extensive profile has been composed which describes in some detail, the composition and metabolic characteristics of skeletal muscle. It was only recently that sport physiologists began to understand the profound influence of various, seemingly obtuse factors on these parameters and on work performance capacity in general.

The fiber type composition and associated metabolic properties of a number of muscles in various species, including man, have been elucidated (Lindholm, 1973; Ariano, 1973; Gollnick, 1973). Accordingly, it has been shown (Bass, 1969) that fiber types tend to be enzymatically predisposed to metabolize specific substrates in a specific manner. Neurotrophic influences (Engel, 1966) and specific training regimens (Staudte, 1973; Exner, 1973; Gollnick, 1972, 1973) tend to modify the metabolic characteristics and predominant substrate utilization of the whole muscle by affecting the metabolism of the fiber type(s) of which it is composed. As well the nature of the work performed by the muscle has a direct bearing on the fiber type recruited and subsequent utilization of substrate. Several authors (Piehl, 1974; Costill, 1973; Gollnick, 1974; Baldwin, 1974) have shown that during long term low intensity work there is selective depletion of glycogen in the slow twitch high oxidative skeletal muscle fibers. This depletion was shown to occur at a higher rate in slow twitch fibers than in fast twitch

fibers during this type of exercise. Evidence from glycogen depletion studies suggests also that during prolonged work, as exhaustion approaches, more fast twitch fibers are recruited (Gollnick, 1974). Thus it is thought that the availability of substrate may directly or indirectly limit the work capacity of a fiber type. Alterations in substrate availability were in fact shown to affect work capacity (Costill, 1973). Bergstrom and Hultman (1967) have well documented the case for "glycogen supercompensation" and improved performance capacity for endurance work. After depletion of glycogen by exhaustive exercise there appeared to be an increased capacity for glycogen storage in the muscle as a direct result of a high carbohydrate diet. This capacity seemed to be further enhanced if the subjects were deprived of carbohydrate after exercise and prior to carbohydrate loading. This supercompensatory effect was subsequently shown to increase capacity for prolonged submaximal work (in, 1971; Gollnick, 1971). Piehl (1974) and Terjung (1974) have, as well, studied the repletion of muscle glycogen stores in skeletal muscle under varied diet and exercise conditions. It has been suggested (Bar et al., 1965) that glucose uptake in skeletal muscle is not due to differences in fiber type characteristics, but rather it is a function of the absolute depletion in the fiber. Hence the exercise program which results in the recruitment of specific fiber type and subsequent glycogen depletion also determines the relative uptake of glucose in each fiber (Gollnick, 1973).

Piehl (1974) presented an apparent contradiction to this theory in demonstrating after prolonged exercise (slow twitch fiber recruitment) higher repletion rates in the fast twitch fibers. However, Gollnick (1974) showed increased fast twitch fiber recruitment at near exhaustion following prolonged work. Therefore, one might expect high rates of repletion in fast twitch fiber. The rationale for this preferential uptake of glycogen in the fast glycolytic fibers is still equivocal since Piehl (1974) noted that this repletion continues at an elevated rate in these fibers in spite of higher I-form synthetase activity in the slow oxidative fibers.

A relatively small portion of the research in this area deals with the effects of high intensity short term work on fiber type recruitment, metabolism, substrate depletion or the effect of diet manipulation or performance capacity. Exner et al. (1973) showed higher depletion of glycogen in fast twitch skeletal muscle fibers in rats during sprint exercise. Gollnick (1973) demonstrated this same effect in humans. Armstrong in 1973 showed the adaptations of fiber enzyme composition to sprint training. Later (1974), he demonstrated that at running speeds above those which should elicit $M\dot{V}O_2$ for a rat, there was a rapid decrease in PAS staining intensity occurring primarily in the fast glycolytic fibers. He suggested a major dependence on anaerobic fibers during high intensity work output or during low intensity work when aerobic fibers are depleted of glycogen.

Gollnick (1973) considered that this selective depletion of glycogen in fast glycolytic muscle during high intensity interval work could limit the capacity of the muscle to continue work.

Since supercompensation is not always practical and the depletion of glycogen stores could be limiting in high intensity interval work, the benefits of an acute supplemental dose of glucose prior to work should be researched. In 1973, Costill attempted to demonstrate the utilization of glucose using an isotope tracer technique in endurance type work. However, the results were reported as counts per minute and this leads one to question the validity of the results. Too, it must be kept in mind that this was long term endurance work. If carbohydrate is one of the major substrates for fast twitch high glycolytic fibers and these fibers are depleted during high intensity work (Gollnick et al., 1973b) then a dietary glucose supplement could be an important factor in the performance of this type of work.

It was therefore of interest and practical value to study the effects of a glucose supplement on glycogen storage, substrate utilization and the performance capacity of rats during repeated bouts of high intensity short term work. It was necessary to determine the fate of the glucose supplement in such a system. In doing so, it was necessary to elucidate the extent to which it contributed to a muscle glycogen sparing effect, was stored in liver and different muscle fiber types, or utilized as a direct metabolic energy source both

on the day of ingestion or the day following ingestion. Having established the degree to which these factors were implicated under these conditions it was imperative that one determine the benefit, if any, on the performance capacity for this type of work.

Specifically the aims of this study were threefold: To demonstrate the effect of glucose supplement administered prior to anaerobic work performance on day 1.

To demonstrate the effect of glucose supplement administered on day 1 on the anaerobic work performance and glucose metabolism and storage parameters on day 2.

To demonstrate the effect of glucose administration on day 1 and day 2 on the various parameters measured during work performance on day 2.

DEFINITION OF TERMINOLOGY

The classification of muscle fibers were made on the basis of the observations of Peter et al. (1972). Although the general classification system of Peter et al. was used, it is emphasized that a glycolytic stain was not done. Strictly speaking, the classification of fibers used in this study could only reflect the extent of myosin ATPase activity and the oxidative capacity as indicated by NADH diaphorase. Thus:

Fast Glycolytic (F.G.) - Those fibers with a high glycolytic capacity, low oxidative capacity, high myosin ATPase activity and fast twitch contractile characteristics.

Slow Oxidative (S.O.) - Those fibers showing low glycolytic capacity, high oxidative capacity, low myosin ATPase

activity and slow twitch contractile characteristics.

Fast Oxidative-Glycolytic (F.O.G.) - Those fibers demonstrating high glycolytic capacity, high oxidative capacity, high myosin ATPase activity and fast twitch characteristics.

Other definitions and abbreviations include:

Short-term High Intensity Work - Interval work involving repeated high intensity work output over a short period of time, followed by a similar period of rest. In this case a running speed of 85 m/min. at a 30% grade for 15 seconds followed by a 20 second rest period.

PAS - Periodic Acid Schiff stain for glycogen after Pearse 1961 (Dubowitz and Brooke, 1973).

Myosin ATPase Activity - Adenosine Triphospatase Activity as demonstrated by the intensity of a stain for ATPase presence in a tissue (Padykula and Herman) as modified by Guth and Samaha, 1969.

Glucose Dose - 2 mls. of 50% glucose solution.

^{14}C-glucose (Uniformly labelled glucose, labelled glucose) - D-glucose^{14}C (U).

^{14}C-glucose Dose - 2 mls. of a 50% glucose solution to which has been added 5 μCi of ^{14}C-glucose.

Cpm - Counts per minute. The number of disintegrations per minute of a radioactively decaying element registered in a counting system.

Dpm - Disintegrations per minute. The absolute number of disintegrations per minute of a radioactive element in a compound sample; equivalent to the Cpm corrected for sample

preparation recovery, background counts and the efficiency of the counting system.

Specific Activity - The total radioactivity of a given isotope (in this case, ^{14}C) per unit weight of the compound, eg. dpm/mg or dpm/mM.

METHODOLOGY

ANIMAL CARE

A total of 106 male rats of the Wistar strain (specific pathogen free CFN rats) weighing approximately 160 grams were obtained through the Director of the Health Animal Science Centre at The University of Alberta. Upon arrival the animals were weighed, housed separately in self-cleaning cages, and provided with Purina chow (Appendix 6) and water ad libitum. Immediately they were subjected to a reversal of the day/night cycle (light 6 pm. to 6 am.) which was maintained until the end of the experimental program (the rest of their lives). The animals were allowed a period of 7 days of orientation to the new environment before the treadmill program was begun. Each day the cages were shifted systematically by one position on the rack such that no animal remained in the same position for two consecutive days or more. At a specified time each morning (0630 hours) the animals were weighed and their food and water replenished. The soiled papers were changed, the trays swabbed, and cages rotated at this time. After the first week, this phase was done after the daily morning exercise period. Once each week all the cages were washed and sterilized. At the

beginning of the second week the animals began their orientation to treadmill running (Appendix 2). Inevitably, some animals were bruised and cut in the area of the lower hind limbs while running: in these cases the injured area was treated externally with Blue Lotion (MTC Pharmaceuticals, London, Ontario). Before each running session the animals' hindquarters were soaked with water and after each session each animal was thoroughly dried and inspected before being returned to its cage.

The treadmill orientation period in all cases lasted for 22 days beginning and ending on a Monday. On the evening of the 22nd day (17 hours before experimental treatment) the animals were deprived of chow and randomly assigned to treatment groups. After the first treatment day, the animals on a two day treatment protocol were allowed chow until 1600 hours at which time they were again deprived of chow in preparation for the day 2 treatment of the 2nd day.

All animals were sacrificed by decapitation. At this time the samples of blood and tissue were taken.

Of the 106 animals destined for this study, data from 81 animals were included in the final analysis. This figure accounts for the inclusion of 15 animals in pilot experiments and an experimental attrition rate of 10.9%.

ORIENTING PROCEDURE/ACUTE EXERCISE PROTOCOL

Generally speaking, laboratory rats are not accustomed to running at these speeds and it was found necessary to orient the animals to short term high intensity work on a

treadmill. The animals were found to perform satisfactorily after 20 days of orientation.

All the animals were exercised on a motor driven treadmill consisting of a wide endless belt on rollers divided into 10 compartments (75 x 10 cm.). Each compartment was fitted with a shock grid across which was maintained a potential of 65 volts (Staudte, 1963). The animals were "oriented to running" by strictly adhering to the program outlined in Appendix 2. Briefly, this procedure included a maximum of 2 exercise bouts daily beginning at 0800 hours and at 1500 hours 5 days per week. Each day the speed and/or the number of repetitions was increased (per schedule Appendix 2) and the duration of exercise and rest intervals decreased until the animals could complete 10 repetitions (15 seconds running, 20 seconds rest) at a speed of 85 m/min. and a grade of 30%. The animals were maintained at this criterion level until the end of the orientation period. During this period they were exercised in groups of 5 animals at a time. Seriously injured and "non running" animals were eliminated from the study.

The acute exercise test administered on designated treatment days consisted of having the animals run at the criterion rate until fatigue (defined as the point at which the animal rested against the shock grid for 8-10 seconds with prodding). During the acute exercise test the animals were run individually.

EXPERIMENTAL DESIGN

At the end of the 20 day orientation period the animals were randomly assigned to one of 14 treatment groups or 2 non treated control groups. The protocol of each group is outlined in Figure 1. Animals designated as having to receive glucose or labelled glucose were dosed with 2 mls. of 50% glucose. The administration of the glucose was intragastrally by gavage 1 to 1.5 hours before the next step in the group protocol. The exercise phase was described earlier as the acute exercise test. Sacrifice was by decapitation. The designation "MET CAGE ^{14}C" means that the animal was placed in a restrainer and placed inside a cylindrical metabolism cage through which room air was drawn at 400 ml/minute. By bubbling the effluent gas through an ethanolamine solution all the $^{14}CO_2$ was trapped. In cases that the animal was exercised, the collection of the $^{14}CO_2$ was begun within 20 seconds after completion of exercise. The $^{14}CO_2$ gas samples were collected in scintillation vials containing 5 mls. of the ethanolamine/ethylene glycol monomethyl ether (1:2) solution. Samples were collected for three minutes every 10 minutes for 40 minutes.

Those animals designated to groups involving "MET CAGE CO_2" were restrained and placed in the metabolism cage through which air was being drawn at a rate of 1150 mls/minute. Samples of the well mixed effluent gas were drawn through an oxygen and CO_2 analyzer. Readings were noted at 3 minutes, 5 minutes and each 10 minutes after that for the

	TREATMENT	EXPERIMENTAL GROUP															
		1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16
Day 2	Glucose		X		X			X	X					X			
	^{14}C Glucose	X		X		X	X					X	X				
	Exercise	X	X	X^2	X	X	X	X	X	X	X			X			
	Sacrifice					X				X		X				X	
	Met Cage ^{14}C			X^1			X										
	Met Cage CO_2							X	X		X						
Day 1	Glucose				X										X		
	^{14}C Glucose							X	X					X			
	Exercise	X	X		X^2		X	X			X			X	X^2		
	Sacrifice						X	X	X		X		X				X
	Met Cage ^{14}C	X												X			
	Met Cage CO_2		X		$X^{1,3}$										$X^{1,3}$		
	n	5	5	4	5	6	5	5	5	5	5	6	5	5	5	5	5

Figure 1: The Assignment of Animals to Treatment Groups
-Indices indicate the sequence of treatments

first 40 minutes post exercise.

In all cases the time for the absorption of the administered glucose was not greater than 1.75 hours and not less than 1.0 hours before the next step in the group protocol. When the animals were placed in the metabolism cage after exercise, the air flow was started within 20 seconds of cessation of exercise.

In attempting to provide evidence as a basis for the resolution of the previously stated proposals, certain aspects of performance and glucose metabolism and storage were investigated. Briefly, the study involved evaluation of certain dependent variables at various points in treatment protocol as outlined in Figure 2:

1) the absolute running time (seconds);

2) the lactate concentration in the blood (mg%);

3) the percent of the total lactate in the blood derived from the dose of glucose;

4) the volume of carbon dioxide produced in the first 40 minutes post exercise (mls.);

5) the percent of the total CO_2 produced 40 minutes post exercise derived from the dose;

6) the concentration of glucose in the blood (mg%);

7) the concentration of glycogen in the plantaris and soleus muscle of the right hind leg (mg%);

8) the percent of this total glycogen in these muscles derived from the dose of glucose;

9) the concentration of glycogen in the liver (mg%);

DEPENDENT VARIABLE	GROUP MEANS TESTED (n)	TEST
Performance	5+7+6 vs 9+10 (16) (10)	t-test
Blood Lactate Concn	5 vs 9 (6) (5)	t-test
% Total Lactate from dose	5 (6)	
CO_2 Production	7 vs 10 vs 14 vs 14[1] (5) (5) (5) (5)	anova, t-test
% Total CO_2 from dose	6 vs 3 (5) (4)	t-test
Blood Glucose Concn	5 vs 9 vs 11 vs 15 (6) (5) (6) (5)	anova, t-test
Muscle Glycogen Concn M. Soleus	5 vs 9 vs 11 vs 15 (6) (5) (6) (5)	anova, t-test
% Total Glycogen from dose	5 vs 11 (6) (6)	t-test
M. Plantaris	5 vs 9 vs 11 vs 15 (6) (5) (6) (5)	anova, t-test
% Total Glycogen from dose	5 vs 11 (6) (6)	t-test
Liver Glycogen Concn	5 vs 9 vs 11 vs 15 (6) (5) (6) (5)	anova, t-test
% Total Glycogen dose	5 vs 11 (6) (6)	t-test

Figure 2: Assignment of Animals to Demonstrate the Effect of Supplement Administered on Day 1 on the Various Parameters on Day 1 and the Tests Used to Compare Group Means

10) the percent of the total liver glycogen derived from the dose of glucose.

To demonstrate the effect of the glucose supplement administered day 1 prior to exercise the comparison of group means were reported as in Figure 2.

To demonstrate the effect of glucose administered prior to exercise on day 1 on anaerobic work performance and glucose storage and metabolism parameters on day 2, the procedure was as outlined in Figure 3.

To demonstrate the effect of administration of the glucose supplement on day 1 and day 2 on the respective parameters during anaerobic work performance on day 2, the comparisons were carried out as in Figure 4.

PREPARATION BLOOD EXTRACTS AND ANALYSES

Immediately post exercise or as otherwise specified, the animals were decapitated using a small animal guillotine and 5-6 mls. of mixed arterial/venous blood were collected from the wound in heparinized flasks. Aliquots of this blood were taken for subsequent glucose and lactate analysis.

ASSAYS - The glucose analysis was accomplished using the method of Feteris (1965) (Sigma Kit 635). The blood sample assigned to lactate analysis was deproteinized, cleared and the perchloric extract was subsequently used to determine enzymatically the lactate concentration by the method of Noll (Lowry and Passonneau, 1972). In cases of the animal having received labelled glucose, a 1 ml. aliquot

DEPENDENT VARIABLE	GROUP MEANS TESTED (n)	TEST
Performance	6+1+2 vs 10 (15) (5)	t-test
	10_1 vs 10_2 (5) (5)	t-test
	6+1+2 vs $8_2+1_2+10_2$ (15) (15)	t-test
Blood Lactate Concn	6 vs 5 6 vs 10 (5) (6) (5) (5)	t-test
% Total Lactate - Dose	6 vs 5 (5) (6)	t-test
CO_2 Production	7 vs 2 (5) (5)	t-test
% Total CO_2 - Dose	6 vs 1 (5) (5)	t-test
Blood Glucose Concn	6 vs 10 vs 16 (5) (5) (5)	anova, t-test
	6+10+16 vs 5+9+15 (15) (16)	
	5 vs 6 9 vs 10 15vs16 (6) (5) (5) (5) (5) (5)	
Muscle Glycogen Concn M. Soleus	6 vs 10 vs 12 vs 16 vs 3 (5) (5) (5) (5) (4)	anova, t-test
% Total Glycogen - Dose	6 vs 12 vs 3 6 vs 5 (5) (5) (4) (5) (6)	anova, t-test
	11 vs 12 3 vs 5 (6) (5) (4) (6)	
M. Plantaris	6 vs 10 vs 12 vs 16 vs 3 (5) (6) (5) (5) (4)	anova, t-test
% Total Glycogen -Dose	6 vs 12 vs 3 6 vs 5 (5) (5) (4) (5) (6)	anova, t-test
	11 vs 12 3 vs 5 (6) (5) (4) (6)	
Liver Glycogen Concn	6 vs 10 vs 12 vs 16 vs 3 (5) (5) (5) (5) (4)	anova, t-test
% Total Glycogen -Dose	6 vs 12 vs 3 6 vs 5 (5) (5) (5) (5) (6)	anova, t-test
	11 vs 12 3 vs 5 (6) (5) (5) (6)	

Figure 3: Assignment of Animals to Demonstrate the Effect of Supplement Given Day 1 on Various Parameters on Day 2 and the Tests Used to Compare Group Means

DEPENDENT VARIABLE	GROUP MEANS TESTED (n)	TEST
Performance	7+13+4 vs 6+1+2 vs 10 (14) (15) (5)	anova, t-test
Blood Lactate Concn	6 vs 7 vs 10 (5) (5) (5)	anova, t-test
% Total Lactate -Dose	6 vs 7 vs 12 (5) (5) (5)	anova, t-test
CO_2 Production	4 vs 2 (5) (5)	t-test
% Total CO_2 -Dose	13 vs 1 (5) (5)	t-test
Blood Glucose Concn	7 vs 6 vs 3 vs 8 (5) (5) (4) (5)	anova, t-test
Muscle Glycogen Concn M. Soleus	7 vs 6 vs 3 vs 8 (5) (5) (4) (5)	anova, t-test
% Total Glycogen -Dose	7 vs 6 vs 3 vs 8 (5) (5) (4) (5)	anova, t-test
M. Plantaris	7 vs 6 vs 3 vs 8 (5) (5) (4) (5)	anova, t-test
% Total Glycogen -Dose	7 vs 6 vs 3 vs 8 (5) (5) (4) (5)	anova, t-test
Liver Glycogen Concn	7 vs 6 vs 3 vs 8 (5) (5) (4) (5)	anova, t-test
% Total Glycogen -Dose	7 vs 6 vs 3 vs 8 (5) (5) (4) (5)	anova, t-test

Figure 4: Assignment of Animals to Demonstrate the Effect of Glucose Supplement on Both Days Prior to Analysis on Day 2 and the Tests Used to Compare Group Means

of the extract was set aside for ^{14}C-lactate analysis by the method of Freminet et al. (1972). The original counting solution was modified such that the hyamine hydroxide was eliminated. (See Preparation for ^{14}C Counting).

TISSUE PREPARATION

Immediately following exsanguination, the plantaris and soleus muscles of the right hind leg were isolated and excised. Each muscle was divided into samples of sufficient size for histochemical (50-70 mg.) and biochemical (40-60 mg) analysis. In the case of those animals having received ^{14}C-glucose an additional 35-50 mg. sample was cut. Samples of similar size were taken from the liver for glycogen and, where applicable, ^{14}C analysis. After recording the weight of each sample, the tissue was immersed in liquid nitrogen cooled isopentane and stored at -60°C.

GLYCOGEN ASSAY - The glycogen was isolated from the muscle and liver tissue by the method of Lo et al. (1970) and analyzed colorimetrically for glycogen content. In the situations that animals were dosed with ^{14}C-glucose, a 1 ml. aliquot of the aqueous glycogen extract was frozen and saved at -30°C for subsequent counting.

PREPARATION FOR ^{14}C COUNTING

All samples were counted using liquid scintillation counting techniques. The scintillation cocktail suggested by Freminet et al. (1972) for the counting of the lactate solution was modified such that 0.5 mls. of the final

filtrate were pipetted into the vial with 3 mls. of methanol (instead of hyamine hydroxide and ethanol) and the 10 mls. of the PPO-POPOP in toluene system. This decreases the chemiluminescence due to the presence of the hyamine hydroxide. The basic premise of this method was the difference in counting rate of two similar samples from one of which the ^{14}C-lactate has been removed. The authors fail to mention that because of the addition of NAD^+ and LDH to one sample (for the extraction of lactate) the quench characteristics are no longer similar for both systems. Therefore, in doing the calculations, one must allow for differences in counting efficiency in each sample.

The 1 ml. aliquots allocated to ^{14}C glycogen counting were added to vials containing 10 mls. of fluor (PPO 6 gm/l., POPOP 50 mg/l. in toluene), 5 mls. of methanol and 1 ml. of ethylene glycol monomethyl ether.

The ^{14}C carbon dioxide collected from the metabolism cage gas effluent in 5 ml. of the ethanolamine trapping solution (ethanolamine/ethylene glycol monomethyl ether; 1:2 - methyl cellosolve) was counted in 12 mls. of a 50% solution of PPO-POPOP fluor in methyl cellosolve. All counting was done in Searle Mk III and Picker Liquimat 220 LSC machines.

HISTOCHEMICAL ANALYSIS

The frozen muscle plugs obtained from the sacrificed animals were mounted on a chuck and maintained at -25 to -30°C in the cryostat. Serial sections 10μ thick were cut

and mounted on microscope slides in preparation for glycogen myosin ATPase and NADH diaphorase staining. The samples were allowed to dry at least 3 hours before being stained. The method used for glycogen staining was the PAS stain suggested by Dubowitz and Brooke (1973). The procedure followed for the estimation of myosin ATPase was the method of Padykula and Herman as modified by Guth and Samaha (1969). The tetrazolium blue reaction procedure for staining for NADH diaphorase was taken from Dubowitz and Brooke (1973).

All muscle plugs from all the animals sacrificed were cut serially and stained as outlined above. Sections of muscle from 2 animals representing each treatment group were photographed and representative areas of the photographs ($\simeq$ 75 fibers) were analyzed for fiber type composition and glycogen depletion.

CALCULATIONS

^{14}C-Lactate: To calculate the percent of the total blood lactate derived from the dose the following equations were used:

$$\frac{\dfrac{(Cpm_A - Cpm_B) \times CE}{Sp\ Act_{(1)}} \times D}{[Lact]_{Bl} \times Vol_{Bl}} \times 100 = \%\ \text{from dose}$$

where,

$Cpm_{(A)}$ and $Cpm_{(B)}$ are the count rates of the two samples (Δ Cpm $\equiv$ Cpm of ^{14}C Lactate)

CE is the counting efficiency of the system

Sp. $Act_{(1)}$ is the specific activity of the ^{14}C-glucose dose as lactate.

D is a dilution factor (in this case 6).

$[Lact]_{BL}$ is the blood lactate concentration in mM/unit volume.

Vol_{BL} is the estimated blood volume (65 ml/kg), 1974 ARS/Sprague Dawley Calendar.

^{14}C Carbon Dioxide - The calculations for % CO_2 produced derived from the dose were similar in form to the above:

$$\frac{\frac{DPM_{CO_2}}{Sp\ Act_{(2)}}}{[CO_2]} \times 100 = \%\ CO_2 \text{ Total from dose}$$

where,

DPM_{CO_2} was the count rate produced in the first 40 mins. post exercise calculated from means of the measured values.

Sp. $Act_{(2)}$ is the specific activity of the dose as carbon dioxide.

$[CO_2]$ is the amount of CO_2 (mM) produced in the first 40 minutes post exercise calculated from similarly treated groups in the gas analysis cage. Example:

$$\frac{\%\ CO_2 \times Vol./min._{(STP)} \times 40}{22.4} = mM\ CO_2/\ 40 \text{ minutes}$$

where,

% CO_2 is the mean percent CO_2 over 40 minutes post exercise.

Vol/min. is the air flow rate through the cage.

X 40 is the volume in 40 minutes adjusted to STP.

Knowing that 1 mole CO_2 occupies 22.4 l. at STP volume then divide by 22.4 and derive the number mM produced in 40 minutes post exercise.

^{14}C-Glycogen - Similarly for glycogen:

$$\frac{\frac{DPM_{glyc} \times D_1}{Sp\ Act_{(3)}}}{[glyc] \times WT} \times 100 = \%\ \text{Muscle glycogen from dose}$$

where,

DPM_{glyc} is the corrected count rate of the glycogen sample multiplied by a dilution factor D_1.

$Sp\ Act_{(3)}$ is the specific activity of the dose as glycogen.

[glyc] is the glycogen concentration in the muscle in mg/mg wet weight.

WT is the estimated total weight of the muscle (from average weights of the total muscle from the contralateral leg).

It must be kept in mind that these calculations are theoretical and are based on a number of assumptions and estimations. For example, in the calculation of the percent lactate derived from the dose, it is assumed that blood volume remains constant during the exercise.

APPARATUSES

Quinton Rodent Treadmill Model 21-40

Small animal guillotine

Dewar Flask fitted to cool isopentane in N_2 (liq)

Metabolism cage with restrainer

Vacuum pump

Flow meter (air)

Vacuum Bell jar with A pore size bubbler

Beckman O_2 analyzer (constant flow)

Capnograph CO_2 analyzer

Beckman Dynograph (graphic readout)

Cryostat

Timer

Dye baths

Overhead microscope slide projector

Leitz microscope with photographic adaptor

Packard Tri Carb Sample Oxidizer Model 306

Searle Mk III LSC

Picker Liquimat 220 LSC

RESULTS

The treatment of each group is summarized using a maximum of four symbols: (G) - glucose supplement; (E)- acute exercise test; (N)- no treatment.

The first symbol in the series concerns the administration of glucose on treatment day 1; the third, glucose administration on day 2. The second symbol in the series concerns exercise on treatment day 1 and the fourth symbol, exercise on day 2. Thus GENE represents the treatment of an animal that received glucose and exercise on day 1 and exercise on day 2.

The outcome of the comparisons of treatment group means are tabulated according to the dependent variable tested in Tables I through IX.

WORK PERFORMANCE - Table I represents the effect of treatment combinations on work performance measured on treatment day 1 and day 2. There was no statistically significant difference in the mean running time of those rats represented by the comparison of groups 6+5+7(GE) and 9(NE)+10(NE). Similarly there was no significant difference in the mean running times of groups 6+1+2(GENE) and 10(NENE) when evaluated on day 2, thus signifying no benefit in terms of performance from having received glucose on day 1. Groups that received glucose supplements on both days had a significantly higher mean running time on day 2 than those that did not receive any glucose supplement on either day and those that had glucose on day 1 only. No statistically significant

difference in running time existed between the day 1 and day 2 means of those groups that received none or one glucose supplement over the treatment period. However, animals that were fed glucose on both treatment days ran significantly longer ($p < .05$) on the second day than on the first day of treatment.

BLOOD LACTATE CONCENTRATION - The results of the blood lactate analyses are outlined in Table VIII. The mean blood lactate concentration of group 5(GE) was significantly higher than those animals of group 9(NE). A significant difference was not present between the means of group 5(GE) and group 6(GENE). There was no significant difference between the group mean blood lactate concentration of groups 6(GENE), 7(GEGE), and 10(NENE) which were all evaluated after exercise on day 2.

The estimated percent of the total blood lactate being derived from the glucose dose was significantly lower on day 2 than post exercise on day 1 as demonstrated by the comparisons of the means of group 5(GE) and 6(GENE). The mean value of group 6(GENE) was compared as well to group 7(GEGE). Again the mean percent of the total blood lactate derived from the dose was significantly lower in group 6(GENE).

EXPIRED CO_2 - This data is presented in Table IX. In groups of animals evaluated for CO_2 production over the first 40 minutes post exercise on day 1, the mean CO_2 production of group 7(GEGE) which received a pre-exercise glucose supplement was not significantly different from group 10(NENE)

which did not get the glucose supplement. This mean CO_2 production was significantly higher in group 7(GEGE) than in group 14_1(GN). Similarly, the group 14_1(GN) mean value was significantly lower than that of group 10(NENE). There was no significant difference in mean post exercise CO_2 production between groups that were given glucose on day 1, exercised and evaluated on day 2, and those which were tested on day 1 or those which were again given glucose and exercise on day 2. This is shown in the comparisons of group 3(GN) with groups 7(GEGE) and 4(GEGE).

The mean percent total CO_2 produced in the first 40 minutes post exercise derived from the glucose dose was significantly higher in group 6(GENE) than in groups 3(GN) and 1(GENE). Group 13(GEGE) as well had a significantly higher mean % CO_2 derived from the glucose dose than did group 2 (GENE).

BLOOD GLUCOSE CONCENTRATION - The results of the blood analyses for glucose concentration are outlined in Table II. Mean blood glucose concentration in group 9(NE) animals were significantly lower than those of control animals (Group 15 (NN)), Group 11(GN), and Group 5(GE). Group 5(GE) and group 11(GN) means were not significantly different from the control (NN) mean nor was the group 5(GE) mean significantly less than the group 11 (GN) mean glucose concentration.

The group 6(GENE) post exercise levels of glucose in the blood on day 2 were not significantly different from those of group 10(NENE) or control group 16 (NNNN). Group 10(NENE)

mean glucose concentration was not significantly lower than control levels (Group 16(NNNN)), group 6(GENE) mean blood glucose concentration was not significantly different from that of group 7(GEGE). However, it was significantly lower than group 8(GEGN). The group 3(GENN) mean glucose concentration was found to be significantly higher than that of group 7(GEGE) and significantly less than group 8(GEGN). Group 3(GENN) was not significantly different from group 6 (GENE). Group 7(GEGE) had significantly lower blood glucose concentration than group 8(GEGN).

MUSCLE GLYCOGEN CONCENTRATION - The muscle glycogen concentration was analyzed in 2 muscles, soleus and plantaris, and the results are presented in Tables IV and VI. There was no significant difference between group 11(GN) in both plantaris and soleus muscles and the control group 15 (NN). However, there was significantly higher glycogen concentration in both muscles in group 11(GN) than in groups 9(NE) and 5(GE). In the plantaris muscle there was a significant difference between these two groups in mean glycogen concentration. The plantaris muscle glycogen of groups 5(GE) and 9(NE) was also significantly less than the control; but this was not different in the soleus muscles of these groups. Group 6(GENE) muscle glycogen concentration was analyzed on day 2. It was found not to be significantly different in either muscle from that of group 10(NENE). There was significant difference in mean glycogen concentrations between group 6(GENE) and group 12(GNNN) and the control group

16(NNNN), in both plantaris and soleus muscles. Group 3 (GENN) mean glycogen levels in plantaris and soleus were significantly lower than the control (16) and group 12(GNNN), but not significantly greater than group 6(GENE). In the soleus muscle the glycogen concentration was significantly higher in group 3(GENN) than in group 10(NENE). The group 10(NENE) muscle glycogen levels were lower in plantaris and soleus muscles than those in the control group or in group 12(GNNN). The group 12(GNNN) mean muscle glycogen concentration was not significantly different from control levels. There were also no significant differences between groups 7(GEGE), 6(GENE), 8(GEGN) and 3(GENN) in either muscle.

PERCENT TOTAL MUSCLE GLYCOGEN DERIVED FROM DOSE - The estimated percent of the total muscle glycogen in plantaris and soleus muscle derived from the glucose dose was calculated and presented in Tables V and VII. The mean values for group 5(GE) were significantly higher in plantaris than in group 11(GN). When group 5(GE) was compared with group 6(GENE) there was no significant difference between them, nor was there any significant difference between groups 11 (GN) and 12(GNNN). There was however, a significantly greater percent of total glycogen derived from the dose in the plantaris muscle in group 3(GENN) than group 5(GE). However, the group 3(GENN) mean for soleus muscle was not significantly greater than that of group 5(GE). In comparing the means of groups 3(GENN), 6(GENE) and 12(GNNN), no significant difference in the percent of glycogen derived from the dose was found in

the soleus muscle, however, the plantaris muscle showed a significantly higher percent in group 3(GENN) than in both groups 12(GNNN) and 6(GENE). There was no significant difference in either muscle between the means of groups 6(GENE) and 12(GNNN). In the plantaris muscle there was a significantly higher percent of the total glycogen derived from the dose in group 3(GENN) than in group 8(GEGN). In both muscles there was significantly higher mean values for group 7 (GEGE) than group 8(GEGN), however, there was no significant difference of the means of groups 8(GEGN) and 6(GENE) in either muscle. In the soleus muscle, group 7(DXDX) animals had significantly high percent incorporation of glucose from the dose than did groups 6(GENE) or 3(GENN). In plantaris this was also higher for groups 7(GEGE) than 6(GENE) but not between groups 7(GEGE) and 3(GENN). Only in plantaris of group 3(GENN) muscle was the percent of the total glycogen derived from the dose significantly higher than that of group 6(GENE).

LIVER GLYCOGEN - The summary of the liver glycogen concentration in the experimental groups is outlined in Table III. There were no significant differences in the mean values of glycogen concentration in all the groups compared. These groups included; 11(GN), 15(NN), 5(GE) and 9(NE) for day 1 analyses and 6(GENE), 10(NENE), 12(GNNN), 16(NNNN) and 3(GENN) for analysis on day 2. There were also no significant differences in the mean values of liver glycogen concentration of groups 7(GEGE), 6(GENE), 3(GENN) and 8(GEGN).

In the same table are presented the mean estimated percent of the total glycogen derived from the glucose supplement. The group 5(GE) mean percent was not significantly different from group 11(GN) nor were the means of group 6 (GENE) and group 12(GNNN) significantly different. The group 6(GENE) mean however, was found to be significantly lower than the mean value calculated for group 3(GENN). This same relationship held for the comparison of the group 7(GEGE) mean with group 3(GENN). The mean percent incorporation of the glucose dose to glycogen in liver in group 6(GENE) was compared to the mean of group 5(GE). The mean of group 6(GENE) was significantly less than that of group 5(GE). The mean of group 11(GN) was significantly higher than group 12(GNNN). There was no significant differences in the mean percent glucose incorporation from the dose to liver glycogen between groups 5(GE) and 3(GENN), 8(GEGN) and 3(GENN), or [redacted]nd 8(GEGN). However, the mean of group 6(GENE) was [redacted]antly less than groups 8(GEGN), 7(GEGE), and 3(GENN). [redacted]ean of group 7(GEGE) was not significantly different fro[redacted]e group 3(GENN) mean.

HISTOCHEM[redacted]RY - The results of the tissue staining experiment are o[redacted]lined in Table XII. On the extreme right side of the ta[redacted]e are the percent fiber types in soleus and plantaris muscles. The treatment groups are listed in the vertical columns on the right side of which is the percent of the fiber types having a relative PAS stain intensity of dark, medium or light. The percent fiber type is listed on the left side of the column for both muscles.

PARAMETER	(n)	GROUP	TREATMENT DAY 1 GLUCOSE	DAY 1 EXERCISE	DAY 2 GLUCOSE	DAY 2 EXERCISE	MEAN ± S.E. (seconds)	t-TEST α=0.05
	(16)	5+6+7	X	X			276 ± 39	
	(10)	9+10	--	X			236 ± 24	
	(15)	6+1+2	X	X	--	X	259 ± 22	
	(5)	10	--	X	--	X	249 ± 36	
WORK PERFORMANCE	(5)	10	--	X	--	X	207 ± 36	
							249 ± 42	
	(15)	6+1+2	X	X	--	X	258 ± 22	
							259 ± 40	
	(14)	$7+13+4_2$	X	X	X	X	392 ± 56	
	(15)	6+1+2	X	X	--	X	259 ± 40	**
	(14)	$7+13+4_2$	X	X	X	X	392 ± 56	
	(5)	10	--	X	--	X	249 ± 42	**
	(14)	7+13+4	X	X	X	X	276 ± 25	
							384 ± 33	

TABLE I THE EFFECT OF TREATMENT COMBINATIONS ON WORK TIME TO EXHAUSTION

**Denotes significance at the α=0.05 level

PARAMETER	(n)	GROUP	TREATMENT DAY 1 GLUCOSE	DAY 1 EXERCISE	DAY 2 GLUCOSE	DAY 2 EXERCISE	MEAN ± S.E. (mg%)	t-TEST ∝=0.05
BLOOD GLUCOSE CONCENTRATION	(5)	9	--	X			61 ± 6	**
	(5)	15	--	--			106 ± 4	
	(5)	9	--	X			61 ± 6	**
	(6)	11	X	--			114 ± 19	
	(6)	5	X	X			99 ± 13	**
	(5)	9	X	--			61 ± 6	
	(6)	11	X	--			114 ± 19	
	(5)	15	--	--			106 ± 4	
	(6)	5	X	X			99 ± 13	
	(5)	15	--	--			106 ± 4	
	(6)	5	X	X			99 ± 13	
	(6)	11	X	--			114 ± 19	
	(5)	6	X	X	--	X	105 ± 8	**
	(5)	10	--	X	--	X	86 ± 11	
	(5)	6	X	X	--	X	105 ± 8	
	(5)	16	--	--	--	--	108 ± 6	
	(5)	10	--	X	--	X	86 ± 11	
	(5)	16	--	--	--	--	108 ± 6	
	(5)	7	X	X	X	X	93 ± 5	**
	(5)	6	X	X	--	X	105 ± 8	
	(5)	6	X	X	--	X	105 ± 8	
	(5)	8	X	X	X	--	131 ± 10	
	(5)	7	X	X	X	X	93 ± 6	
	(4)	3	X	X	--	--	109 ± 5	

CONT'D

PARAMETER	(n)	GROUP	TREATMENT DAY 1 GLUCOSE	DAY 1 EXERCISE	DAY 2 GLUCOSE	DAY 2 EXERCISE	MEAN ± S.E. (mg%)	t-TEST α=0.05
BLOOD GLUCOSE CONCENTRATION (CONT'D)	(5)	8	X	X	X	--	131 ± 10	
	(4)	3	X	X	--	--	109 ± 5	
	(5)	6	X	X	--	X	105 ± 8	
	(4)	3	X	X	--	--	109 ± 5	
	(5)	7	X	X	X	X	93 ± 5	**
	(4)	8	X	X	X	--	131 ± 10	

TABLE II THE EFFECT OF TREATMENT COMBINATIONS ON BLOOD GLUCOSE CONCENTRATION

** Denotes significance at the α = 0.05 level

PARAMETER	(n)	GROUP	TREATMENT DAY 1 GLUCOSE	DAY 1 EXERCISE	DAY 2 GLUCOSE	DAY 2 EXERCISE	MEAN ± S.E. (mg%)/%	t-TEST ∝=0.05
	(6)	11	X	--			.16 ± .05	
	(5)	15	--	--			.12 ± .01	
	(5)	5	X	X			.11 ± .03	
	(5)	9	--	X			.07 ± .01	
LIVER	(5)	6	X	X	--	X	.20 ± .08	
GLYCOGEN	(5)	10	--	X	--	X	.13 ± .04	
CONCENTRATION	(5)	12	X	--	--	X	.69 ± .32	
	(5)	16	--	--	--	--	.77 ± .33	
	(4)	3	X	X	--	--	.27 ± .16	
	(5)	7	X	X	X	X	.11 ± .01	
	(5)	6	X	X	--	X	.20 ± .08	
	(4)	3	X	X	X	--	.27 ± .16	
	(5)	8	X	X	--	--	.17 ± .03	
	(6)	5	*X	X			42 ± 8	
	(6)	11	*X	--			36 ± 3	
	(5)	6	*X	X	--	X	15 ± 3	
% TOTAL LIVER	(5)	12	*X	--	--	--	18 ± 6	
GLYCOGEN DE-								
RIVED FROM	(5)	6	*X	X	--	X	15 ± 3	
GLUCOSE DOSE	(4)	3	*X	X	--	--	45 ± 3	**
	(5)	12	*X	--	--	--	18 ± 6	
	(4)	3	*X	X	--	--	45 ± 3	**
	(5)	6	*X	X	--	X	15 ± 3	
	(6)	5	*X	X			42 ± 8	**
	(6)	11	*X	--			36 ± 3	
	(5)	12	*X	--	--	--	18 ± 6	**

CONT'D

PARAMETER	(n)	GROUP	TREATMENT DAY 1 GLUCOSE	DAY 1 EXERCISE	DAY 2 GLUCOSE	DAY 2 EXERCISE	MEAN ± S.E. (mg%)/%	t-TEST α=0.05
	(4)	3	*X	X	--	--	45 ± 3	
	(5)	5	*X	X			42 ± 8	
	(5)	8	X	X	*X	--	56 ± 17	
	(4)	3	*X	X	--	--	45 ± 3	
	(5)	7	X	X	*X	X	63 ± 11	
% TOTAL LIVER GLYCOGEN	(5)	8	X	X	*X	--	56 ± 17	
DERIVED FROM	(5)	8	X	X	*X	--	56 ± 17	
GLUCOSE DOSE	(5)	6	*X	X	--	X	15 ± 3	**
(CONT'D)	(5)	7	X	X	*X	X	63 ± 11	
	(5)	6	*X	X	--	X	15 ± 3	**
	(5)	6	*X	X	--	X	15 ± 3	
	(4)	3	*X	X	--	--	45 ± 3	**
	(5)	7	X	X	*X	X	63 ± 11	
	(4)	3	*X	X	--	--	45 ± 3	

TABLE III THE EFFECT OF TREATMENT COMBINATIONS ON PERCENT OF LIVER GLYCOGEN DERIVED FROM GLUCOSE DOSE

*Denotes ^{14}C administration

**Denotes significance at the α = 0.05 level

PARAMETER	(n)	GROUP	TREATMENT DAY 1 GLUCOSE	DAY 1 EXERCISE	DAY 2 GLUCOSE	DAY 2 EXERCISE	MEAN ± S.E. (mg%)	t-TEST $\alpha = 0.05$
	(6)	11	X	--			.18 ± .03	
	(5)	15	--	--			.10 ± .02	
	(6)	11	X	--			.18 ± .03	
	(5)	9	--	X			.07 ± .01	**
	(6)	5	X	X			.08 ± .03	
	(6)	11	X	--			.18 ± .03	**
	(6)	5	X	X			.08 ± .03	
	(5)	9	--	X			.07 ± .01	
	(6)	5	X	X			.08 ± .03	
	(5)	15	--	--			.10 ± .02	
MUSCLE	(5)	9	--	X			.07 ± .01	
GLYCOGEN	(5)	15	--	--			.10 ± .02	
CONCENTRATION								
M. SOLEUS	(5)	6	X	X	--	X	.06 ± .01	
	(5)	10	--	X	--	X	.05 ± .01	
	(5)	6	X	X	--	X	.06 ± .01	
	(5)	12	X	--	--	--	.18 ± .02	**
	(5)	6	X	X	--	X	.06 ± .01	
	(5)	16	--	--	--	--	.20 ± .02	**
	(5)	6	X	X	--	X	.06 ± .01	
	(4)	3	X	X	--	--	.10 ± .01	
	(5)	16	--	--	--	--	.20 ± .03	
	(4)	3	X	X	--	--	.10 ± .001	**

CONT'D

PARAMETER	(n)	GROUP	TREATMENT DAY 1 GLUCOSE	DAY 1 EXERCISE	DAY 2 GLUCOSE	DAY 2 EXERCISE	MEAN ± S.E. (mg%)	t-TEST α=0.05
	(5)	12	X	--	--	--	.18 ± .02	
	(4)	3	X	X	--	--	.10 ± .01	**
	(5)	10	--	X	--	X	.05 ± .01	
	(4)	3	X	X	--	--	.10 ± .01	**
MUSCLE	(5)	10	--	X	--	X	.05 ± .01	
GLYCOGEN	(5)	12	X	--	--	--	.18 ± .02	**
CONCENTRATION	(5)	10	--	X	--	X	.05 ± .01	
M. SOLEUS	(5)	16	--	--	--	--	.20 ± .03	**
(CONT'D)	(5)	12	X	--	--	--	.18 ± .02	
	(5)	16	--	--	--	--	.20 ± .03	
	(5)	7	X	X	X	X	.07 ± .00	
	(5)	6	X	X	--	X	.06 ± .01	
	(5)	8	X	X	X	--	.14 ± .04	
	(4)	3	X	X	--	--	.10 ± .01	

TABLE IV THE EFFECT OF TREATMENT COMBINATIONS ON GLYCOGEN CONCENTRATION IN THE SOLEUS MUSCLE

** Denotes significance at the α= 0.05 level

PARAMETER	(n)	GROUP	TREATMENT DAY 1 GLUCOSE	DAY 1 EXERCISE	DAY 2 GLUCOSE	DAY 2 EXERCISE	MEAN ± S.E. (%)	t-TEST α=0.05
% TOTAL MUSCLE GLYCOGEN DERIVED FROM GLUCOSE DOSE M. SOLEUS	(6)	5	*X	X			27 ± 7	
	(6)	11	*X	--			23 ± 7	
	(5)	6	*X	X	--	X	27 ± 7	
	(6)	5	*X	X			27 ± 7	
	(6)	11	*X	--			23 ± 7	
	(5)	12	*X	--	--	--	18 ± 3	
	(4)	3	*X	X	--	--	39 ± 7	
	(6)	5	*X	X			27 ± 7	
	(5)	6	*X	X	--	X	27 ± 7	
	(5)	12	*X	--	--	--	18 ± 3	
	(4)	3	*X	X	--	--	37 ± 7	
	(5)	8	X	X	*X	--	46 ± 9	
	(4)	3	*X	X	--	--	39 ± 7	
	(5)	7	X	X	*X	X	65 ± 4	**
	(5)	8	X	X	*X	--	46 ± 9	
	(5)	8	X	X	*X	--	46 ± 9	
	(5)	6	*X	X	--	X	27 ± 7	
	(5)	7	X	X	*X	X	65 ± 4	**
	(5)	6	*X	X	--	X	27 ± 7	
	(5)	6	*X	X	--	X	27 ± 7	
	(4)	3	*X	X	--	--	37 ± 7	
	(5)	7	X	X	*X	X	65 ± 4	**
	(4)	3	*X	X	--	--	39 ± 7	

TABLE V THE EFFECT OF TREATMENT COMBINATIONS ON PERCENT OF THE TOTAL SOLEUS MUSCLE GLYCOGEN CONCENTRATION DERIVED FROM THE GLUCOSE DOSE
*Denotes ^{14}C administration, **Denotes significance at the α = 0.05 level

PARAMETER	(n)	GROUP	TREATMENT DAY 1 GLUCOSE	DAY 1 EXERCISE	DAY 2 GLUCOSE	DAY 2 EXERCISE	MEAN ± S.E. (mg%)	t-TEST α=0.05
	(6)	11	X	--			.10 ± .02	
	(5)	15	--	--			.11 ± .01	
	(6)	11	X	--			.10 ± .02	
	(5)	9	--	X			.03 ± .00	**
	(6)	5	X	X			.06 ± .03	
	(6)	11	X	--			.10 ± .02	**
	(6)	5	X	X			.06 ± .03	
	(5)	9	--	X			.03 ± .00	**
MUSCLE GLYCOGEN CONCENTRATION M. PLANTARIS	(6)	5	X	X			.06 ± .03	
	(5)	15	--	--			.11 ± .01	**
	(5)	9	--	X			.03 ± .00	
	(5)	15	--	--			.11 ± .01	**
	(5)	6	X	X	--	X	.07 ± .02	
	(5)	10	--	X	--	X	.07 ± .01	
	(5)	6	X	X	--	X	.07 ± .02	
	(5)	12	X	--	--	--	.19 ± .02	**
	(5)	6	X	X	--	X	.07 ± .02	
	(5)	16	--	--	--	--	.21 ± .03	**
	(5)	6	X	X	--	X	.07 ± .02	
	(4)	3	X	X	--	--	.09 ± .06	
	(5)	16	--	--	--	--	.21 ± .03	
	(4)	3	X	X	--	--	.09 ± .06	**
	(5)	12	X	--	--	--	.19 ± .02	
	(4)	3	X	X	--	--	.09 ± .06	**

CONT'D

PARAMETER	(n)	GROUP	TREATMENT DAY 1 GLUCOSE	DAY 1 EXERCISE	DAY 2 GLUCOSE	DAY 2 EXERCISE	MEAN ± S.E. (mg%)	t-TEST ∝=0.05
	(5)	10	--	X	--	X	.02 ± .01	
	(4)	3	X	X	--	--	.09 ± .06	
	(5)	10	--	X	--	X	.07 ± .01	**
MUSCLE	(5)	12	X	--	--	--	.19 ± .02	
GLYCOGEN	(5)	10	--	X	--	X	.07 ± .01	**
CONCENTRATION	(5)	16	--	--	--	--	.21 ± .03	
M. PLANTARIS	(5)	12	X	--	--	--	.19 ± .02	
(CONT'D)	(5)	16	--	--	--	--	.21 ± .03	
	(5)	7	X	X	X	X	.03 ± .00	
	(5)	6	X	X	--	X	.07 ± .02	
	(5)	8	X	X	X	--	.10 ± .00	
	(5)	3	X	X	--	--	.09 ± .06	

TABLE VI THE EFFECT OF TREATMENT COMBINATIONS ON THE PLANTARIS MUSCLE GLYCOGEN CONCENTRATION

**Denotes significance at the $\alpha = 0.05$ level

PARAMETER	(n)	GROUP	TREATMENT DAY 1 GLUCOSE	DAY 1 EXERCISE	DAY 2 GLUCOSE	DAY 2 EXERCISE	MEAN ± S.E. (%)	t-TEST α=0.05
	(6)	5	*X	X			28 ± 6	
	(6)	11	*X	--			15 ± 5	**
	(5)	6	*X	X	--	X	23 ± 9	
	(6)	5	*X	X			28 ± 6	
	(6)	11	*X	--			15 ± 5	
	(5)	12	*X	--	--	--	12 ± 2	
	(4)	3	*X	X	--	--	54 ± 8	
% TOTAL	(6)	5	*X	X			28 ± 6	**
MUSCLE GLY-	(5)	6	*X	X	--	X	23 ± 9	
COGEN DERIVED	(5)	12	*X	--	--	--	12 ± 2	
FROM GLUCOSE								
DOSE	(5)	6	*X	X	--	X	23 ± 9	
M. PLANTARIS	(4)	3	*X	X	--	--	54 ± 8	**
	(5)	12	*X	--	--	--	12 ± 2	
	(4)	3	*X	X	--	--	54 ± 8	**
	(5)	8	X	X	*X	--	25 ± 7	
	(4)	3	*X	X	--	--	45 ± 3	**
	(5)	7	X	X	*X	X	54 ± 10	
	(5)	8	X	X	*X	--	25 ± 7	**
	(5)	8	X	X	*X	--	25 ± 7	
	(5)	6	*X	X	--	X	23 ± 9	

CONT'D

PARAMETER	(n)	GROUP	TREATMENT DAY 1 GLUCOSE	DAY 1 EXERCISE	DAY 2 GLUCOSE	DAY 2 EXERCISE	MEAN ± S.E. (%)	t-TEST α=0.05
% TOTAL	(5)	7	X	X	*X	X	54 ± 10	
MUSCLE GLYCO-	(5)	6	*X	X	--	X	23 ± 9	**
GEN DERIVED FROM GLUCOSE	(5)	6	*X	X	--	X	23 ± 9	
DOSE	(4)	3	*X	X	--	--	54 ± 8	**
M. PLANTARIS	(5)	7	X	X	*X	X	54 ± 10	
(CONT'D)	(5)	3	*X	X	--	--	54 ± 8	

TABLE VII THE EFFECT OF TREATMENT COMBINATIONS ON PERCENT OF THE TOTAL PLANTARIS MUSCLE GLYCOGEN CONCENTRATION DERIVED FROM THE GLUCOSE DOSE

*Denotes ^{14}C administration

**Denotes significance at the α= 0.05 level

PARAMETER	(n)	GROUP	TREATMENT DAY 1 GLUCOSE	DAY 1 EXERCISE	DAY 2 GLUCOSE	DAY 2 EXERCISE	MEAN ±S.E. (mg %)/%	t-TEST α=0.05
	(6)	5	X	X			170 ±37	**
	(5)	9	--	X			123 ±18	
LACTATE CONCENTRATION IN BLOOD	(5)	6	X	X	--	X	167 ±37	
	(6)	5	X	X			170 ±37	
	(6)	10	--	X	--	X	186 ±17	
	(5)	6	X	X	--	X	167 ±37	
	(5)	7	X	X	X	X	207 ±15	
	(5)	6	X	X	--	X	167 ±37	
	(5)	10	--	X	--	X	186 ±17	
PERCENT LACTATE DERIVED FROM GLUCOSE DOSE	(5)	6	*X	X	--	X	9 ± 1	**
	(5)	5	*X	X			61 ± 6	
	(5)	7	X	X	*X	X	36 ± 9	**
	(5)	6	*X	X	--	X	9 ± 1	

TABLE VIII THE EFFECT OF TREATMENT COMBINATIONS ON LACTATE CONCENTRATION IN THE BLOOD AND PERCENT OF TOTAL LACTATE FROM THE DOSE

*Denotes Labelled Glucose Administration
**Denotes significance at the α = 0.05 level

PARAMETER	(n)	GROUP	TREATMENT DAY 1 GLUCOSE	DAY 1 EXERCISE	DAY 2 GLUCOSE	DAY 2 EXERCISE	MEAN ± S.E. (mls) (%)	t-TEST α=0.05
TOTAL VOLUME OF CARBON DIOXIDE PRODUCED IN THE FIRST 40 MINUTES POST-EXERCISE	(5)	7	X	X			386 ± 12	
	(5)	10	--	X			384 ± 13	
	(5)	7	X	X			[illegible] ± 12	**
	(5)	14	--	--			295 ± 16	
	(5)	10		X				**
	(5)	14		--				
	(5)	2	X	X	--	X	395	
	(5)	7	X	X			386 ± 12	
	(5)	4	X	X	X	X	375 ± 14	
	(5)	2	X	X	--	X	395 ± 14	
PERCENT OF THE TOTAL CO_2 PRODUCED IN THE FIRST 40 MINUTES POST-EX. DERIVED FROM THE GLUCOSE DOSE	(5)	6	*X	X			69 ± 6	
	(4)	3	*X	--			26 ± 3	**
	(5)	1	*X	X	--	X	15 ± 4	**
	(5)	6	*X	X			69 ± 6	
	(5)	13	X	X	*X	X	57 ± 10	*
	(5)	1	*X	X	--	X	15 ± 4	

TABLE IX THE EFFECT OF TREATMENT COMBINATIONS ON CO_2 PRODUCTION IN THE FIRST 40 MINUTES POST EXERCISE AND THE PERCENT DERIVED FROM THE GLUCOSE DOSE
* Denotes ^{14}C Administration
** Denotes significance at the α= 0.05 level

DISCUSSION

The importance of carbohydrate stores in muscle and liver to endurance work has been well elucidated (Hultman, 1967; Bergstrom et al., 1967; Gollnick et al., 1973). Similarly, the role of blood glucose in long term work has been outlined. The concensus of opinion is that there is a shift in the muscle metabolism to increased fatty acid uptake and oxidation (Keul et al., 1973). There is evidence that carbohydrate utilization is drastically increased during heavy exercise (Wahren et al., 1971; Costill et al., 1973). It was attempted in the present study to evaluate using various techniques, the effect of an ingested glucose supplement at different times prior to performance of high intensity interval work. The only situation in which an increase in performance was noted was the case of rats receiving two glucose supplements 24 and 1.5 hours before the exercise test. The performance was enhanced but not significantly so in those animals that received the glucose 1.5 hours before exercise performance. If it is true that blood glucose is important as a substrate during sprint exercise as is the case during prolonged work, (Keul et al., 1974; Baldwin et al., 1973), then the data of this study corroborates this idea. The post exercise blood glucose in the non treated animals was less than that of glucose dosed and control animals. The rats that were exercised and dosed had significantly higher blood glucose than exercised animals but not significantly higher than control groups. This indicates

that if the performance is dependent on blood glucose then the dosed animals have a greater capacity for this type of exercise. There was no significant difference between any of the groups that were sacrificed on day 1. Therefore, it is possible that exercise of this type does not depend on pre-exercise glucose dose or liver glycogenolysis to a large extent. It appears that, in the groups of animals with somewhat depleted liver glycogen and given the glucose supplement and exercised, the glucogenesis mechanisms are functioning as 42% of the total liver glycogen was derived from the glucose dose. Based on the non-significant differences of the mean blood glucose values in the different groups one must assume that liver glycogenolytic mechanisms must be working at a comparable rate such that equilibrium is maintained. Neither exercise of this nature nor the dose appear to shift the equilibrium either way. The muscle glycogen levels of the rats used in this study are somewhat lower than those reported for resting, sprint trained, control rats (Armstrong et al., 1974). The discrepancy of mean values could be ascribed to the orientation that was used in the study. Armstrong et al. (1974) specify the training program: the running speed was 80.5 m/minute for 30 seconds followed by 30 seconds followed by 30 seconds rest. This was done each day until exhaustion for 10 weeks prior to the experimental period. This type of training is known to augment the carbohydrate store in the muscle. However, they do not specify the time period between the end of training and beginning

of the experimental period. In this study the animals attained a higher intensity of work (85 m/minute, 30% grade) after three weeks of twice daily work periods. This training or orientation period is probably not sufficiently long to allow any augmentation that would take place due to training. The time between the final orientation bout and the experimental treatment was only 17 hours during which time they were deprived of chow. The fact the repletion of muscle glycogen was not complete was also evident in the histochemical analysis. There was evidence of depletion in all muscle fibers control and exercised, in that the PAS stain was never uniform in colour intensity. The muscle glycogen was further depleted to some degree in the plantaris and soleus muscles as a result of exercise. All fibers in the soleus muscle were stained lightly indicating depletion of glycogen stores in that muscle of animals that were only exercised on day 1. The plantaris muscle from these same animals did not appear (Appendix 5) to be depleted to the same extent and all three fiber types were comparably depleted (50%). The levels of glycogen depletion were significantly greater in the soleus muscle than in the plantaris muscle (Table IV, VI). Although the glycogen concentration in the muscle of animals given glucose were not significantly higher than control levels, the muscle glycogen concentration was significantly higher (not significant in soleus muscle) in animals fed the glucose and exercised than those just exercised (Table IV, VI). This mean value was significantly lower than those animals that

were just fed glucose in both the plantaris and soleus muscles. These differences indicate a "sparing" effect of muscle glycogen or preferential use of ingested glucose in these muscles in this type of exercise. It must be kept in mind as well, that the initial glycogen levels are quite low and the work intensity quite high and therefore one would expect a greater reliance on blood glucose as a substrate. The uptake of the dose glucose to muscle glycogen is quite high in non-exercised animals, further supporting the idea of initially low glycogen levels in the muscle. This uptake was significantly less than in the exercised and dosed animals.

The lactate concentration in the blood of exercised animals was quite high and attests to the extent to which this type of work relies on anaerobic metabolism. Armstrong et al. (1974) recorded higher blood lactate concentrations in sprint exercised rats than had previously been reported. The mean values obtained in this study are generally similar to his results. However, the mean blood lactate concentration of one group of animals studied rose to 207 mg%, somewhat higher than the maximum 190 mg% reported by Armstrong et al. The exercise intensity used in this study, as was pointed out earlier, was more severe than the one they used and as well their rats were trained 10 weeks prior to the test.

The rats that received glucose prior to exercise had a significantly higher blood lactate concentration than the exercised animals that did not receive glucose (Table VIII).

This is particularly noteworthy since there was no significant difference in the mean running times of the two groups. This might imply that anaerobic catabolism of the ingested glucose dose partially pre-empted the use of other substrates used by the non dosed animals or that some of the lactate produced by the non dosed animals is utilized aerobically by oxidative tissue to a greater extent than in the dosed animals. An estimated 61% of the total lactate produced was derived from the dose glucose either as blood glucose or as muscle glycogen. This information further supports the theory that during this type of work under these experimental conditions, there is extensive utilization of the dose glucose. In light of previously reported blood glucose and liver glycogen data and the finding that as much as an estimated 61% of the total blood lactate was derived from the glucose supplement, one is led to believe in this case, the substantial anaerobic utilization of the dose glucose.

The manner in which the CO_2 data was collected and reported makes it more difficult to interpret. The time over which the CO_2 production was measured necessitates the consideration of a number of additional sources of CO_2. As a gross measure it gives a relative idea of the eventual aerobic fate of the dose glucose. The total CO_2 production in the first 40 minutes post exercise in dosed and exercised animals was significantly greater than the animals that were dosed and not exercised and essentially equal to non-dosed exercised animals. An estimated 69% of this volume of CO_2

was derived from the glucose dose and this is significantly greater than the mean value of the dosed non-exercised group. This data reinforces the idea of the increased utilization of the dose blood glucose during this type of work under these conditions. Although the dose glucose made up substantial portions of lactate produced and had a glycogen sparing effect in exercised plantaris and soleus muscles as well as the liver, the results of this study indicate that there is no significant enhancement of performance or increased running time in rats that received a pre-exercise glucose supplement.

There was no significant difference in the day 2 performance in groups of animals that received the supplement on day 1 and those that did not. As well, there was no significant improvement of the performance on day 2 over day 1 in those animals that were dosed on day 1. Thus this data does not confirm a significant augmentation of performance capacity in rats having received glucosed and exercised 24 hours prior to day 2 exercise performance. These findings support the hypothesis that under these conditions the dose glucose on two successive days is an important substrate for performance of sprint work. This fact was born out directly by the data of this study previously described. If the glucostatic humoral mechanisms are functioning normally only a transient hyperglycemia over 24 hours would be expected and the blood glucose concentration would not be elevated before exercise performance on the second day. This is shown by

the high percentages of uptake of the glucose dose in liver and muscle tissue (Table III, V, VII). The blood glucose concentration data for these groups indirectly support this theory in that there was no significant difference in post exercise blood glucose levels in the animals dosed on day 1 and those not dosed with glucose. The significantly lower blood glucose in exercised animals compared with controls should confirm a substantial blood glucose utilization in sprint exercise. However, the mean glucose concentrations in acutely exercised animals having received glucose on day 1 and those not having received glucose are not significantly lower than control levels. However, the mean performance times in these groups are low (Table X) and this may account for the inconsistency. The blood lactate levels in these two groups (groups 7,8) although not significantly different, are elevated (Table VIII) indicating a high level of anaerobic work.

The post exercise muscle glycogen concentration in the two muscles measured were not significantly different in the animals dosed and not dosed with glucose on day 1. Since the work performance of these animals were not significantly different, it follows therefore, that glucose administration followed by exercise 24 hours prior to day 2 exercise did not increase muscle glycogen concentration to a large extent. This is signified by the finding that there was no significant difference in groups 12(GNNN) and 16(NNNN). The finding (Tables IV, VI) that the muscle glycogen levels of day 1

glucose dosed exercised and non glucose dosed, exercised animals were significantly lower than day 1 glucosed dosed, non exercised and control animals, suggests that muscle glycogen might seem, under these conditions of initially low muscle glycogen, a secondary source of energy for this type of work. However, it is possible that the dose glucose prior to exercise was incorporated into muscle glycogen in the 1.5 hour interval and then used as the substrate for the exercise. The groups that received glucose and exercise on day 1 and were sacrificed on day 2 were significantly lower in muscle glycogen than control values but higher than those of acutely exercised animals (Table IV, VI). These data indicate that the pre-exercise glucose dose of day 1 does not fully replenish the muscle glycogen stores and that day 2 exercise utilizes stored glycogen remaining in the muscle tissue.

The interpretation of the estimated percent of the total glycogen present in a muscle is difficult because it does not relate the absolute amounts of glycogen concerned. Thus an acutely exercised group of animals may have a higher percent of the total muscle glycogen derived from the dose than a control group but may still have a lower absolute uptake of glucose from the dose. Therefore, these results should only be considered in light of similar exercise conditions. There was no significant difference in the percent total glycogen derived from the dose in groups that received glucose and were sacrificed on day 1 and those that were

sacrificed on day 2 (Table V, VII). This data would suggest that there is no additional glucose uptake between day 1 and day 2 in these groups. This is perhaps due to the prior depletion of blood glucose to the extent that significant muscle uptake is impaired. The post exercise repletion of glycogen with dose glucose is indicated by the higher percent (not significant in soleus) in animals sacrificed on day 2 after glucose supplement and exercise on day 1 than in those receiving glucose and exercise and sacrificed on day 1 (Table V, VII). These data suggest that under these conditions the glucose dose was stored as muscle glycogen only to the extent determined by some other factor than absolute depletion--perhaps this is the availability of glucose. A significant improvement in performance of this type of work would not be expected if the performance capacity was a function of muscle glycogen alone, as there is no increased storage over control levels due to prior glucose supplement administration.

Although not significantly so, the liver glycogen concentration in control and non-exercised but glucose dosed animals was higher than that of exercised animals (Table III). This information suggests that although the liver is not implicated to an appreciable extent in the acute exercise situation, the liver does compensate for utilization of blood glucose and muscle glycogen over a 24 hour period. The data also show a significant liver uptake of the day 1 dose in exercised animals in that the percent of the total liver

glycogen on day 2 derived from the day 1 dose is higher in day 1 exercised than non exercised animals.

The percent of the total CO_2 produced in the first 40 minutes post exercise derived from the day 1 dose was less after exercise on day 2 than after exercise on day 1. Thus, probably a large amount of the dose has been metabolized in the interim between and during exercise bouts.

These data imply that not only does the glucose supplement of day 1 not improve the performance of this type of sprint exercise to a large extent on day 2, it is also used to a much lesser extent on day 2 than on day 1. It indicates as well that a large part of the dose glucose is not accessible for metabolism during exercise on day 2, perhaps because it has been catabolized or stored before day 2 exercise.

The animals that were dosed with glucose on both treatment days showed significant increases in performance capacity over those that did not receive any glucose on either day and those that received glucose on day 1 only (Table I). Further, there was a significant improvement in day 2 performance of animals dosed on both days over day 1 performance of dosed animals. One can only speculate as to the mechanisms by which this occurs. The fact that the dose glucose is used to a large degree as a substrate for this type of exercise under these conditions emphasizes the importance of the day 2 glucose supplement. Whether it was used as blood glucose or incorporated into muscle glycogen and then used as substrate is unclear. Further, it seems that endogenous

muscle glycogen is important as well and therefore fully repleted muscle glycogen stores would be beneficial to performance of this type of work. Since the second glucose dose could serve to replete the glycogen stores, the necessity of day 2 dose for improved performance is indicated. The groups of animals that received glucose supplement on both days before the acute exercise test did not have significantly different blood glucose concentrations than those that did not receive the glucose on the second day. This might imply that the dose glucose was used directly or was incorporated rapidly into muscle or liver glycogen.

As mentioned previously, the running times for the group which received glucose and exercise on both days was significantly better than the group which did not receive glucose on day 2. Therefore, in light of similar liver glycogen levels (Table III) and non-significantly different muscle glycogen (Table IV) and blood glucose levels (Table II) in the animals which were dosed and exercised on both days versus those that received the dose on day 1 only, one could reason that the improved work performance of the day 2 dosed animals was due to the additional dose of glucose received on day 2. This is further implied in that the animals which were dosed and exercised on both days had significantly lower blood glucose levels than animals which were dosed both days but exercised only on day 1 (Table III). Also, this latter group had higher blood glucose levels than animals which were dosed and exercised on day 1 and had no treatment on

day 2 (Table III). Although non-significant, the mean muscle glycogen values of groups 7(GEGE) and 8(GEGN) show a difference between them (soleus 0.06 $\pm$ 0.00 and 0.14 $\pm$ 0.04; plantaris 0.03 $\pm$ 0.00 and 0.10 $\pm$ 0.01). There is an implicationperhaps that performance of this type of work under these conditions depends to a limited extent on the muscle glycogen stores.

In interpreting the percent of the total glycogen derived from the dose it must be realized that although differences in percent values may not be evident, the absolute amount of dose glucose incorporated may be quite different. In groups that were dosed and exercised on day 1 about 30% and 54% of the muscle glycogen in soleus and plantaris, respectively, analyzed on day 2 was derived from the dose of glucose dosed, exercised and glucose dosed again on day 2 incorporated glucose from the day 2 dose such that 47% and 25% of the total muscle glycogen was incorporated from the dose in the soleus and plantaris muscles, respectively (Tables V, VII). Since the total glycogen in these two groups in both muscles was not significantly different, it seems that greater uptake of the second dose occurred in soleus muscle but not in plantaris. The reason might be related to the initial levels of glycogen on day 2 but these were shown to be similar. Therefore the reason for the preferential uptake in soleus as a result of the additional dose but not in plantaris is unclear. Although it is possible that since soleus is solely oxidative, this type of tissue

may have a faster rate of glycogenesis than glycolytic tissue. The post exercise percent of the total muscle glycogen derived from the dose was significantly higher in those animals that were dosed and exercised on both days (label on day 2) than those that were dosed and exercised on day 1. Also, since both the total muscle glycogen and the percent glycogen from dose were greater (though not significantly) in the group which had additional glucose on day 2 (Table IV), it would further indicate that the day 2 dose does play a role in increasing muscle glycogen levels.

There was essentially no change in the liver glycogen concentration whether the animals were given glucose and/or exercised or not. This data might imply that liver glycogenolysis does not contribute significantly to the substrate pool required in the performance of this sprint type work. However, it is possible that the liver may indirectly contribute to day 2 exercise by partially replenishing the previously depleted muscle glycogen store or supplying glucose to the blood as a substrate. Data from the percent of the total liver glycogen derived from the supplement might suggest that the liver could play this role in that the percent liver glycogen derived from the dose was found to be significantly higher in animals that were glucose dosed and exercised again on day 2 (Table VIII). This data indicates that a large part of the day 1 dose which was incorporated into the liver was utilized either before exercise on the second day through basal metabolism and/or replenishment of glycogen

stores, or used during exercise as blood glucose. There is also an indication that there is some additional uptake by the liver of the day 2 glucose dose after the animals have received glucose and exercise on day 1 (Table III). These data also indicate that there is a large uptake of the day 2 glucose dose after glucose dose and exercise on day 2 since 56% of the liver glycogen was from the ingested day 2 dose. Since there is no difference in the liver glycogen levels in groups which had exercise and glucose and those which did not on day 2, it seems that the liver glycogen from the day 2 dose does not contribute extensively to the performance of this type of work (Table III).

The post exercise lactate concentration after day 2 glucose supplement was not different from that of animals that were not dosed on day 2. However, 36% of the total blood lactate was derived from the day 2 dose and this is significantly higher than that reported value from the day 1 dose (9%) after exercise on day 2. It is interesting to note that the contribution of day 1 dose to day 1 lactate (61%) is significantly higher than the contribution of day 2 lactate (36%) after having had glucose on day 1. This implies a "sparing" effect of the day 1 glucose dose on the day 2 dose glucose utilization. Again this fact emphasizes the important role of the day 2 glucose administration in the performance of sprint work in this study.

HISTOCHEMISTRY - The histochemical analyses of the plantaris and soleus muscles indicate that there was essentially

no alteration of the fiber type composition of these muscles over the treadmill orientation period (Appendix 7). The fiber type composition of soleus and plantaris muscles of the animals in this study were similar to those reported in normal animals in the literature (Baldwin et al., 1972; Ariano et al., 1973).

The glycogen content, as indicated by the intensity of the PAS stain, of the muscle of control animals was somewhat depleted in that some fibers appeared light compared to others. In both muscles analyzed there was no uniform dark stain throughout the section that is expected from a saturated PAS stain (Appendix 7). This corroborates the biochemical data indicating lower levels of muscle glycogen in these animals. Generally it is noted that the animals that were exercised before sacrifice show a high percent lighter staining fibers than those that were not exercised, dosed and not exercised and the rats that were glucose dosed and exercised. Depletion after day 1 exercise is demonstrated in group 5 in the soleus muscle but the F.G. and F.O.G. of the plantaris are not extensively depleted of their glycogen. This suggests recruitment of both of the oxidative fibers in soleus muscles and certainly more slow oxidative in the plantaris. In the other acutely exercised groups it appears that all fibers are generally depleted and to the same extent after exercise. Gollnick et al. (1973) suggested increasing recruitment of all fibers at or near the end of exhaustive exercise in humans and perhaps this is the explanation for

the severe depletion in this study.

CONCLUSIONS

A summary of the group mean values (±S.E.) of the parameters measured is presented in Table XIV. High intensity work of the type used in this study was shown to utilize carbohydrate metabolism as an energy source to a large extent. The high post exercise blood lactate concentration attests to the predominantly anaerobic nature of this sprint work. It seems, from the data presented, that the primary sources of this carbohydrate substrate are the blood glucose and muscle glycogen pools. The liver glycogen pool is perhaps a secondary source of carbohydrate under these experimental conditions being implicated more in a restorative role after the exercise is completed. Thus it was shown that pre-exercise glucose administration on the day before performance does augment the performance capacity, presumably by reinforcing depleted or partially depleted muscle glycogen stores. This initial dose is in itself not sufficient to significantly affect the performance but must be followed by another glucose supplement just before day 2 exercise. Glucose supplements given immediately before (1.5 hours) sprint exercise is anaerobically metabolized to a degree but does not in itself seem to be sufficient to increase the capacity for sprint work in rats. Thus it is noteworthy that a combination of both glucose treatments used in the study did produce a significant improvement in the performance of high intensity work in rats. It was shown that the orientation

TREATMENT	WORK PERFORMANCE (secs)	$[LACTATE]_{BL}$ (mg%)	% LACTATE/ DOSE	CO_2 (40 mins) (mls)	% CO_2/ DOSE	$[GLUCOSE]_{BL}$ (mg%)	MUSCLE GLYCOGEN					
							SOLEUS		PLANTARIS		LIVER	
							[GLYCOGEN] (mg%)	% GLYCOGEN/ DOSE	[GLYCOGEN] (mg%)	% GLYCOGEN/ DOSE	[GLYCOGEN] (mg%)	% GLYCOGEN/ DOSE
GE	276±39	170±37	61±6	386±12	69±6	99±13	.08±.03	27±7	.06±.03	28±6	.11±.03	42±8
GN		13±1		295±16	26±3	114±9	.18±.03	23±7	.01±.02	15±5	.12±.05	36±3
NE	236±24	123±18		384±13		61±6	.07±.01		.03±.00		.07±.01	
NN		13±2				106±4	.10±.02		.11±.01		.16±.05	
GENE	258±22 259±40	167±37	9±1	395±14	15±4	105±8	.06±.01	27±7	.07±.02	23±9	.20±.08	15±3
NENE	207±36 249±42	186±17		384±13		86±11	.05±.01		.07±.01		.13±.04	
GEGE	276±25 384±33	207±15	36±7	375±14	57±10	93±5	.06±.01	65±4	.07±.03	54±1	.11±.01	63±11
GEGN	267±30	21±6		381±13		131±10	.14±.02	46±9	.10±.06	25±7	.27±.16	56±7
GENN	225±25	22±4				109±5	.10±.01	39±7	.09±.06	54±8	.17±.03	45±3
GNNN		17±6	33±8			109±12	.18±.02	18±3	.19±.02	12±2	.69±.32	18±6
NNNN		23±3				108±6	.20±.03		.21±.03		.77±.16	

TABLE XIV SUMMARY TABLE OF THE GROUP MEAN VALUES OF THE PARAMETERS DISCUSSED

to sprint treadmill running did not induce changes in the fiber type composition of the rat soleus and plantaris muscles.

RECOMMENDATIONS - In view of the low tissue glycogen concentration levels observed in the animals of this study, it is recommended that the time interval between the final orientation exercise bout and the beginning of the food deprivation be extended. A period of 36 to 48 hours during which the animals are fed ad libitum purina chow is thought to be sufficient to replete glycogen stores. Since there are significant diurnal changes in liver glycogen concentration it is important to begin the starvation period late in (8-12 hours) the wake phase, and thus ensure maximal tissue glycogen levels before the beginning of experimental treatment.

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APPENDIX 1

REVIEW OF THE LITERATURE

HISTORICAL BACKGROUND[1]

Researchers have differentiated skeletal muscle fiber types for well over a century. The basis for this differential analysis has been modified through time. The first report of a variation in the colour of certain muscles of the rabbit was published by Lorenzini in 1678. At that time he assumed that the appearance of red in some muscle was due to a richer supply of blood to these muscles. A hypothesis advanced by Kuhne in 1865 suggested that the red colour of some fibers was due to the presence of a pigment intimately associated with the contractile proteins of the muscle. This substance termed myocrome by Morner in 1897 later became known as myohemoglobin or myoglobin. In 1871 Lankester noted that the most active muscles contained greater amounts of this pigment. Subsequently, Ranvier in 1873 using electrical stimulation of "red" and "white" muscles described some of the contractile characteristics of these different fibers: "red" muscle contracted and relaxed at a slower rate than "white" muscle. Grutzner in 1887, while studying the properties of different muscle during single twitch and during tetanus confirmed the work of Ranvier and suggested that muscles of higher animals contained a mixture of the different fiber types. He also theorized that the ratio of the "red" to "white" fibers in a particular muscle governed

[1]Much of the information and reference material are contained in Piehl (1974a).

its contractile properties. In that same year, Gliess suggested a different metabolism for "red" and "white" muscle fibers. In 1891 Knoll classified the fibers as "protoplasma-arm" and "protoplasma-reich". It wasn't until 1927 that Denny Brown noted that fibers displaying similar contractile properties were contained in discrete bundles within the muscle. The concensus of these early reports was that "red" muscles were associated with sustained repetitive type work while "white" muscle was associated with rapid vigorous contractions of briefer duration.

The modern histochemical and biochemical characterization of skeletal muscle fiber is based on these ideas that were first reported before the turn of the century. Extensive examination of many different muscles of a variety of species, including horses (Lindholm; 1974), pigs, and humans (Gollnick, 1973; Dalrymple, 1974; Ariano, 1973). Ogata (1969) described three distinct fiber types in humans based on the staining intensity of several oxidative enzymes. Barnard et al. (1971) using histochemical techniques as well described three fiber types in guinea pigs. Barany et al. (1965) found a higher ATPase activity in man in white muscle than in red muscle and later (1967) showed a high correlation between speed of contraction and ATPase activity. For this reason, some researchers now term the fibers as being fast twitch and slow twitch based on ATPase staining intensity (Gollnick, 1973). Other methods of fiber characterization are based on atypical constant proportion enzyme groups

(Bass, 1969), a concept which recently has been questioned (Dalrymple, 1974), and respiratory capacity of the different fibers (Baldwin, 1972). Today there is not 100% agreement, but is is generally construed that human muscle contains only two distinct fiber types; fast-twitch glycolytic and slow-twitch oxidative (Gollnick, 1974) based on ATPase activity and NADH diaphorase. Differences in resting membrane potentials and $[K^+]_i$ in different fiber types has been demonstrated (Campion, 1974).

MUSCLE GLYCOGEN DEPLETION DURING EXERCISE

The fact that the body's carbohydrate supply is stored as glycogen principally in the liver, in muscle and in the kidney has been recognized for many years. So too, has the importance of muscle glycogen to the metabolism of muscle. Muscle glycogen was first described by Claude Bernard in 1859. Since that time a number of studies of human muscle have been carried out, aided by the muscle biopsy technique of Bergstrom (1962). This method provides a means to accurately determine the glycogen content of muscle. Hermanson (1967) reported that during prolonged heavy exercise (78% $\dot{V}O_2$ max) there is progressive decrease in muscle glycogen concentration from 1.6 g/100 gww to less than 0.5 g/100 gww in the quadriceps femoris of human subjects exercising on a bicycle ergometer. This glycogen depletion was found to closely parallel the calculated rate of carbohydrate combustion using RQ and $\dot{V}O_2$. This suggests that at high workloads it is primarily the glycogen stores that supply the

substrate. Low glycogen content was found to accompany the point of exhaustion beyond which the subjects could no longer continue exercise. On the basis of ATPase and PAS staining techniques, Gollnick (1973) showed primary depletion of the slow twitch fibers during prolonged exercise. However, as exercise continued and exhaustion was eminent, fast twitch fibers were recruited to a larger extent, as shown by their glycogen depletion. These subjects worked on a bicycle ergometer at moderate workloads (60-70% $\dot{V}O_2$ max). He noted in these subjects greater rates of depletion of the fast twitch fibers than in the slow twitch fibers. This demonstrates the inefficiency of ATP production and the greater consumption of ATP per unit of contractile force in the fast twitch fibers. The energy turnover however is much higher than in fast twitch fibers.

The absolute amount of glycogen stored in muscle is dependent basically on two enzyme systems; that for the synthesis of glycogen and that system involved in its breakdown. One of the enzymes for glycogen synthesis was found to be rate limiting (Leloir, 1957): glycogen synthetase. The enzyme phosphorylase is responsible for the breakdown of glycogen. The max rate of glycogen synthesis in vitro is about 1/100 that of breakdown using these enzyme systems. It has been reported that the amount of glycogen as well as the activity of this enzyme varies in the different human skeletal muscle fiber types (Engel, 1962; Edstrom, 1969). This was also shown to be the case in rats (Kugelberg, 1968). White

fiber has been found to have higher phosphorylase activity. It is reasonable to assume that the activity of this enzyme and the amount of glycogen present in a fiber reflects the ability to utilize glycogen and further it is likely that fibers with a high level of this enzyme predominantly use glycogen as substrate in energy production. The PAS stain has been used to follow the depletion of glycogen in the fibers of skeletal muscle (Edgerton, 1970a) and it was found during electrical stimulation of nerve and muscle that there was a preferential loss of glycogen in white fibers. This was found not to be the case in the depletion of glycogen in guinea pig skeletal muscle in prolonged exhaustive exercise (Edgerton, 1970b; Armstrong, 1973). Costill (1973) also on the basis of PAS staining, studied glycogen depletion patterns in human skeletal muscles during long distance running. They demonstrated marked depletion of slow-twitch fibers. This was not in total agreement with the work of Gollnick (1973) who showed greater depletion of slow-twitch fibers at the outset of bicycle exercise with increasing fast-twitch fiber depletion as exhaustion became apparent. This was perhaps so because at the end of the race the runners tended to walk the difficult sections and hills, in which case the fast twitch fibers may not have been recruited. They concluded from this study that there is a primary reliance on slow twitch fibers in prolonged running. They also point out the limitations of assessing substrate utilization or availability in working fibers from muscle samples with mixed

fiber populations.

Baldwin (1973) determined the time course depletion of substrate stores in three skeletal muscle fibers of rats during prolonged running. They showed a difference in depletion patterns under different types of running exercise programs in rats. One group ran at 1 mph, 8% grade, and speed increased to 1.5 mph for 1 minute every ninth minute for 2 hours; another group ran alternate 30 second periods of 0.5 mph and 1.5 mph for 2 hours; a third group ran at one mph for 2 hours at an 8% grade. Changes in muscle glycogen content in the different fibers was noted at various points during exercise. There was minimal depletion in the white (vastis lateralis) in all exercise programs when compared to depletion in the red fibers (deep vastis lateralis) and intermediate fibers (soleus) over the 2 hour period. They concluded that when exercise is of an intensity that can be maintained for 2 hours of longer, the work is performed primarily by the red and intermediate fibers with little involvement of the white fibers. This is in keeping with Gollnick's work and Costill's work (1973). They also noted that during the 2 hour period, 85% of the glycogen store in liver was depleted in all groups. Thus they emphasized the importance of liver glycogen as a substrate for muscle metabolism. In a subsequent study (Baldwin et al., 1975) this group demonstrated in rats a "sparing effect" of endurance swim training on muscle glycogen depletion during an acute treadmill run. The F.G., F.O.G., and S.O. fibers were depleted significantly more slowly in the trained than in the

untrained animals. This was also the case in the depletion of liver glycogen. Terjung (1974) also emphasized the importance of liver glycogen in swimming rats to exhaustion. The contribution of glucose by the liver was found in both cases to be considerably larger than that of muscle glycogen. In a follow-up study Gollnick (1973c) reported the muscle glycogen depletion pattern in human skeletal muscle during short high intensity exercise on a bicycle ergometer. The exercise was estimated to require the equivalent of 150% $\dot{V}O_2$ max and was performed in 6 one-minute sprints with 10 minutes of rest between work bouts. Biopsies were taken from the vastis lateralis at various times during exercise and using PAS staining they showed increasing total glycogen depletion in muscle with increasing number of work bouts. They demonstrated also that at this intensity of work the low oxidative, high glycolytic fast-twitch fibers were first to become depleted of glycogen stores suggesting greater recruitment of these fibers. Staudte et al. (1973) showed a similar response in rats during sprint training and they too suggest greater gast-twitch fiber recruitment in short-term high intensity work. This has earlier been shown by Barnard (1970) in the electrical stimulation of guinea pig skeletal muscle. The results of studies of muscle fibers during prolonged work are exactly the opposite in that the slow-twitch fibers were first to be depleted. A study by Gollnick (1974a) demonstrated this differential recruitment at different exercise intensities. The study involved human subjects pedalling a

bicycle ergometer at different rates and different work loads from 30-150% $\dot{V}O_2$ max. They demonstrated a dramatic increase in total muscle glycogen depletion with increasing workload. PAS staining revealed that the high oxidative slow-twitch fibers were first depleted at submax workloads; ie. after prolonged low intensity work there was still high glycogen content in the fast-twitch fibers. Progressive depletion of high glycolytic fast twitch fibers as work time increased; further there was pronounced depletion of fast-twitch fibers at workloads above $\dot{V}O_2$ max. On the basis of these results they suggested increased recruitment of the fast twitch fibers during high intensity exercise. Exner et al. (1973) also suggests a preferential recruitment of fast twitch muscle during short-term high intensity (isometric) work in rats. This effect of high intensity, intermittent or continuous, work to exhaustion was further corroborated by Gollnick (1974b) in humans. He showed that during isometric contractions involving less than 20% mvc there is a greater and greater depletion in fast-twitch than in slow-twitch fibers. Thus they concluded that this selective glycogen depletion is indicative of a differential recruitment of muscle fibers during isometric exercise of varying intensity.

GLYCOGEN REPLETION AND GLYCOGEN SYNTHETASE ACTIVITY

Much of the information we have today regarding muscle glycogen supercompensation was contributed by Bergstrom and Hultman. In the original studies (1966) they found that after decreasing glycogen stores to near zero values in muscle

through exhaustive exercise, a high carbohydrate diet enhancement drastically glycogen storage in the exercise muscle. In the non-exercised muscle there was minimal increased storage comparatively. They concluded that exercise with concommittant local glycogen depletion enhances glycogen resynthesis and that the factor involved acts locally in the exercised muscle. This effect last for 2-3 days. Earlier, Bar (1965) had shown that oral administration of glucose to fasted rats three hours before sacrifice resulted in the increased glycogen storage in red (diaphragm) muscle but not in white (ext. oblique) muscle. When the muscles were incubated in ^{14}C-glucose medium there was an increase in glycogen in all samples except in red muscle with high initial glycogen levels. They concluded that the differences in net glycogen change and glucose incorporation into glycogen was secondary and not specific to fiber type. Bocek et al. (1966a,b) on the basis of in vitro studies on the incubation of red and white fibers interpreted their data as indicating that the pathway glucose to glycogen is more active in red muscle and that glycogen metabolism is different in both fiber types. From their work on isolated rat soleus muscle, Moorthy and Gould (1969) implied that glycogen synthesis was limited by the availability of glucose and not by the level of glycogen itself.

More recently attention has centered on the activities of enzymes responsible for the resynthesis of glycogen. UDPG-transglucosylase or glycogen synthetase has been found

to have the lowest activity of the enzymes involved. It is therefore thought to be the rate-limiting enzyme in the series. Activity of this enzyme is regulated by the interconversion of 2 isomers of the protein: the 'D' form (dependent on the presence of glucose-6-phosphate for activity) and the 'I' form (independent of glucose-6-phosphate for activity). The conversion from the 'I' form to the 'D' form is mediated by an enzyme synthetase kinase which is activated in the presence of C-AMP. Synthetase D is dephosphorylated to the I form in the presence of synthetase D phosphatase:

$$\text{mATP} + \text{Synthetase I} \xrightarrow{\text{Protein Kinase}} \text{Synthetase D} + \text{mADP}$$

$$\text{Synthetase 'D'} \xrightarrow[\text{Synthetase D phosphatase}]{} \text{Synthetase I} + \text{mP}_i$$

Under physiological conditions glycogen synthesis is controlled largely by the I isomer owing to the inhibition of the D form by ADP, ATP and P_i (Bergstrom, 1972). Activity of the I-form is inversely related to the concentration of glycogen in muscle; ie. when glycogen content is low there is a higher concentration of the enzyme in the I-form (Danforth, 1968) in rats. In man at rest the I-form represents 10-25% of the total synthetase activity, however, after exercise depletion of glycogen this activity is about 75% of the total (Bergstrom, 1972). This confirmed earlier observations of an inverse relationship of glycogen and I-form activity in rats. Taylor (1974) showed that glycogen synthesizing enzyme activity was related to resting muscle

glycogen content and the relative fitness of the subject.

Terjung (1974) studied the rate of glycogen repletion in rat skeletal muscle and liver after an exhaustive swim. They used soleus, superficial vastis lateralis and deep vastis lateralis as being typical of each fiber type. They found that the exhaustive swim almost totally depleted the liver but skeletal muscle was depleted by 75%. They suggested that hypoglycemia secondary to liver depletion, rather than depleted muscle glycogen was responsible for the physical exhaustion in swimming. The peak rates of glycogen resynthesis in fast-twitch fibers (red) were three times greater than peak rates in white muscle and two times greater than those in slow red muscle. The liver repletion rate was many times greater than in muscle. They also suggested that the synthetase I form is not the rate limiting step in glycogen resynthesis because repletion continued at a high rate even when synthetase I form activity decreased in all muscle fiber types. Piehl (1974), using human subjects, showed no difference in glycogenesis of the two fibers.

DIET AND GLYCOGEN STORAGE

The gross effects of diet and exercise on glycogen storage in human skeletal muscle have been studied in depth (Bergstrom, 1967, 1972; Hultman, 1967; Pernow, 1971, Gollnick, 1972).

Bergstrom (1967), studying human subjects during bicycle exercise reported that muscle glycogen content in man can be manipulated by exercise depletion and diet. The enhancement

of glycogen synthesis by carbohydrate rich diet is localized to the exercise depleted muscle. When a high fat or protein diet was administered, incomplete and slow glycogen synthesis occurred. On the other hand, only moderate increases in muscle glycogen occurs when a carbohydrate rich diet is given without previous depletion of glycogen. The average work capacity was also influenced by diet after depletion, indeed the longest work times were recorded by those subjects on the high carbohydrate diet. Muscle glycogen levels after depletion and diet were 3.7 g/100 gww. After subsequent depletion, these values were 0.45 g/100 gww. Similar results were also reported by Saltin (1967). Hultman (1967) also reported maximum glycogen storage values up to 4-5 g/100 gww after exercise depletion and subsequent carbohydrate diet. He also noted longer work times in these subjects as opposed to those in subjects on fat and protein diets. He showed too, that synthetase I form activity decreased in spite of high levels of glycogen resynthesis. Pernow (1971) demonstrated that work capacity was highly correlated with depletion of muscle glycogen stores. It was found that exercise depleted glycogen drastically. When subjects were kept on a carbohydrate diet the work capacity increased significantly. Bergstrom (1972) showed that following exhaustive work glycogen in the muscle was almost totally depleted in the quadriceps femoris of human subjects. The synthetase I form activity increased accordingly from 12-70% of the total synthetase activity. During the administration of a high

carbohydrate diet after exercise the glycogen resynthesis was recorded at a high rate when synthetase I activity was decreasing. Glycogen repletion continued when synthetase I form was back to normal levels. Gollnick (1972) studied the effects of diet and exercise on glycogen changes in fiber types of human skeletal muscle. His observations were based on PAS staining and ATPase activity. In subjects performing at 74% of $\dot{V}O_2$ max for 30 minutes on a bicycle ergometer the effect of diet variation was studied. It was found that glycogen depletion during exercise was less in fast-twitch fibers than in slow-twitch after the mixed and carbohydrate diets but much higher after the fat-protein diet. Thus it would seem that high carbohydrate diet has a sparing effect on fast-twitch fibers during prolonged sub-maximal work.

Glucose administration during prolonged submaximal work tends to minimize the fall in muscle glycogen (Hultman, 1967). Curtis-Prior (1967) studied the assimilation of orally administered glucose in rats. It was found that many tissues were actively involved in the uptake of glucose but that muscle because of its larger proportions in terms of body weight (38%) showed the greatest absolute uptake. After 1.5 hours of post-administration skeletal muscle uptake represented 17% of the total dose. Nuttall (1972) also studied the effect of oral glucose administration to rats (4 g/Kg in 34% solution) and showed over a two hour period increased synthetase I form activity with hyperglycemia. The glucose absorption rate was also found to be first order with the

amount of glucose remaining in the gastrointestinal tract. Costill (1973) showed that intestinal absorption of glucose in man was not affected by exercise but that orally administered glucose was used at a much higher rate during exercise than at rest.

APPENDIX 2

DAY		GRADE (%)	SPEED (m/sec)	INTERVAL 'ON' (secs)	INTERVAL 'OFF' (secs)	REPETITIONS
(MON) 1	AM	30	20	60	60	3
	PM	30	25	60	60	3
(TUE) 2	AM	30	30	45	45	3
	PM	30	30	45	45	4
(WED) 3	AM	30	35	30	30	5
	PM	30	35	30	30	5
(THU) 4	AM	30	40	30	30	5
	PM	30	45	20	20	5
(FRI) 5	AM	30	50	20	20	5
	PM					
(MON) 8	AM	30	45	20	20	5
	PM	30	50	20	20	5
(TUE) 9	AM	30	55	15	20	5
	PM	30	55	15	20	7
(WED) 10	AM	30	60	15	20	8
	PM	30	60	15	20	8
(THU) 11	AM	30	65	15	20	8
	PM	30	70	15	20	8
(FRI) 12	AM	30	75	15	20	8
	PM					
(MON) 15	AM	30	70	15	20	8
	PM	30	75	15	20	8

DAY			GRADE (%)	SPEED (m/sec)	INTERVAL 'ON' (secs)	INTERVAL 'OFF' (secs)	REPETITIONS
(TUE)	16	AM	30	80	15	20	8
		PM	30	80	15	20	9
(WED)	17	AM	30	85	15	20	10
		PM	30	85	15	20	10
(THU)	18	AM	30	85	15	20	10
		PM	30	85	15	20	10
(FRI)	19	AM	30	85	15	20	10
		PM					
(MON)	22	AM	30	85	15	20	10
		PM	30	85	15	20	10
TREATMENT (DAY 1)		(TUE) AM	30	85	15	20	TO EXHAUSTION
TREATMENT (DAY 2)		(WED) AM	30	85	15	20	TO EXHAUSTION

Figure 5 Rat Orientation Protocol to Treadmill Sprint Running. 22 Days Pre-Treatment

APPENDIX 3

GROUP #		RUNNING TIME (seconds) DAY 1	DAY 2	[LACTATE]$_{BL}$ CONCN (mg%)	% FROM DOSE	TOTAL CO_2	% FROM DOSE
1	$\bar{X}$	246	214				15
	S.E.	24	17				10
2	$\bar{X}$	261	212			395	
	S.E.	14	22			14	
3	$\bar{X}$	225		22	39		26
	S.E.	25		4	9		3
4	$\bar{X}$	372	498			375	
	S.E.	32	27			4	
5	$\bar{X}$	307		170	61		
	S.E.	48		37	6		
6	$\bar{X}$	267	351	167	9		69
	S.E.	33	62	37	1		6
7	$\bar{X}$	255	330	207	36	386	
	S.E.	26	39	15	9	12	
8	$\bar{X}$	267		21	23	381	
	S.E.	30		6	5	13	
9	$\bar{X}$	264		123			
	S.E.	15		18			
10	$\bar{X}$	207	249	186		384	
	S.E.	36	42	17		13	
11	$\bar{X}$				13	47	
	S.E.				1	5	
12	$\bar{X}$			17	33		
	S.E.			2	6		
13	$\bar{X}$	210	348				57
	S.E.	16	83				10
14$_2$	$\bar{X}$		285			387	
	S.E.		11			25	
14$_1$	$\bar{X}$					295	
	S.E.					16	
15	$\bar{X}$			13			
	S.E.			2			

GROUP #		RUNNING TIME (seconds) DAY 1	DAY 2	$[LACTATE]_{BL}$ CONCN (mg%)	% FROM DOSE	CO_2 TOTAL	% FROM DOSE
16	$\bar{X}$			23			
	S.E.			3			

TABLE X MEAN VALUES OF RUNNING TIME, $[LACTATE]_{BL}$ AND CO_2 PRODUCTION FOR EACH GROUP

APPENDIX 4

GROUP #		MUSCLE GLYCOGEN SOL CONCN (mg%)	SOL %FROM DOSE	PLT CONCN (mg%)	PLT % FROM DOSE	LIVER GLYCOGEN CONCN (mg%)	LIVER % FROM DOSE	[GLUCOSE]BL (mg%)
1	$\bar{X}$							
	S.E.							
2	$\bar{X}$							
	S.E.							
3	$\bar{X}$	0.10	39	0.09	54	0.27	45	109
	S.E.	0.01	7	0.06	8	0.16	3	5
4	$\bar{X}$							
	S.E.							
5	$\bar{X}$	0.08	27	0.06	28	0.11	42	105
	S.E.	0.03	7	0.03	6	0.03	8	8
6	$\bar{X}$	0.06	27	0.07	23	0.20	15	105
	S.E.	0.01	7	0.02	9	0.08	3	8
7	$\bar{X}$	0.06	65	0.03	54	0.11	63	93
	S.E.	0.00	4	0.00	10	0.01	11	5
8	$\bar{X}$	0.14	46	0.10	25	0.17	56	131
	S.E.	0.04	9	0.00	7	0.03	17	10
9	$\bar{X}$	0.07		0.03		0.07		61
	S.E.	0.01		0.00		0.01		6
10	$\bar{X}$	0.05		0.07		0.13		86
	S.E.	0.01		0.01		0.04		11

GROUP #		MUSCLE GLYCOGEN SOL CONCN (mg%)	SOL % FROM DOSE	PLT CONCN (mg%)	PLT % FROM DOSE	LIVER GLYCOGEN CONCN (mg%)	LIVER % FROM DOSE	$[GLUCOSE]_{BL}$ (mg%)
11	$\bar{X}$	0.18	23	0.10	15	0.12	36	114
	S.E.	0.03	4	0.02	5	0.01	3	19
12	$\bar{X}$	0.14	18	0.19	12	0.69	18	109
	S.E.	0.02	3	0.01	2	0.32	6	5
13	$\bar{X}$							
	S.E.							
142	$\bar{X}$							
	S.E.							
141	$\bar{X}$							
	S.E.							
15	$\bar{X}$	0.10		0.11		0.16		106
	S.E.	0.02		0.01		0.01		4
16	$\bar{X}$	0.25		0.21		0.77		108
	S.E.	0.02		0.03		0.33		6

TABLE XI TISSUE GLYCOGEN CONCENTRATION FOR EACH TREATMENT GROUP

APPENDIX 5

GROUP	PLANTARIS FG	% PAS D	M	L	FOG	% PAS D	M	L	SO	% PAS D	M	L
1												
2												
3	36	18	62		50	40	60		14	95	5	
4												
5	26	42	58		60	23	57	20	14		100	
6	19			100	68	7	9	84	13			100
7	11			100	44			100	15			100
8	30	49	51		60	44	56		10	21	79	
9	48	14	37	49	40		50	50	12		50	50
10	6			100	78		55	45	16			100
11	53	50	50		28	70	30		19		50	50
12	25	50		50	61	41	50	9	14		50	50
13												
14												
15	48	53	47		43		50	50	9		50	50
16	26	50	50		59	75	25		13		100	
MEAN	33				54				13			
S.E.	± 4				± 4				± 1			

TABLE XII SUMMARY OF FIBER TYPE COMPOSITION OF PLANTARIS MUSCLE AND PAS STAINING INTENSITY FOR EACH FIBER TYPE

D - Dark M - Medium L - Light

	SOLEUS									
GROUP	FG	% PAS			FOG	% PAS			SO	% PAS		
		D	M	L		D	M	L		D	M	L
1												
2												
3					22	22	78		78	40	60	
4												
5					38		100		62		100	
6					39		100		61		100	
7					24	50	50		76	64	36	
8					43	87	13		57	61	34	5
9					28		100		72		100	
10	7				33		100		60	8	37	55
11					34	26	74		66	12	88	
12					21	46	54		79	24	56	20
13												
14												
15	2	100			38	100			60	22	78	
16					41	43	59		59	29	61	10
MEAN	1				33				66			
S.E.	± .64				± 2				± 2			

TABLE XIII SUMMARY OF FIBER TYPE COMPOSITION OF SOLEUS MUSCLE AND PAS STAINING INTENSITY FOR EACH FIBER TYPE

D - Dark M - Medium L - Light

APPENDIX 6

PURINA CHOW:

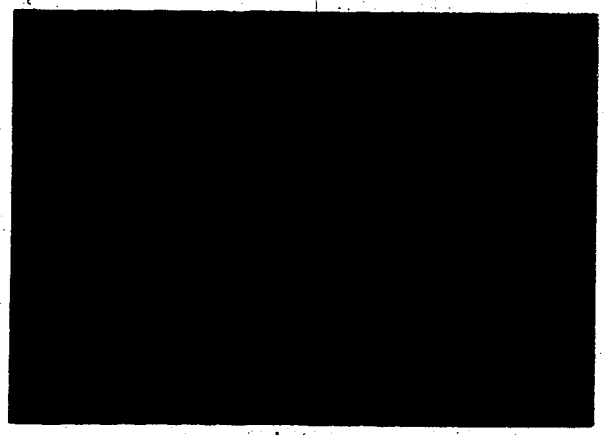
Crude protein not less than . . .	23.0%
Crude fat not less than	4.5%
Crude fiber not more than	6.0%
Ash not more than	9.0%

Meat and bone meal, dried skimmed milk, wheat germ meal, fish meal, animal liver meal, dried beet pulp, ground extruded corn, ground oat groats, soybean meal, dehydrated alfalfa meal, can molasses, animal fat preserved with BHA, vitamin B_{12} supplement, calcium pantothenate, choline chloride, folic acid, riboflavin supplement, brewers' dried yeast, thiamin, niacin, vitamin A supplement, D activated plant sterol, vitamin E supplement, calcium carbonate, dicalcium phosphate, iodized salt, iron sulfate, iron oxide, manganous oxide, cobalt carbonate, copper oxide, zinc oxide.

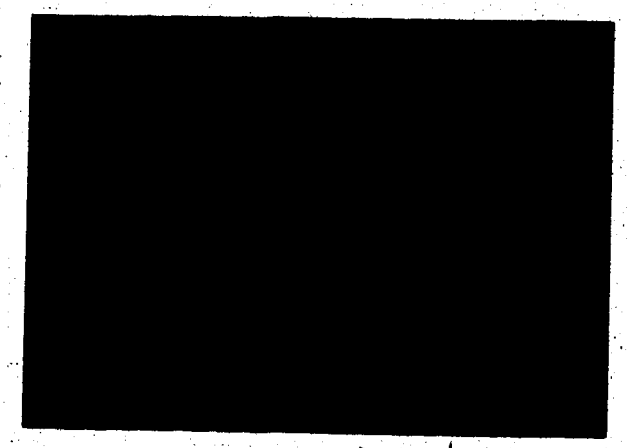
APPENDIX 7

1)

2)



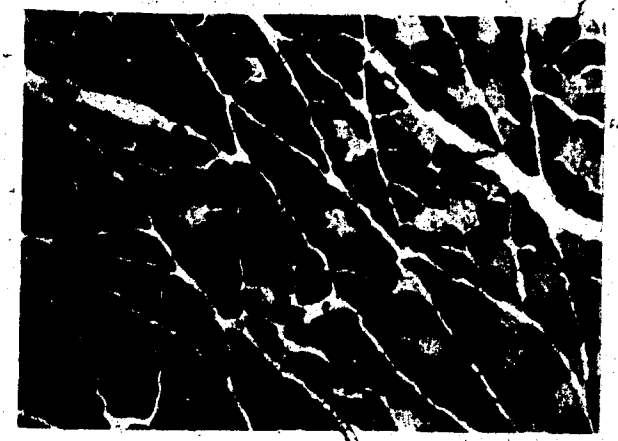
3)



A. SOLEUS HISTOCHEMICAL MICROGRAPHS

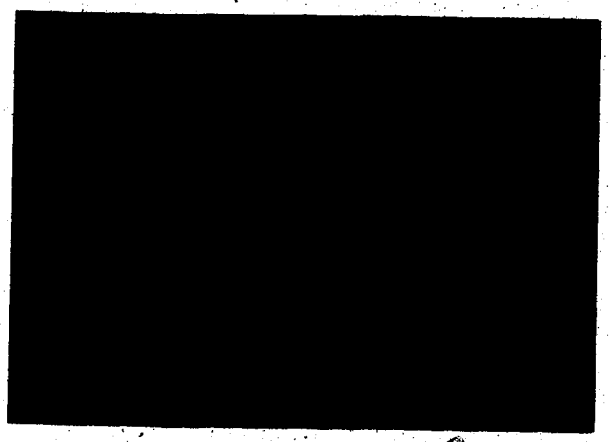
1) Myosin ATPase
2) NADH Diaphorase
3) PAS

1)



2)

3)



B. PLANTARIS HISTOCHEMICAL MICROGRAPHS

1) Myosin ATPase
2) NADH Diaphorase
3) PAS