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THE EFFECTS OF HIGH INTENSITY TRAINING AND EXHAUSTIVE EXERCISE ON THE GLYCOGENIC POTENTIAL OF L-LACTATE IN MAMMALIAM SKELETAL MUSCLE

by

Colin Robert Cory

B.Sc. (Kinesiology), Simon Fraser University, 1977

THESIS SUBMITTED IN PARTIAL FULFILLMENT OF
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THE EFFECTS OF HIGH INTENSITY TRAINING AND EXHAUSTIVE EXERCISE ON THE GLYCOGENIC POTENTIAL OF L-LACTATE IN MAMMALIAN SKELETAL MUSCLE

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ABSTRACT

The ability of skeletal muscle to synthesize glycogen using lactate as the only substrate was assessed in isolated rat hindlimbs following 12 weeks of interval training. Trained animals were subdivided into a Trained Control group (n=10), a group which performed an acute run to exhaustion (Trained Exhausted) (n=10), and a group which performed an acute run to exhaustion superceded by a 12 hour fast (Trained Fasted) (n=10). Soleus and gastrocnemius muscle tissue slices were incubated for one hour in a medium containing 8 millimolar l-lactate and 1 microcurie of uniformly labelled ^{14}C l-lactate. Glycogen content of the soleus and gastrocnemius of Trained Control animals was increased 25% and 16% respectively, following training ($p<0.05$). Exhaustive exercise produced a 75% and 84% depletion of glycogen in both muscles ($p<0.05$). A 12 hour fast following exercise did not maintain the glycogen depleted state since glycogen levels recovered to near Trained Control values in both muscles. During incubation, there was a mean decrease in muscle glycogen of 12 and 18 $\mu mol \cdot gram^{-1}$ in soleus and gastrocnemius muscles in Sedentary Control, Trained Control, and Trained Fasted groups, whereas Trained Exhausted soleus and gastrocnemius muscle resynthesized glycogen to 78% and 66% of Trained Control values. The radiolabelled carbons of lactate were incorporated into the glycogen of all groups in both muscles, although the rate of lactate conversion and the percentage of lactate converted to glycogen were 50% lower in the soleus muscle, for all treatment groups except Trained Exhausted. Acute exhaustive exercise

accelerated conversion of lactate to glycogen in soleus muscle by 100%, such that the rate was not significantly different from Trained Exhausted gastrocnemius muscle. Following exhaustive exercise the percentage of lactate converted to glycogen was increased from 13% to 48% in soleus muscle and from 23% to 53% in gastrocnemius. Activities of glycogenic enzymes were consistantly higher in the gastrocnemius which may explain the higher rates of conversion of lactate to glycogen in this muscle. Both slow and fast skeletal muscle fibres of the rat are capable of rapid intramuscular glycogenesis from lactate. Acute exhaustive exercise and/or the accompanying glycogen depletion appears to be a stimulus for this process. It is suggested that direct intramuscular conversion of lactate to glycogen may represent a strategy for enhancing an exhausted animal's ability to survive "fight or flight" situations.

DEDICATION

This thesis is dedicated to my parents whose patience, love, and understanding contributed more to the completion of this project than words can describe.

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I would like to thank Dr. John Wilkinson for all his help in making this project a reality. John was a constant source of knowledge, advice and encouragement, and always seemed to come through with a "bad" joke at the appropriate moment. I would also like to thank Dr. Wilkinson's lovely wife, June, for tolerating his long absences from home during the experimental phases of this project.

I thank the other members of my committee for their careful and thoughtful reading of the manuscript. It was by virtue of their constructive criticism that I was able to bring this project to completion.

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I. INTRODUCTION

The performance of intense purposeful work, be it occupational or recreational, is often halted by transient localized fatigue. Lactic acid has classically been the factor implicated as directly or indirectly causing this impaired muscular function (Tesch, 1980; Sahlin, 1978; Asmussen et al., 1948; Bang, 1936). Although the causal relationship remains to be established, the inference has existed since 1841 when the Scandanavian chemist, Berzelius, claimed to have found lactic acid in the muscle of exhausted game (cited by Hermansen and Vaage, 1979; Karlsson, 1971a).

Considerable research attention in the last 80 years has been devoted to examining the conditions leading to lactate production and the conditions necessary for removal. Fletcher and Hopkins (1906) demonstrated increased lactic acid production in muscle contractions carried out in an oxygen deficient medium, thus advancing the older hypothesis (Liebig, cited by Karlsson, 1971b) that blood lactate increased as a result of hypoxia. Based on the results of independent studies both Meyerhof (1920, 1925) and Hill (1924) concluded that lactic acid formation during exercise was due to local tissue hypoxia. This hypothesis, however, did not fit with all experimental observations as Cori and Cori (1929) were able to demonstrate that blood lactate concentration increased in rats receiving intravenous glucose.

An examination of the kinetics involved reveals that excess pyruvate would simply, by a mass action effect, lead to lactate

production. The rate of pyruvate production is regulated by the intracellular ratio of adenosine diphosphate/adenosine triphosphate (ADP/ATP). An increase in the ratio of these two nucleotides automatically accelerates glycolysis to match the production of high energy phosphate to that being consumed in metabolic processes, as long as the $NADH+H^+$ (nicotinamide adenine dinucleotide, reduced) produced in the pathway is re-oxidized to NAD^+ (nicotinamide adenine dinucleotide). Under conditions of severe energy demand as imposed by muscle contraction for example, systems responsible for shuttling $NADH+H^+$ into the mitochondria may be exceeded leading to a build up of reducing equivalents in the cytosol. Under these circumstances the active skeletal muscle enzyme lactate dehydrogenase catalyses the redox reaction between pyruvate and $NADH+H^+$. The resulting products of this reaction are lactate and NAD^+ (Wenger and Reed, 1976). Thus the significant benefit of lactic acid production lies in the maintainence of adequate concentrations of the hydride ion acceptor NAD^+ (Wenger and Reed, 1976), and coincidently a cytosolic redox state conducive to a maximum glycolytic rate.

Studies on separated muscle fibre types (Essen et al., 1975) reveal consistent patterns of metabolic profiles and mechanical properties. Extrapolating from observations of animal tissue, Barnard (1971) proposed a fibre type classification system which would incorporate these features. Thus in mammalian skeletal muscle three classes of motor unit pools are identified from which an animal is able to recruit when performing muscular work. The slow oxidative (SO) motor units are relatively slow

contracting and suited for use in prolonged activities. They rely on oxidative metabolism, as indicated by the higher activity of Krebs cycle enzymes (Essen et al., 1975), as well as an LDH isozyme distribution which favors lactate oxidation (Sjodin, 1976).

At the other end of the spectrum are the FG, or fast glycolytic motor units. These units display faster contraction times, higher peak tension (Thorstensson et al., 1977) and are easily fatigued (Burke and Edgerton, 1975; Thorstensson, 1976). Metabolically, these motor units exhibit higher activities of enzymes thought to be rate limiting for glycolysis, ie. phosphorylase (Piehl and Karlsson, 1977), phosphofructokinase (Essen et al., 1975) and lactate dehydrogenase (Sjodin, 1976; Thorstenssen et al., 1977). Between these extremes of the spectrum of motor units are the so called FOG units (fast oxidative glycolytic). Fibres comprising these motor units have the characteristic contraction properties of FG fibres with the fatigue resistant properties of SO fibres (Burke and Edgeton, 1975; Burke et al., 1971).

An analysis of the motor unit pools of skeletal muscle demonstrates consistently higher rates of lactate production in FG fibres compared with SO and FOG fibres in human subjects (Tesch, 1980). Thus, a relationship is implied between physical performance and lactic acid production, dependent on the motor unit distribution pattern within the muscle and the intensity and duration of the performance.

The production of lactate during high intensity work may be considerable. Hermansen and Stensvold (1972) estimated that a

typical post exhaustion blood lactate concentration of 150-200 mg% would represent approximately 70-100 grams of this metabolite in a human subject. The site or sites for removal of such large quantities of lactate has been the subject of debate for several decades. Since lactate is essentially a metabolic dead end, the primary step in its metabolism should be a re-oxidation to pyruvate.

Two schools of thought have produced conflicting evidence regarding the major metabolic fate of lactate during recovery from high intensity exercise (Meyerhof, 1924; Brooks et al., 1973). The traditional view of lactate removal, of Hill and his associates (1924) proposes that oxygen is consumed during recovery from exercise in excess of that during rest ('excess postexercise O_2 consumption' or EPOC) (Brooks et al., 1973; Margaria, et al., 1933). This O_2 debt theory was modified to assign a portion of the EPOC to the rephosphorylation of ADP and creatine, the so called "alactic debt" (Margaria et al, 1933). The O_2 debt theory maintains that the major fraction (80%) of lactate formed during exercise is converted to glycogen in the immediate post exercise period, while the remainder is oxidized to CO_2 and water. The ATP generated by oxidation thus supplies the energy necessary for glycogen repletion.

Much of the recent literature indicates that an insignificant amount of glycogen is resynthesized in the immediate post exercise recovery period (Hultman and Bergstrom, 1967, Piehl, 1974a.). Also, complete restoration of muscle glycogen appears to be dependent on a high dietary intake of carbohydrate. Brooks and Gaesser (1980) were able to demonstrate

skeletal muscle glycogen repletion following pulse injections of lactate but this was not significant nor was it in phase with the time of $\dot{V}O_2$ decline thought to represent EPOC. Radioactive tracer studies using ^{14}C lactate also suggest that the primary fate of lactate at rest, during exercise and recovery is by oxidation (Brooks and Gaesser, 1980; Searle and Calavari, 1972; Depocas et al., 1969).

There are several notable exceptions to these findings, one of which is contained in a report by Hermansen and Vaage (1977). These authors concluded that during recovery from heavy intermittent exercise, 75% of the lactate present in human skeletal muscle was converted to glycogen within the muscle itself, and a much smaller fraction (less than 15%) was oxidized. These findings vindicate a series of much older and often overlooked publications by Meyerhof (1925, 1920) demonstrating in isolated frog skeletal muscle that removal of lactate during aerobic recovery from exhaustion is associated with glycogen resynthesis. His observations also indicated that up to 75% of the removed lactate was converted to glycogen directly within the muscle, while approximately 20% was oxidized to CO_2 and H_2O. This concept was advanced by Hill (1925) who examined the rate of lactate removal in terms of its heat of combustion. The conclusion was that only 20% of the lactate removed during recovery could be accounted for by oxidation, the remaining 80% being resynthesized to glycogen. Meyerhof's original thesis has received recent support from work on isolated skeletal muscle preparations and radioactive tracers (Connett, 1979; Bendall and Taylor, 1970; McLean and Holloszy,

1979). These studies all indicated that under certain circumstances lactate was a significant glycogenic precursor in skeletal muscle.

The actual site or sites of the glycogenic process is controversial. Meyerhof's provocative hypothesis of glycogenesis within the muscle itself, as opposed to a hepatic process, has been severely criticised (Krebs and Woodford, 1965; Krebs, 1965). The unfavorable energetics of a reversal of glycolysis implies the necessity of some additional reactions which overcome several thermodynamic barriers. These reactions are catalysed by the enzymes phospho(enol)pyruvate carboxykinase (EC 4.1.1.32), pyruvate carboxylase (EC 6.4.1.1), and fructose-1,6-diphosphatase (EC 3.3.3.11). Because there is good agreement in the literature that pyruvate carboxylase does not exist in mammalian skeletal muscle (Crabtree, Higgins and Newsholm, 1972), Bendall and Taylor (1970) proposed an extramitochondrial system operating through the so called "malic enzyme" (malate dehydrogenase, $NADP^+$, decarboxylating; EC 1.1.1.40). Recent evidence indicates that mammalian skeletal muscle does possess these proteins, vindicating the biochemical basis of muscle glycogenesis (Mclean and Hollozy, 1979; Pearce and Connett, 1980; Searle and Cavalieri, 1972; Opie and Newsholm, 1967; Krebs and Woodford, 1965).

The fate of lactate in terms of skeletal muscle glycogen formation has great theoretical implications for exercise science. In view of the importance of lactate as a metabolic byproduct of high intensity muscular work and its suggested role in limiting muscular performance, it was necessary to undertake

further experiments to investigate its fate during recovery. The primary purpose of this study was, therefore to determine if mammalian skeletal muscle can directly synthesize glycogen from l-lactate.

To solve this problem it was necessary to determine:

1. if different mammalian skeletal muscle types possess the enzymes necessary for the conversion of lactate to glycogen.
2. what the effects of training were on these enzymes.
3. what the effects of training, acute exhaustive exercise, and nutrient supply during recovery from acute exhaustive exercise were on the potential of mammalian skeletal muscle to syntesize glycogen from lactate.

SCOPE AND LIMITATIONS

The experimental methods used in this thesis represents a unique approach in that an *in vitro* isolated rat skeletal muscle preparation was used. One must use caution in attempting to extrapolate the *in vitro* metabolic potential of muscle to the complex metabolic condition found *in vivo*. The validity of conclusions about the metabolic processes in human tissue drawn from animal experiments are also cautioned.

DEFINITION OF TERMINOLOGY

Definitions and abbreviations used in this thesis include:

1. *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 repeated bouts of one minute of running at 1 meter per

second up an 8% grade, followed by one minute of rest.

2. l-lactate (l-lactic acid). Lactic acid is often referred to as lactate since it is largely dissociated at physiological pH. Both terms will be used interchangeably in this thesis.
3. ^{14}C lactate. Commercially prepared sodium l-lactate in which all three carbons labelled as carbon 14.
4. Cpm or counts per minute. The number of disintigrations per minute of a radioactively decaying element registered in a counting system.
5. Dpm or disintigrations per minute. The absolute number of disintigrations per minute of a radioactive element in a compound sample: equivalent to the cpm corrected for sample preparation and recovery, background and the efficiency of the counting system.
6. Specific activity. The total radioactivity of a given isotope (in this case ^{14}C), per unit weight of the compound or tissue e.g. $Dpm \cdot mg^{-1}$, or $Dpm \cdot mM^{-1}$.

II. METHODOLOGY

2.1 ANIMAL CARE AND SELECTION

Forty male rats of the Wistar strain (specific pathogen free) were obtained at approximately 12-15 weeks of age (300-350 grams) through the director of animal care at Simon Fraser University, Burnaby, British Columbia. Upon arrival the animals were weighed, housed individually in self cleaning cages kept on racks in an air conditioned room (22°C) and provided with commercial rat feed (Purina Lab Chow) and water ad libitum. Immediately, the animals were subjected to a reversed light/dark cycle (light from 1600 h to 0800 h) to allow training sessions to be performed during the animals' normal peak activity period.

Each morning all animals were handled, the cages were rotated on the cage rack, soiled papers were changed, and food and water were replenished. The cages were washed and sterilized every two weeks and the rats were weighed weekly. After one week of orientation to the laboratory the animals were randomly assigned to one of four groups, one sedentary (N=10) and three exercise training groups (N=3·10). Due to injury and poor running ability five animals were eliminated from the trained groups. Thus, the final number of experimental animals used in the study was 35.

2.2 INTERVAL TRAINING

Training for short term, high intensity work was performed four days per week (e.g. Mon., Tues., Fri., Sat.) at approximately 1200 hours. Prior to each running session the animals hind quarters were soaked with water and after each session the animals were thoroughly dried and inspected before being returned to their cages.

The first week of training was essentially a treadmill orientation programme consisting of one 10 minute running session each day at .33 $m \cdot sec^{-1}$ up an 8% grade. Following this preliminary exposure the training programme was initiated, consisting of 5 intervals of one minute running at .33 $m \cdot sec^{-1}$, up an 8% grade followed by one minute rest.

The treadmill speed was progressively increased by .083 $m \cdot sec^{-1}$ each week until all animals were running 5 intervals at 1 $m \cdot sec^{-1}$, up an 8% grade. The number of intervals performed each training session was increased by one each day until all animals were running a total of 10 bouts at 1 $m \cdot sec^{-1}$, 8% grade, four times per week.

The running speed, % grade, and interval protocol was maintained until time of sacrifice. This work intensity initially represented approximately 120% of the maximum oxygen uptake of the rat (Bedford, 1980; Gollnick et al., 1974). Furthermore previous investigators have shown that this running programme resulted in significant aerobic and anaerobic training effects, and when carried to exhaustion caused significant, immediate post-exercise skeletal muscle glycogen depletion (Gaboriault, 1977).

2.3 EXPERIMENTAL DESIGN

After a 12 week period the three training groups were paired with the age matched sedentary controls. The sedentary control group (SC) provided data on normal rat skeletal muscle biochemical parameters. The trained control (TC) group provided data on the effect of chronic interval training on skeletal muscle. The trained exhausted group (TE) provided data on the effects of acute exhaustive work on skeletal muscle, and the trained fasted group (TF) allowed collection of data on the effects on skeletal muscle of an acute exhaustive run followed by a 12 hour fast.

To examine the previously stated problems several aspects of lactic acid metabolism were investigated. This study involved the evaluation of the following dependent variables at various points in the experimental protocol.

1. Body Weight (grams)
2. Muscle Weight (grams, wet weight)
3. Acute exercise performance (total distance, meters)
4. Fructose-1,6-diphosphatase activity ($umole \cdot g^{-1} \cdot min^{-1}$)
5. Phospho(enol)pyruvate carboxykinase activity ($umole \cdot g^{-1} \cdot min^{-1}$)
6. Malic enzyme activity ($umole \cdot g^{-1} \cdot min^{-1}$)
7. Muscle glycogen content, pre and post-incubation ($umol \cdot gram^{-1}$)
8. Muscle lactate content, pre and post-incubation ($umol \cdot gram^{-1}$)
9. Rate of ^{14}C uptake into muscle glycogen ($Dpm \cdot g^{-1} \cdot hour^{-1}$)

10. Rate of lactate uptake (umol·gram^{-1}·hour^{-1})
11. Rate of lactate oxidation to $^{14}CO_2$ (Dpm·g^{-1}·hour^{-1}).
12. Rate of lactate conversion to glycogen (umol·g^{-1}·hour^{-1})
13. Rate of lactate oxidation to carbon dioxide (umol·g^{-1}·hour^{-1})
14. Rate of lacate metabolism (umol·gram^{-1}·hour^{-1})
15. Percentage of total metabolized lactate converted to glycogen (%)
16. Percentage of total metabolized lactate oxidized to carbon dioxide (%)

2.4 STATISTICAL CONSIDERATIONS

Group means +/- the standard error of the mean (SEM) were calculated for all the dependent variables listed. One way analyses of variance (S.P.S.S.7 ANOVA Programme) were performed between treatment conditions with respect to muscle metabolite contents, incorporation of ^{14}C lactate into tissue glycogen or CO_2, and with respect to muscle glycogenic enzyme activity. When the F ratio indicated significance ($P<0.05$), post hoc Neuman-Keuls analyses were used to locate significant ($P<0.05$) group differences. Varying N's for the dependent variables in various treatment groups arise out of methodological error in laboratory preparation of experimental samples.

2.5 PRE SACRIFICE PROTOCOL

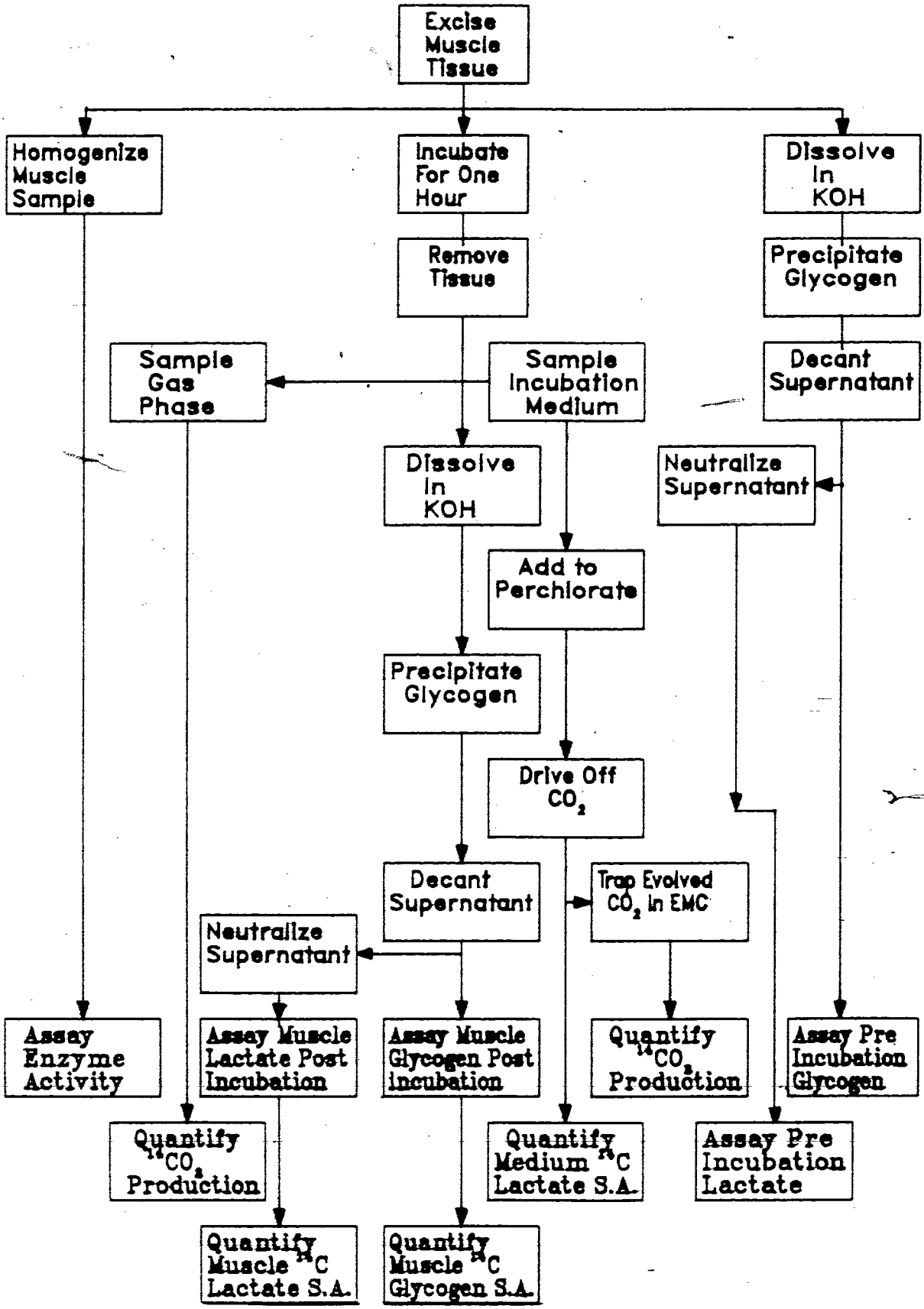
Seventy two hours prior to the acute run or sacrifice all training was terminated to reduce the chances of carry over effects confounding the experimental results. Five hours prior to the acute run or sacrifice all animals were placed on a fast to control for potential dietary effects by diminishing the output of exogenous fuels from the gut. The fast also served to diminish liver glycogen in an effort to reduce the time necessary for the acutely exercised animals to reach exhaustion (Clarke and Conlee, 1979).

The acute exercise test was administered to individual runners from TE and TF groups on designated treatment days and consisted of the animals running one minute work/rest intervals at 1 $m \cdot sec^{-1}$, up an 8% grade, to exhaustion. Exhaustion was defined as the point at which the animal could not keep pace with the treadmill speed, repeatedly fell back on the shock grid, and could not right himself when placed on his back.

2.6 TISSUE PREPARATION

The general experimental procedure is outlined in the flow chart on the following page. All animals were killed by a blow to the head followed by cervical dislocation. The left and right lower limbs were skinned and the achilles tendon severed at the musculotendon junction. The soleus and gastrocnemius muscles were excised, trimmed of connective tissue, blotted, and weighed on a Mettler balance. A 50 mg tissue sample was taken from the soleus muscle and from a full cross section of the medial gastrocnemius muscle and placed in hot KOH in preparation for

Fig. 1. Experimental Procedure



initial glycogen and lactate determinations. An additional 150 mg tissue sample was taken from the soleus and cross section of the head of the medial gastrocnemius muscle of the two control groups (SC and TC), quick frozen in liquid nitrogen and stored at -80°C for subsequent assay of glycogenic enzyme activity. Two hundred mg of soleus and medial gastrocnemius muscle tissue was excised from each animal for incubation studies.

2.7 TISSUE INCUBATION

The 200 mg muscle tissue samples from soleus and gastrocnemius muscles were cut into 1 mm slices and suspended in 2 ml of a 37°C buffered Ringers medium (pH 7.3). The 2 ml of incubation buffer (Appendix 1) contained 8 mM l-lactate and 1 μCi uniformly labelled lactic acid (Na salt: (U-^{14}C)L(+), 138.6 mCi·mmole-1, New England Nuclear, Boston, MA). Radiochemical purity of the tracer was certified by the vendor to exceed 98%. The buffered incubation medium was freshly bubbled with a standard gas mixture of known composition (20% O_2, 5% CO_2, balance nitrogen).

The incubations were carried out for 60 minutes in sealed 15 ml Vacutainer tubes. The space above the incubation medium was well gassed with the mixture described previously. The temperature of the incubations was maintained at 37°C by a circulating water bath. The time from sacrifice to onset of the incubation period averaged 10 minutes.

Immediately prior to commencement of the incubations an aliquot of the medium was drawn off for initial determination of the ^{14}C specific activity of the medium. Following incubation a

5 ml sample of gas was drawn off in a sealed hydrated syringe from above the incubation medium for determination of radiolabelled $^{14}CO_2$ which may have escaped to the gas phase above the incubation medium. One 500 µl aliquot of the incubation medium was anaerobically withdrawn in a tuberculin syringe for determination of dissolved $^{14}CO_2$ specific activity and residual ^{14}C lactate in the medium.

Following incubation the muscle samples were removed, washed in cold (4°C) non radiolabelled buffer, blotted and immediately emmersed in hot KOH for determination of glycogen and lactate content and for glycogen and lactate ^{14}C specific activity.

2.8 TISSUE METABOLITE DETERMINATIONS

2.8.1 MUSCLE GLYCOGEN

All biochemical determinations were performed in duplicate unless otherwise stated. The non incubated tissue samples for glycogen and lactate analysis were placed in screw capped culture tubes and digested in 30% KOH at 100°C for 30 minutes. Cold 95% ethanol was added to the cooled samples which were then placed on ice for 20 minutes in order to precipitate glycogen. Following centrifugation of the sample at 2000·g for 10 minutes, the supernatant was decanted and retained frozen at -80°C for subsequent lactate determination. After the supernatant was decanted the glycogen pellet was dissolvded in 1 ml of distilled water for assay. Muscle glycogen content was analyzed colorimetrically according to Lo et al., (1970) (Appendix 2).

All muscle samples incubated in radiolabelled lactate were treated similarly. However, the glycogen precipitate was washed with cold 66% ethanol and centrifuged at 2000·g for 10 min, a procedure which was repeated 3 times to ensure constant specific activity of the radioisotope incorporated into glycogen (Gaboriault, 1977). All supernatants from each centrifugation were decanted, added together and frozen for subsequent lactate determination.

The radiolabelled glycogen pellets were similarly dissolved in 1 ml of distilled water; 500 µl of this suspension was used for glycogen determination (Lo et al., Appendix 2), while the remaining 500 µl was used for determination of the ^{14}C specific activity of glycogen.

2.8.2 MUSCLE LACTATE

The thawed KOH/ethanol supernatants and washings from the radiolabelled glycogen extraction procedure were neutralized with 60% $HClO_4$ (Bendall and Taylor, 1970). The supernatant was decanted from the $KClO_4$ precipitate, and following appropriate dilution was assayed spectrophotometrically for lactate concentration according to Lowry and Passaneau (1973) (Appendix 3). A 250 µl aliquot of the KOH extract from incubated muscle samples was assayed for intramuscular ^{14}C lactate specific activity.

2.9 PREPARATION FOR ^{14}C COUNTING

Carbon 14 beta emission was quantified in a Beckman LS 8000 liquid scintillation counter using 15 ml of Aquasol liquid scintillation cocktail (New England Nuclear, Boston, MA) as the fluor. Counting efficiency was assessed using an external standard, and corrected for dpm using the APL computer programme NUBEC.

Radiolabelled carbon dioxide ($^{14}CO_2$) dissolved in the incubation medium was prepared for liquid scintillation counting by injecting a 500 µl aliquot of the medium into a center well created in a stoppered liquid scintillation vial. The center well contained perchloric acid, while the scintillation vial contained 2 mls of ethanolamine methylcellusolve (EMC), saturating a strip of Whatman #1 filter paper. The $^{14}CO_2$ liberated by the acid treatment of the incubation medium was trapped in EMC. Following an overnight equilibration period the center well was removed and 15 ml of Aquasol was added to the scintillation vial containing the trapped $^{14}CO_2$ and EMC-filter paper and β-emission was quantified.

Five hundred µl of the solution from the center well of the CO_2 trap was added to 15 ml of Aquasol in a scintillation vial for determination of residual radiolabelled lactate within the incubation medium.

Radiolabelled CO_2 which may have escaped into the gas phase above the incubation medium was recovered by flushing the gas sample through a blood/gas analyser, trapping the exhaust in a scintillation vial containing EMC and quantifying the beta emission. Specific activity of the evolved CO_2 was determined

from trap activity.

Two hundred and fifty µl of the glycogen extract solution was added to 15 ml of Aquasol in scintillation vials and the specific activity of the radiolabelled glycogen (dpm·gram^{-1}) was determined.

Two hundred and fifty µl of the supernatant collected from the washing of the radiolabelled glycogen pellet was added to 15 ml of Aquasol for determination of the uptake of the radiolabel into the tissue metabolite pool.

2.10 MUSCLE LACTATE UPTAKE AND METABOLISM

The rate at which the incubated muscle tissue took up exogenous lactate from the medium during one hour of incubation was determined from the following equation:

$$\text{LACTATE UPTAKE} = \text{tracer S.A.} \times (\text{Medium activity}_{\text{initial}} - \text{medium activity}_{\text{final}})$$

(μmol/gram/hour) (μmol/dpm) (dpm) (dpm)

...2.1

The amount of lactate transported into the muscle per gram of tissue per hour when added to the initial pre incubation lactate content of the muscle gave the total lactate pool available to the various metabolic pathways during the one hour incubation. Thus by subtracting the post incubation lactate content of the muscle from this value the total amount of *in vitro* lactate

metabolism was determined.

2.11 UPTAKE OF ^{14}C LACTATE INTO GLYCOGEN AND OXIDATION TO CARBON DIOXIDE

The rate of uptake of the radiolabelled carbon atoms into glycogen, expressed as ($dpm \cdot 10^{-3} \cdot gram^{-1}$ wet weight of tissue $\cdot hour^{-1}$) was calculated from the following formula:

$$\underset{\text{(dpm/gram/hour)}}{\text{INCORPORATION OF TRACER INTO TISSUE GLYCOGEN POOL}} = \underset{\text{(}\mu\text{mol/gram)}}{\text{concentration}} \times \underset{\text{(dpm/}\mu\text{mol)}}{\text{S.A.}} \times \underset{\text{(hours)}}{\text{time}^{-1}} \qquad \ldots 2.2$$

The rate of lactate converted to glycogen expressed as µmol lactate $\cdot gram^{-1} \cdot hour^{-1}$ was calculated according to the following equation:

$$\underset{\text{(}\mu\text{mol/gram/hour)}}{\text{LACTATE CONVERTED TO GLYCOGEN}} = \frac{\text{incorporation of tracer into tissue glycogen pool (dpm/gram/hour)}}{\text{total incorporation of tracer into tissue (dpm/gram/hour)}} \times \underset{\text{(}\mu\text{mol/gram/hour)}}{\text{total lactate metabolized}} \qquad \ldots 2.3$$

2.12 ENZYME ASSAYS

One hundred and fifty milligram muscle samples obtained from the soleus and cross section of the medial gastrocnemius muscle of TC and SC animals were separated into three fractions and assayed for glycogenic enzyme activity. The tissue samples were then homogenized in the appropriate medium according to McLane and Holloszy (1979) (Appendices 4, 5, 6). After homogenization and centrifugation the muscle fractions were assayed for fructose-1,6-diphosphatase activity (Opie and Newsholm, 1967) phospho(enol)pyruvate carboxykinase activity (Opie and Newsholm, 1967), and for malate dehydrogenase ($NADP^+$, decarboxylating) enzyme activity (Stickland, 1959) (Appendices 5, 6, 7). All enzyme activities are reported as rate expressed in µmoles of product formed per hour per gram wet weight of muscle tissue according to the following formula:

$$\text{ENZYME ACTIVITY } (\mu\text{mol/hour/g}) = \frac{\Delta \text{O.D.} \times V \times 10^3}{\text{E.coeff.} \times (W \times V_2/V_1)} \qquad \ldots 2.4$$

where:

Delta O.D. is the change in optical density of the appropriate pyridine nucleotide at 340 nm.

V is the total volume in the reaction cuvette.

10^3 is a transformation factor.

E. coeff. is the extinction coefficent of NADH or NADPH at 340 nm.

W is the wet weight of the muscle tissue sample.

V_1 is the volume of the buffered homogenizing medium.

V_2 is the volume of the tissue homogenate used in the enzyme reaction.

III. RESULTS

3.1 BODY WEIGHTS, MUSCLE WEIGHTS AND PERFORMANCE TIMES

Group means for body weights, muscle weights, and running performance are shown in Table 1. In all groups there was a significant ($P<0.05$) growth over time. Interval training induced a significant suppression in weight gain (mean difference, 88 grams). This type of training however, did not result in muscle hypertrophy as no significant differences were demonstrated in whole gastrocnemius or soleus weights, and there were no differences when these values were expressed as a percentage of total body weight.

The average number of one minute running bouts performed during the acute performance test for TE and TF groups was 29.5, corresponding to a mean running distance of 1768 meters.

3.2 PRE-INCUBATION MUSCLE GLYCOGEN

The initial muscle glycogen values prior to *in vitro* incubation treatment are presented in Figure 2. There was a significant increase in resting glycogen in both muscles following twelve weeks of interval training (trained control animals). Soleus muscle glycogen content was increased 25% above sedentary controls (24.8 compared to 18.4 $\mu mol \cdot gram^{-1}$ wet weight). Resting glycogen content in the gastrocnemius muscle of trained animals was 16% greater than in non-trained (31.7 compared to 26.4 $\mu mol \cdot gram^{-1}$ wet weight). For both these control

TABLE 1. Effect of training on body weights, muscle weights, and performance.

	Pre Training Body Weight (grams)	Post Training Body Weight (grams)	Soleus Muscle Weight (milligrams)	Gastroc Muscle Weight (grams)	Soleus as % Body Weight	Gastroc as % Body Weight	Number of Bouts Run
Sedentary Control	361 ±18(10)	* 559 ● ±21(10)	207.21 ±20.29(10)	2.30 ±0.12(8)	0.040 ±0.003	0.43 ±0.01	---
Trained Control	322 ±9(10)	* 466 ±15(8)	186.66 ±3.78(8)	1.90 ±0.05(8)	0.041 ±0.001	0.41 ±0.01	---
Trained Exhausted	342 ±4(10)	* 476 ±7(10)	202.30 ±9.11(10)	2.13 ±0.06(10)	0.042 ±0.002	0.45 ±0.02	29.8 ±4.6(10)
Trained Fasted	341 ±16(10)	* 471 ±25(7)	204.43 ±10.68(7)	1.87 ±0.10(7)	0.048 ±0.002	0.44 ±0.01	29.0 ±6.8(10)

Values are means ± SEM with number of samples in parentheses.

*Significantly different from pre training weight ($p < 0.05$).

●Significantly different from trained groups ($p < 0.05$).

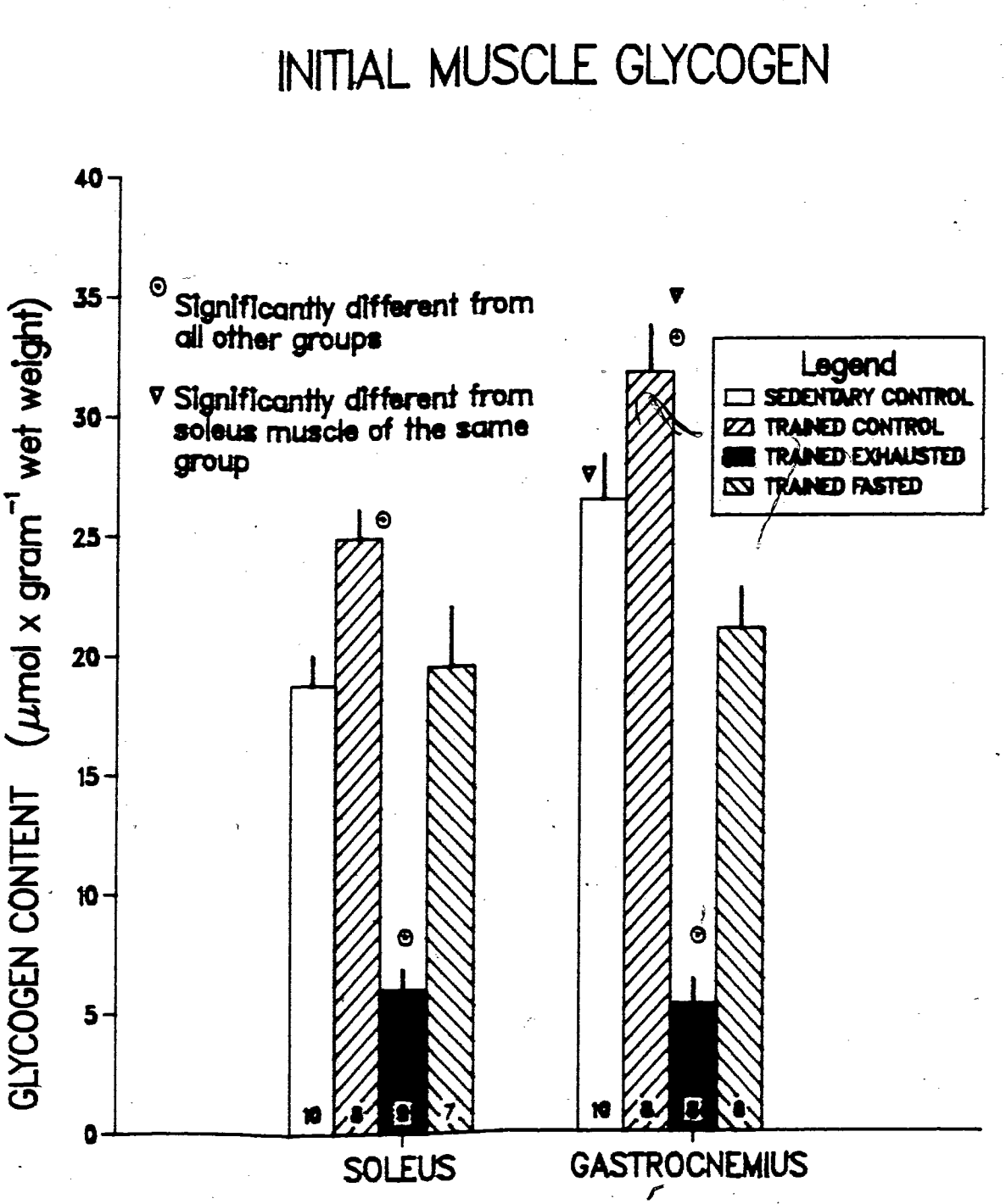


FIG. 2. Muscle glycogen content of rat soleus and gastrocnemius muscle following training, exhaustive exercise, and post exercise fasting. Values are means +/- SEM. Values in the bars are the number of duplicate determinations.

groups (SC and TC) resting glycogen values were significantly higher in gastrocnemius compared to soleus.

The acute run to exhaustion produced decreases ($P<0.05$) in muscle glycogen of 75% in soleus (from 24.9 to 6.0 $\mu mol \cdot gram^{-1}$ wet weight) and 84% in gastrocnemius muscle (from 31.7 to 5.3 $\mu mol \cdot gram^{-1}$ wet weight). At the point of fatigue glycogen content was not significantly different in the two muscle types.

In order to obtain glycogen depleted muscle in a metabolically rested state, the exhaustive run was superceeded by a 12 hour fast. It may be seen in Figure 2 that this procedure was not successful in that a substantial restoration of glycogen occurred in both soleus and gastrocnemius muscles ($p<0.05$). The largest percentage and absolute increase in muscle glycogen under fasting conditions occured in gastrocnemius muscle with an increase of 15.7 $\mu mol \cdot gram^{-1}$ wet weight. This value was significantly less than trained control values but nevertheless represented repletion to 66% of the pre exercise value. Soleus muscle increased its glycogen content by 13.5 $\mu mol \cdot gram^{-1}$ wet weight during fasting recovery from exhaustive exercise. This represented an equivalent restoration of muscle glycogen to 78% of trained control values. Following 12 hours of fasting recovery from exhaustive exercise there was a slightly higher, though non significant (n.s.), glycogen content in the gastrocnemius muscle when compared to soleus.

3.3 POST-INCUBATION MUSCLE GLYCOGEN

Muscle glycogen content measured before and after incubation of soleus and gastrocnemius tissue slices in a medium containing 8 mM l-lactate is shown in Figures 3 and 4. Significant glycogen depletion ($P<0.05$) of the order of magnitude seen in acute exhaustive exercise occured in both muscles from trained control animals during one-hour of incubation. The mean decrease in soleus glycogen content was 11.8 $\mu mol \cdot gram^{-1}$ wet weight, (Fig 2) and 18.1 $\mu mol \cdot gram^{-1}$ wet weight, in gastrocnemius (Fig 3). The degree of depletion in absolute terms was not significantly different in the two muscles. Soleus and gastrocnemius muscle from sedentary animals also depleted glycogen ($P<0.05$) but to a lesser extent.

In contrast to these data, incubated tissue from exhausted animals repleted glycogen. The glycogen content in soleus muscle and gastrocnemius muscle during the one hour incubation, increased 10.5 $\mu mol \cdot gram^{-1}$ wet weight ($P<0.5$) and 12.3 $\mu mol \cdot gram^{-1}$ wet weight ($P<0.05$) respectively. Glycogen content of the soleus muscle from trained fasted animals remained remarkably stable with no significant difference in pre and post incubation glycogen content. This was not the case in the gastrocnemius muscle which showed a slight drop ($P<0.05$) in glycogen content over the course of the incubation.

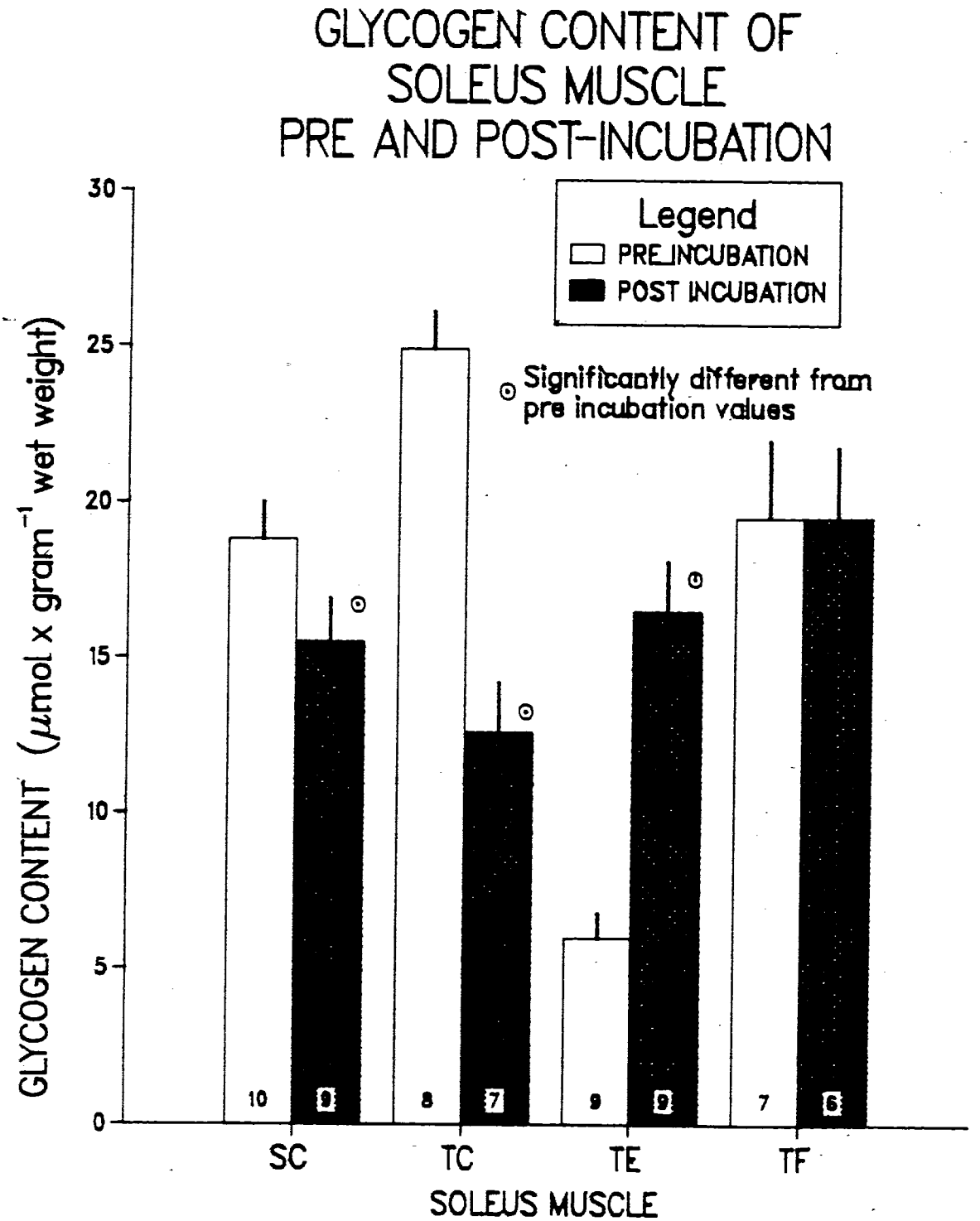


FIG. 3. Glycogen content of soleus muscle from control, exhausted, and post exercise fasted rats following a one hour incubation in an oxygenated bicarbonate buffer containing 8 mM l-lactate. Values are means +/- SEM. The number of duplicate determinations is given in the bars.

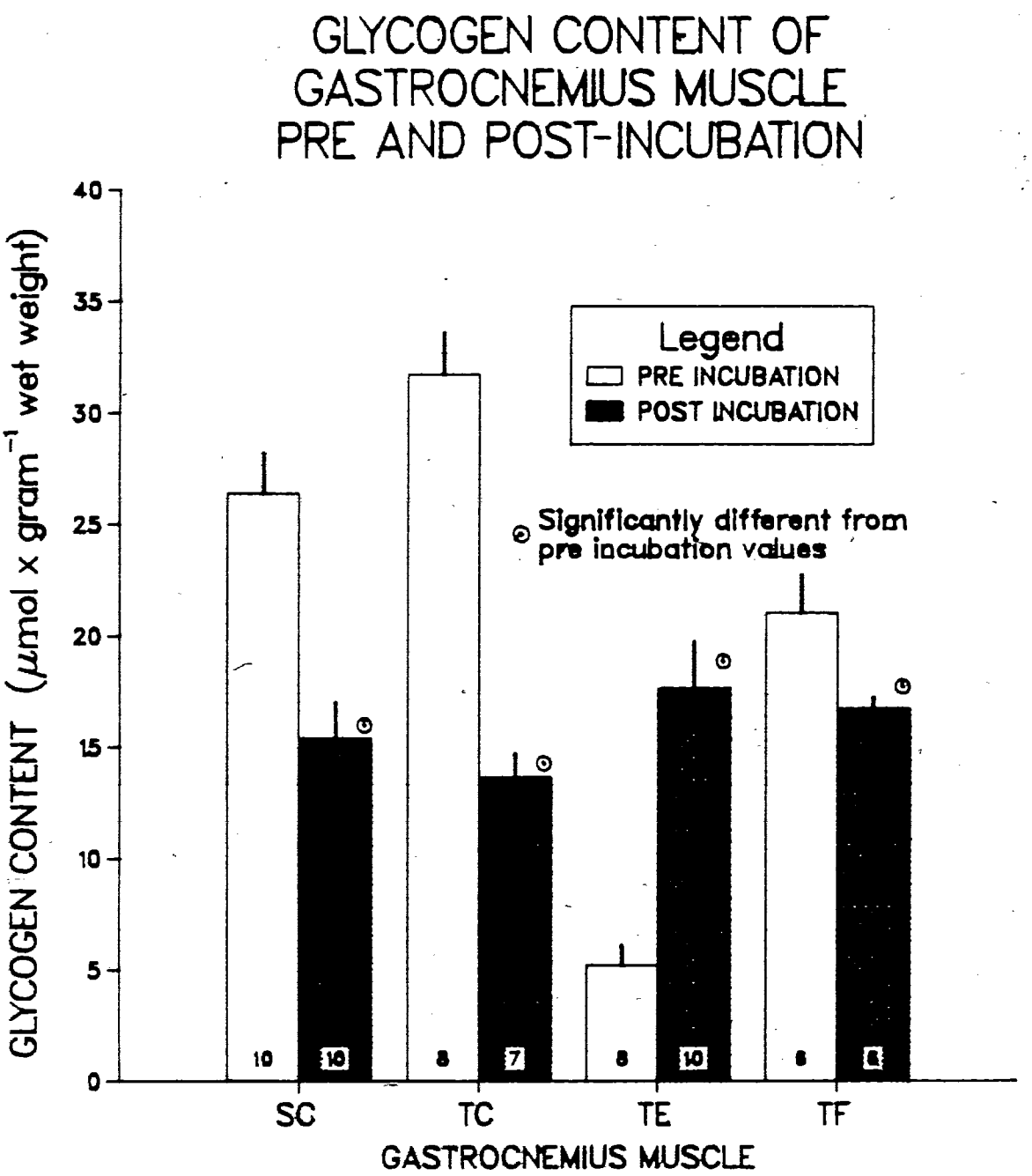


FIG. 4. Glycogen content of gastrocnemius muscle from control, exhausted and post exercise fasted rats following a one hour incubation in an oxygenated bicarbonate buffer containing 8 mM l-lactate. Values are means +/- SEM. The number of duplicate determinations is given in the bars.

3.4 INITIAL MUSCLE LACTATE

The initial pre-incubation muscle lactate content was determined in the neutralized KOH muscle extracts and expressed as $\mu mol \cdot gram^{-1}$ wet weight. As shown in Table 2, the lactate content of gastrocnemius muscle was considerably greater in all treatment groups when compared to soleus muscle ($P<0.05$).

No significant differences were observed in trained control soleus, or gastrocnemius muscle lactate levels when compared to corresponding sedentary control muscles. While lactate content of soleus muscle did not change following acute exhaustive exercise, a decrease ($P<0.05$) was observed in gastrocnemius muscle when compared to the respective control values, with lactate content decreasing from 8.5 ± 0.8 to 5.0 ± 0.7 $\mu mol \cdot gram^{-1}$. Following the exhaustive run, fasting induced a further increase (n.s) in lactate content in the soleus muscle to 3.1 ± 0.7 $\mu mol \cdot gram^{-1}$. The increase was more dramatic in the lactate content of gastrocnemius muscle with mean values reaching 7.6 ± 0.9 $\mu mol \cdot gram^{-1}$ ($P<0.05$).

3.5 MUSCLE LACTATE UPTAKE and METABOLISM

It may be seen in Table 2 that the rate of lactate transport into the muscle remained relatively constant at aproximately 19 $\mu mol \cdot gram^{-1} \cdot hour^{-1}$ regardless of experimental treatment or muscle type. Thus, no significant difference was observed between treatment groups or between muscle types.

The total lactate metabolized ($\mu mol \cdot gram^{-1} \cdot hour^{-1}$) by incubated muscle tissue is shown in Table 2. While gastrocnemius muscle seemed to metabolize more lactate in all treatment groups

TABLE 2. Initial muscle lactate content, and rate of lactate transport and removal

GROUP		SC	TC	TE	TF
INITIAL INTRAMUSCULAR LACTATE ($\mu mol \cdot gram^{-1}$)	Soleus	2.6±0.4 (10)	1.9±0.2 (8)	2.5±0.7 (10)	3.1±0.7 (7)
	Gastroc.	*7.8±0.8 (10)	*●8.5±0.8 (8)	*5.0±0.7 (10)	*●7.6±0.9 (7)
LACTATE UPTAKE BY MUSCLE ($\mu mol \cdot gram^{-1} \cdot hour^{-1}$)	Soleus	20.8±1.7 (10)	17.6±3.0 (8)	21.1±1.1 (10)	18.8±2.0 (7)
	Gastroc.	19.8±1.1 (10)	18.2±1.7 (8)	19.5±0.7 (10)	22.0±1.0 (7)
LACTATE METABOLIZED ($\mu mol \cdot gram^{-1} \cdot hour^{-1}$)	Soleus	18.7±1.0 (10)	19.7±4.0 (8)	17.5±1.0 (10)	18.4±2.0 (7)
	Gastroc.	21.9±0.8 (10)	●22.7±1.8 (8)	18.6±0.8 (10)	*●25.0±1.7 (7)

Values are means +/- SEM. The number of duplicate determinations is given in parenthesis.

○Significantly different from control ($P<0.05$)
*Significantly different from soleus ($P<0.05$)
●Significantly different from TE values ($P<0.05$)

compared with soleus muscle, this was only significant between

trained fasted groups (18.4±2.0 μmol·gram^{-1}·hour^{-1} in soleus, compared with 25.0±1.7 for gastrocnemius) . The interval training programme did not significantly influence the ability of soleus or gastrocnemius muscle to utilize lactate as a substrate under incubation conditions.

In gastrocnemius muscle, exhaustive exercise decreased the total metabolism of lactate ($P<0.05$) from 22.7±1.8 to 18.6±0.8 μmol·gram^{-1}·hour^{-1}. The same general effect was observed in exhausted soleus muscle, however this did not reach statistical significance. After post-exercise fasting, the rate of lactate metabolism showed a 30% ($P<0.05$) increase in gastrocnemius muscle, but not in soleus muscle.

3.6 UPTAKE OF ^{14}C LACTATE INTO GLYCOGEN

Exhaustive exercise increased the uptake rate of ^{14}C into glycogen four fold in soleus muscle (from 10.2±1.8 to 41.3±10.5) and two fold in gastrocnemius muscle (from 15.9±4.0 to 31.4±8.0) when compared to trained control values (Figure 5). The rate of incorporation of tracer lactate carbons was greater in soleus muscle compared to gastrocnemius although this did not reach statistical significance.

The rate of incorporation of ^{14}C carbons into glycogen in the other trained groups (TC and TF) was greater in gastrocnemius muscle ($P<0.05$). It was also noted that soleus muscle ^{14}C incorporation into glycogen exceeded that of gastrocnemius ($P<0.05$) in sedentary controls.

The rate of lactate conversion to glycogen expressed as μmol·gram^{-1}·hour^{-1} was much higher ($P<0.05$) in gastrocnemius

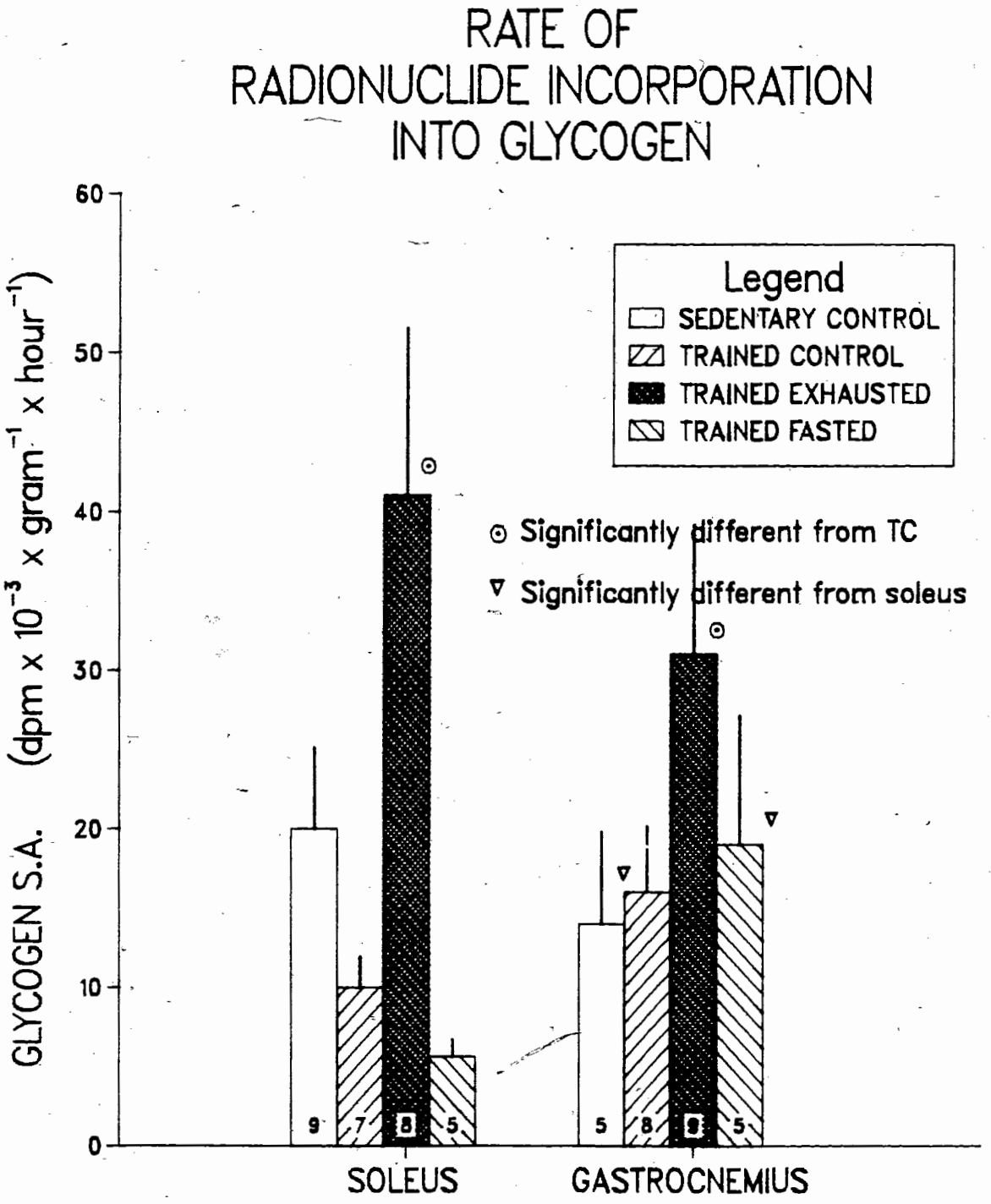


FIG. 5. Incorporation rate of ^{14}C carbons into the glycogen stores of soleus and gastrocnemius muscles of rats. Tissue was incubated for one hour in a medium containing 1 μCi lactate in 8 mM lactate. Groups are as described previously. Values are means +/− SEM. N's are given in the bars.

than in soleus muscle in all treatment groups except TE (Figure 6). Acute exhaustive exercise induced a large significant ($P<0.05$) increase in the rate of lactate conversion to glycogen in soleus muscle and only a slight (n.s.) increase in gastrocnemius muscle when compared to trained control muscle.

In Figure 6 it may be observed that the measured rate of lactate to glycogen conversion accounted for 40% of the actual measured glycogen resynthesis rates in both soleus and gastrocnemius muscle (see Figures 3 and 4).

3.7 OXIDATION OF ^{14}C LACTATE TO CARBON DIOXIDE

The rate of labelled substrate oxidation was determined from $^{14}CO_2$ production and expressed as $dpm \cdot 10^{-3} \cdot gram^{-1}$ wet $weight \cdot hour^{-1}$. As seen in Figure 7, the ability of gastrocnemius muscle in all groups to oxidize the radiolabelled tracer carbons under incubation conditions was less than soleus. These differences were significant in all trained groups ($P<0.05$) but not in the muscle from sedentary animals.

Training increased the ability of resting soleus muscle to oxidize radiolabelled lactate carbons, with oxidation rate increasing from 29.9 ± 4.4 in SC to 44.7 ± 9.5 in TC ($P<0.05$). However, exhaustive exercise had no effect on increasing the rate of lactate oxidation in either muscle when compared to trained controls.

The rate of lactate oxidation was calculated using an equation similar to 2.3, by substituting for the specific activity of carbon dioxide (Figure 8). In both resting sedentary control muscles there was no significant difference in the rate

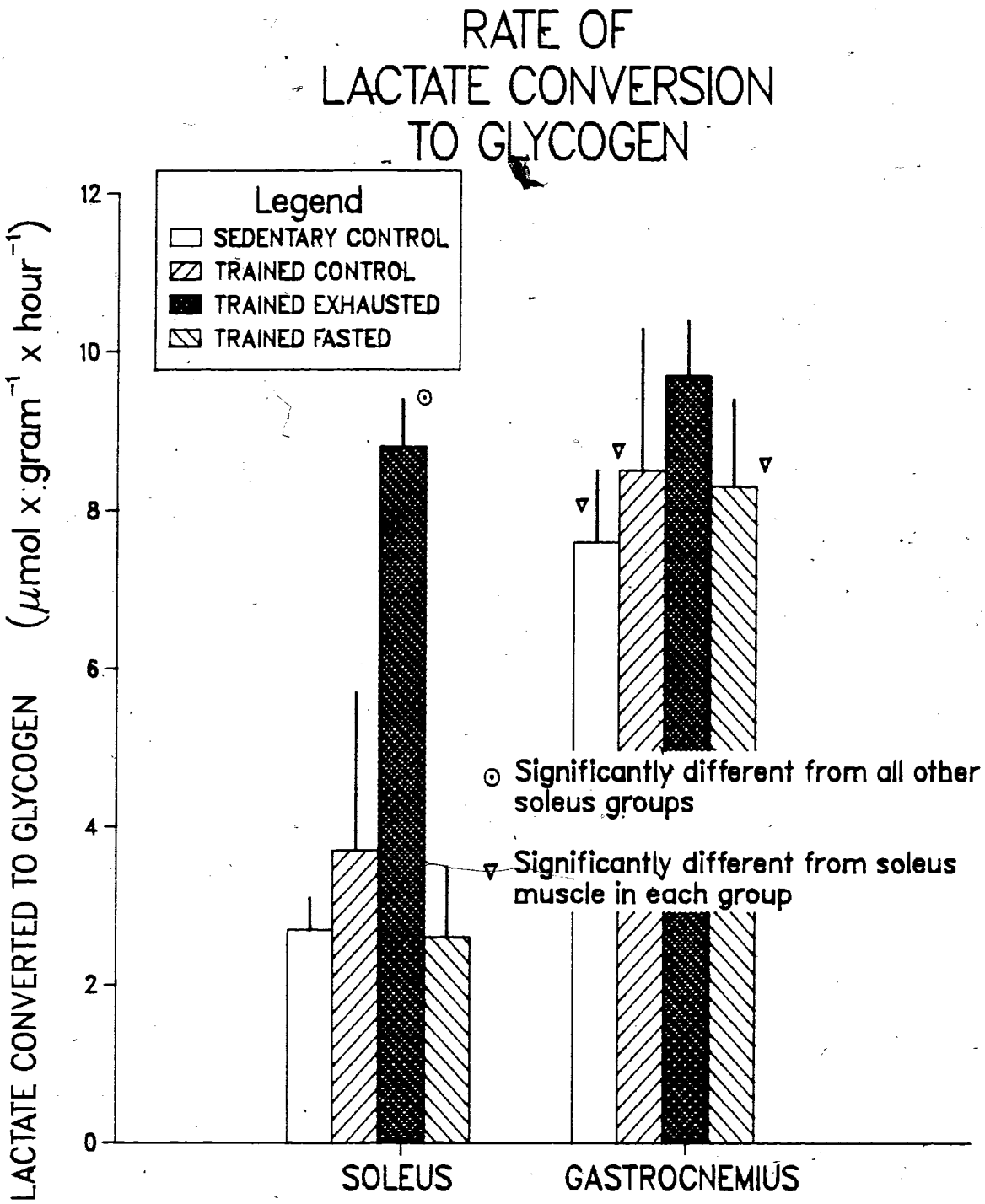


FIG. 6. Rate of lactate conversion to glycogen in the soleus and gastrocnemius muscles of rats during a one hour incubation in a a medium containing 1 μCi lactate in 8 mM lactate. Groups are as described previously. Values are means +/- SEM. N's are given in Fig. 5.

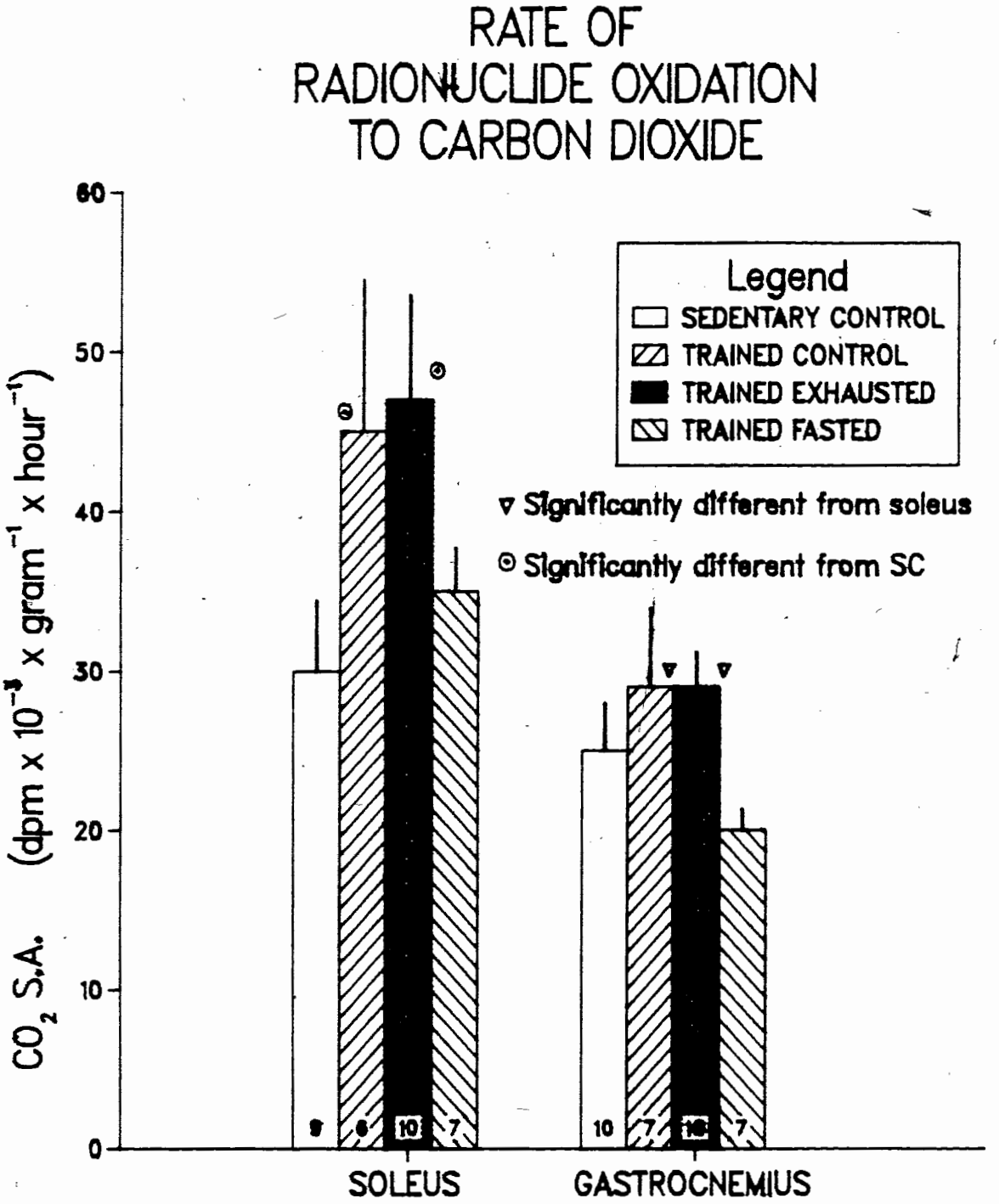


FIG. 7. Oxidation rate of ^{14}C tracer carbons to carbon dioxide in the soleus and gastrocnemius muscles of rats. Tissue was incubated for one hour in a medium containing 1 μCi lactate in 8 mM lactate. Groups are as described previously. Values are means +/- SEM. N's are given in the bars.

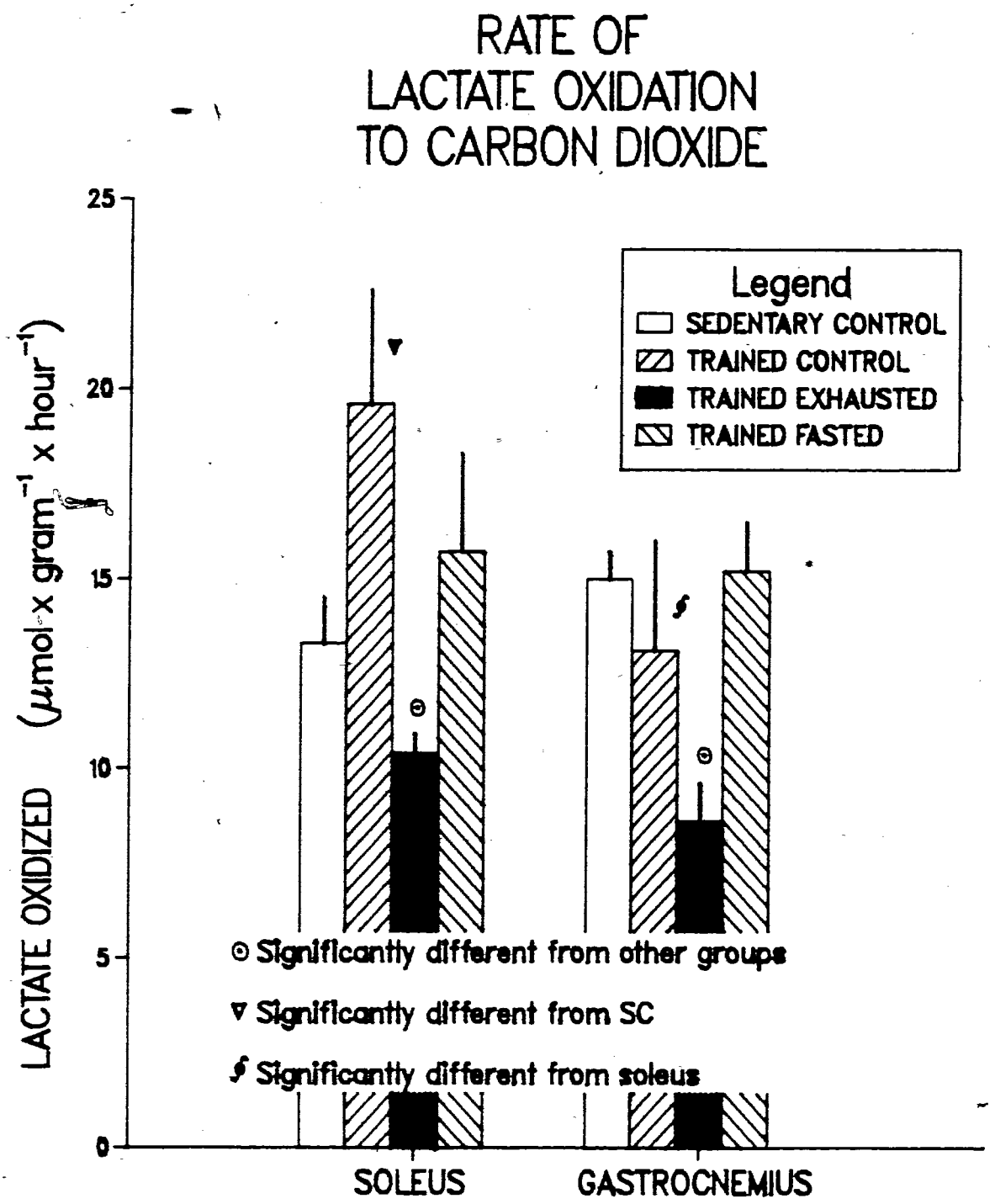


FIG. 8. Rate of lactate oxidation to carbon dioxide in the soleus and and gastrocnemius muscles of rats during a one hour incubation in a medium containing 1 μCi lactate in 8 mM lactate. Groups are as described previously. Values are means +/- SEM. N's are as in Fig. 7.

of lactate oxidation. With interval training a significant ($P<0.05$) inhancement of *in vitro* lactate oxidative capacity was seen in soleus muscle only. It was interesting to note that in both muscles from trained exhausted animals a large significant ($P<0.05$) depression of lactate oxidation was observed. Following fasting, both muscle types increased the rate of *in vitro* lactate oxidation although in soleus muscle this was still lower than rested, trained control values (n.s.).

3.8 TOTAL ^{14}C LACTATE METABOLISM

Figure 9 summarizes the total rate of incorporation of the radiolabelled tracer carbons into glycogen and carbon dioxide expressed as $dpm \cdot 10^{-3} \cdot gram^{-1} \cdot hour^{-1}$. There were no differences in the total incorporation of the labelled tracer between any of the treatment groups with the exception of the increase ($p<0.05$) in the total radionuclide incorporation rate in TE soleus muscle. A significant difference was also observed between the TE soleus and gastrocnemius muscles with soleus muscle demonstrating greater ($P<0.05$) total incorporation of the lactate carbons.

Figures 10 and 11 summarize the rate of lactate conversion to glycogen and oxidation to carbon dioxide expressed as a percentage of the total lactate metabolized. It was generally observed that the greatest percentage of lactate glycogenesis occured in the gastrocnemius muscle ($P<0.05$) of all groups. However, within muscle types a reduced percentage conversion of lactate to glycogen was observed following training. Exhaustive interval exercise was accompanied by a large percentage increase

TOTAL RADIONUCLIDE INCORPORATION INTO GLYCOGEN AND CARBON DIOXIDE

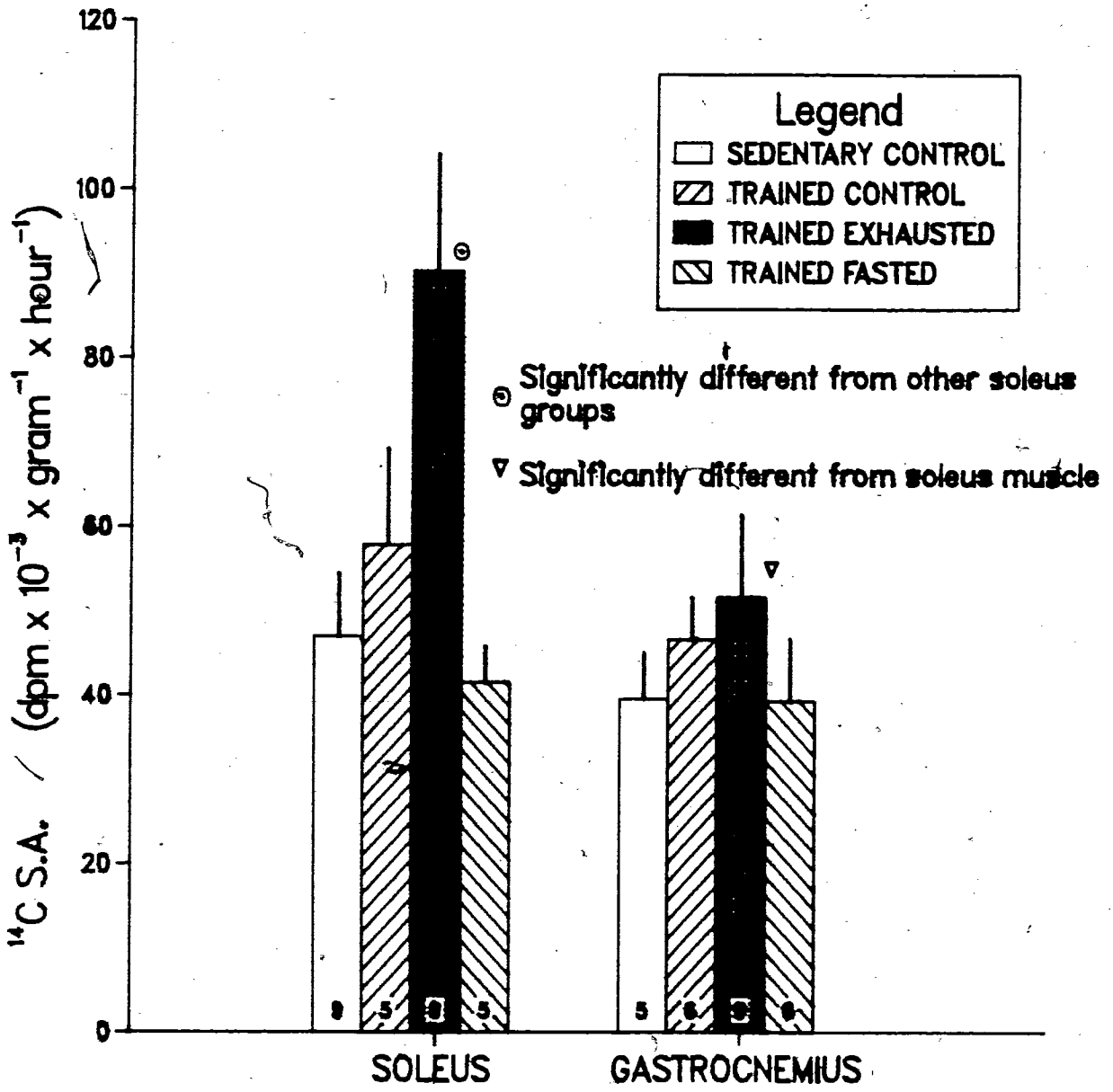


FIG. 9. The total incorporation rate of ^{14}C tracer carbons into glycogen and carbon dioxide in the soleus and gastrocnemius muscles of rats. Tissue was incubated for one hour in a medium containing 1 μCi lactate in 8 mM lactate. Values are means +/- SEM. N's are given in the bars.

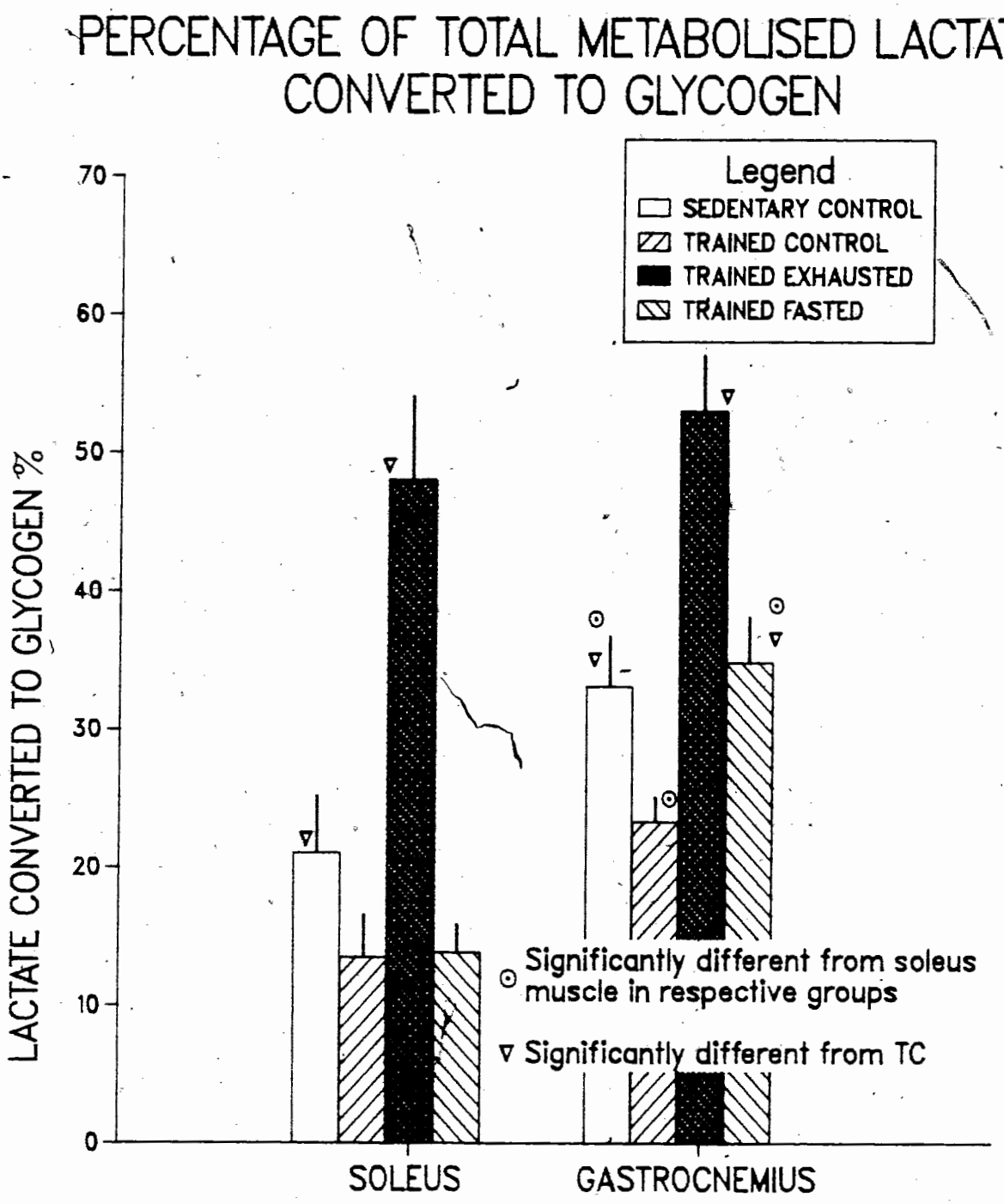


FIG. 10. The percentage of the total lactate metabolized by soleus and gastrocnemius muscle tissue, which was converted to glycogen during a one hour incubation in a medium containing 8 mM lactate. Groups are as described previously. Values are means +/- SEM. N's are as in Fig. 9.

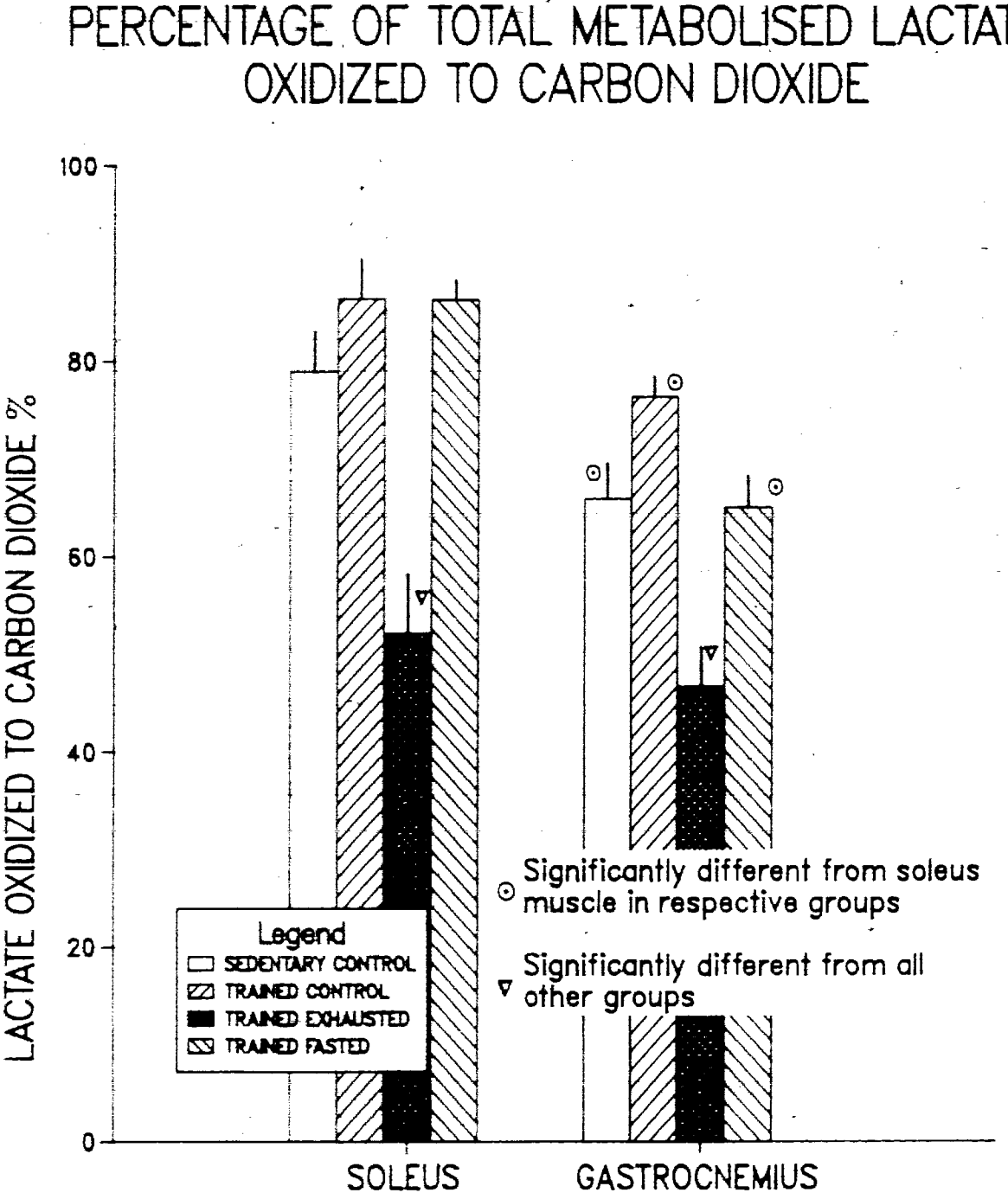


FIG. 11. The percentage of the total lactate metabolized by soleus and gastrocnemius muscle tissue, which was converted to carbon dioxide during a one hour incubation in a medium containing 8 mM lactate. Groups are as described previously. Values are means +/- SEM. N's are as in Fig. 9.

in lactate conversion to glycogen in both soleus and gastrocnemius muscle such that soleus was not significantly different from gastrocnemius.

The total lactate oxidized to carbon dioxide was the mirror image of the previous observations. Of particular interest is the large significant drop in percentage oxidation rate in both tissue types from exhausted animals.

When Figures 10 and 11 are taken together, the general profile is that in sedentary control animals approximately 80% of the lactate metabolized by soleus muscle is oxidized. In gastrocnemius muscle this ratio is approximately 67% oxidized and 33% converted to glycogen. Following 12 weeks of interval training the percentage oxidation rate of lactate increased in both soleus and gastrocnemius muscles and hence lactate conversion to glycogen decreased. Exhaustive exercise shifted the percentage of lactate to glycogen conversion in soleus from 13% in TC animals to 48% in TE and in gastrocnemius muscle from 23% to 53% respectively. Post-exercise fasting returned these fractions to the same order of magnitude as trained control values.

After exhaustive exercise a rather dramatic drop in the percentage oxidation of lactate was observed in both muscle types. In soleus this decrease was from 86% in TC to 52% in TE and in gastrocnemius from 76% to 46% respectively.

3.9 GLYCOGENIC ENZYME ACTIVITIES

Table 3 shows the activity levels of the glycogenic enzymes that catalyse the reactions bypassing pyruvate kinase and phosphofructokinase. The activity of the "malic enzyme" (l-malate dehydrogenase; $NADP^+$, decarboxylating, EC 1.1.1.40) was not significantly different in either of the muscle types and training had no effect on its activity. Phospho(enol)pyruvate carboxykinase (EC. 4.1.132) activity was much lower in soleus muscle when compared to gastrocnemius. In addition, interval training increased the activity of this enzyme by 50% in gastrocnemius muscle and had a slight but not significant effect on activity in soleus muscle.

A major difference was observed in the activity of fructose-1,6-diphosphatase between soleus and medial gastrocnemius muscle. The activity of this enzyme in soleus was quite low under assay conditions. Training had no effect on activity of this enzyme in either muscle type.

TABLE 3. Levels of activity of the glycogenic enzymes malic enzyme, phospho(enol)pyruvate carboxykinase and fructose-1,6-diphosphatase

Muscle	malic enzyme		phospho(enol)-pyruvate carboxykinase		fructose--1,6-di-phosphatase	
Group	SC	TC	SC	TC	SC	TC
Soleus	28±0.6 (6)	29±1.5 (6)	28±4.0 (5)	33±2.0 (5)	8±0.3 (5)	4±0.3 (5)
Gastroc	24±0.6 (5)	22±1.2 (5)	*60±4.5 (6)	*○93±2.4 (6)	*31±0.7 (5)	*32±0.2 (5)

Values are means +/- SEM. The number of duplicate determinations is given in parentheses. Units of activity are $\mu mol \cdot gram^{-1} \cdot hour^{-1}$.

*Significantly different from soleus muscle ($P<0.05$).
○Significantly different from sedentary control ($P<0.05$)

IV. DISCUSSION

4.1 BODY AND MUSCLE WEIGHTS

Increases in total body weight during maturation of sedentary rats have been previously documented (Wilkinson et al., 1978; Pitts and Bull, 1977; Houston and Green, 1975). Body weight changes following high intensity interval training vary. Post training reductions in body weight similar to those observed in the present study have been documented (Wilkinson et al., 1978; Hickson et al., 1976; Houston and Green, 1975; Staudte et al., 1973), whereas non appreciable differences have been reported by Baldwin and co-workers (1975), and Jobin (1977). The pattern of reduced body weight is both intensity and duration related and does not appear to be specific to interval training regimens. It is also observed in isometric training (Exner et al., 1973a, 1973b.) and is most pronounced in endurance training (Baldwin et al., 1975).

Qualitative observations made during the surgical procedures of the present study suppport the suggestion that intense training programmes inhibit the accumulation of large fat deposits, perhaps through increased energy expenditure combined with a suppression of appetite (Houston and Green, 1975). Emperically, exercise inhibits adipocyte proliferation in adult animals and decreases adipocyte size (Bukowiecki et al., 1980). Although this observation has some support in the literature in cases of moderate interval and endurance training programmes (Mackie, 1976; Houston and Green, 1975; Hubbard et

al., 1974), the effects of high intensity interval training on normal growth remains unexplained.

Although whole muscle hypertrophy has been observed following weight training programmes (Jaweed et al., 1974; Gordon et al., 1967) and with endurance running (Jaweed et al., 1974) this was not observed in the present study, in agreement with the findings of others (Baldwin et al., 1975; and Hickson et al., 1976). Similarly, no evidence could be provided to suggest that there was a disproportional hypertrophy of trained muscle when body weights were normalized between sedentary and interval trained animals. The present data suggest that 12 weeks of high intensity interval training has a pronounced effect on normal whole body growth patterns, with little effect on muscle growth itself. These phenomena deserve quantitave description in terms of maturation processes.

4.2 RUNNING PERFORMANCE

Maximal oxygen uptake in the untrained rat is elicited at a running speed of 0.80-0.83 meters per second on a level surface (Patch and Brooks, 1980; Bedford et al., 1979; Shepherd and Gollnick, 1976). Animals used in the present study were trained to run 10 one minute intervals at 1 $m \cdot sec^{-1}$ up an 8% grade. This power output was clearly well above the normal maximal aerobic capacity of the rat (aproximately 120% $\dot{V}O_2max$) which for male Wistar rats is in the range of 75 $ml \cdot kgm^{-1} \cdot min^{-1}$ (Brooks and Gaesser, 1981; Bedford et al., 1979). At the beginning of the training programme the rats in this study had difficulty completing five work bouts at this power output. The average

work time to exhaustion during the acute performance test was 29.47 minutes, corresponding to a mean running distance of 1768 meters. This was an extreme workload and attests to the fact that these animals were highly trained.

4.3 PRE-INCUBATION MUSCLE GLYCOGEN CONTENT

4.3.1 MUSCLE GLYCOGEN CONTENT in CONTROL ANIMALS

Values for pre-incubation muscle glycogen content of sedentary control animals were slightly lower but compare favorably with those previously reported (Fell et al., 1980; Baldwin et al., 1975). Muscle glycogen is influenced by many factors one of which is the method of sacrifice. Killing by a blow to the head and cervical dislocation induces, in many animals, considerable activity of the hindlimbs which may result in reduced muscle glycogen stores enhanced by catacholamine modulated glycogenolysis (Richter et al., 1981). This activity is eliminated in pentobarbital anesthesia but, the effects of barbiturates on muscle carbohydrate metabolism, are not well documented.

Since muscle glycogen content is hormonally modulated and therefore susceptible to diurnal variation, the time of death has also been shown by several research groups (Poland et al., 1980; Clarke and Conlee, 1979; Conlee et al., 1976) to be an important independent variable. Rat soleus muscle for example, shows a 55% change from a peak at 0700 to nadir at 1900 (based on a 12 hour light cycle from 0700-1900) (Conlee et al., 1976).

To ensure that all animals were in a comparable dietary state and to reduce the running time to exhaustion all animals were placed on a five hour fast prior to autopsy. Contrary to previously held views that muscle glycogen is stable and not significantly influenced by fasting (Adrouny, 1969; Russel and Bloom, 1956), Conlee et al. (1976) and others (Poland et al., 1980) have demonstrated that 24 hours of fasting leads to a 49% drop in glycogen content of soleus and 23% drop in glycogen of the gastrocnemius muscles of rats. Experiments in the present study were performed throughout the 12 hour nadir of muscle glycogen content. Combining this effect with a five hour fast, a considerable range of muscle glycogen values and performance times might be expected.

4.3.2 THE EFFECTS of TRAINING on MUSCLE GLYCOGEN CONTENT

Training over 12 weeks resulted in a very significant glycogen supercompensation of 25% in soleus and 16% in gastrocnemius above sedentary controls. Elevated glycogen content in the skeletal muscle of rats is a common response to sprint training (Armstrong et al., 1974) and endurance training (Gollnick et al., 1970) as first described by Proctor and Best (1932; cited by Gollnick et al., 1970). This phenomena has also been observed in well trained humans (Gollnick et al., 1972, 1973a). A significantly higher glycogen content in the gastrocnemius muscle compared to soleus for both sedentary and trained animals occurred as has been observed previously (Beatty and Boceck, 1970). It has been suggested that training may have a local effect on skeletal muscle glycogen, perhaps through

increased activity of glycogen synthetase (EC 2.4.1.11) activity (Taylor et al., 1972). Enhanced glycogen storage is an important training effect in view of the suggestion that at high relative workloads glycogen stores are a decisive factor for maximal work times (Maughan and Poole, 1981; Klausen et al., 1975; Hermansen et al., 1971; Hermansen et al., 1967; Bergstrom et al., 1966).

4.3.3 THE EFFECTS of EXHAUSTIVE EXERCISE on MUSCLE GLYCOGEN CONTENT

Acute exhaustive exercise resulted in pronounced depletion of muscle glycogen in both soleus and gastrocnemius muscles. In both relative and absolute terms glycogen depletion was greater in the gastrocnemius muscle. Based on the numerous studies relating work intensity and glycogen depletion in both man (Gollnick et al., 1973a, 1973c) and rats (Armstrong et al., 1974; Staudte et al., 1973) differential recruitment of motor units may be a possible explanation. However, at workloads approaching 100% of maximal aerobic activity, such as the ones used in this study, it is not reasonable to assume that fast motor units are preferentially recruited over slow motor units.

Soleus muscle is composed of 80% slow oxidative (SO) and 20% fast oxidative glycolytic (FOG) motor units, whereas the medial head of the gastrocnemius muscle of the rat is about 60% fast glycolytic (FG) and 40% FOG motor units (Ariano et al., 1973; Baldwin et al., 1972; Barnard et al., 1971). Based on the original size principle of motor unit recruitment order proposed in 1965 by Henneman and Olsen (Hennamen et al., 1977), and confirmed in electrophysiological (Grimby and Hannerz, 1974;

Grydikov and Kosarov, 1974) and metabolic studies (Gollnick et al., 1973c; Saltin and Karlsson, 1971; Armstrong et al., 1974) all three motor unit types are recruited at high work intensities. Slow oxidative fibres would, by virtue of high oxidative potential (Saltin et al., 1977) make more economical use of stored glycogen. Gastrocnemius muscle, with its high glycolytic capacity (Piehl and Karlsson, 1977; Essen et al., 1975; Thorstesson et al., 1977) would, on the other hand, be somewhat less economical. However, the greater glycogen depletion observed in gastrocnemius muscle suggests a proportionally greater contribution to tension development at rapid speeds of contraction, as demonstrated in sudies of isokinetic leg exercise (Thorstensson et al., 1977,1976; Tesch, 1980).

4.3.4 THE EFFECTS of FASTING on MUSCLE GLYCOGEN CONTENT

Following exhaustive exercise, one group of animals was subjected to a 12 hour fast designed to provide a glycogen depleted state in metabolically rested muscle. The results indicated that this procedure was not successful in that post fasting glycogen was increased by up to 600% over exhausted glycogen depleted values. This represented a return to 78% of resting trained control values in the soleus muscle and 66% of resting trained control values for the gastrocnemius muscle. This is an interesting finding in light of the commonly held view that the restoring process for muscle glycogen is relatively slow, taking up to 46 hours with carbohydrate feeding, and being virtually non existent if carbohydrate is

excluded from the diet (Piehl, 1974b; Piehl et al., 1974; Bergstrom and Hultman, 1966).

Recent exceptions to this view of essential carbohydrate feeding for muscle glycogen repletion have been provided by Maehlum, Felig and Wahren (1978) and by MacDougal and co-workers (1977). Both groups of investigators reported rapid and significant glycogen repletion in quadriceps muscle of fasting men recovering from exhausting bicycle leg work. It was concluded that in the absence of carbohydrate feeding muscle glycogen stores were preferentially replaced at the expense of hepatic glycogenolysis and gluconeogenesis. Direct supportive evidence is provided by Poland et al., (1980) who showed that muscle glycogen increases after exercise with insignificant liver glycogen recovery.

McLean and Holloszy (1979) studied rats made to swim to exhaustion and subsequently maintained on a minimal carbohydrate diet of liver sausage. As found in the present study, muscle glycogen recovered to 77% and 69% of resting fed controls in plantaris and soleus muscles respectively. Gaesser and Brooks (1980) observed a repletion of muscle glycogen concentration of 51.1% and 51.6% in quadriceps of rats recovering from exhausting continuous and intermittent exercise within the first four hours of a fast. In a protocol similar to that in the present study Fell and co-workers (1980) observed a similar phenomena.

During heavy, intense exercise epinephrine levels rise sharply (Bloom et al., 1976). Concurrently, sympathetic alpha-adrenergic stimulation of pancreatic cells results in decreased insulin levels (Galbo et al., 1977; Galbo and Holst,

1976). Plasma glucagon levels are observed to increase during exercise (Winder et al., 1979), possibly via a sympathetic beta-adrenergic pathway (Samols and Weir, 1979). As a result, a decrease in the insulin/glucagon ratio during intense exercise is observed.

Glucagon and epinephrine are powerful stimulators of hepatic glycogenolysis and gluconeogenesis. Low insulin/glucagon and high cortisol levels permit increased release of gluconeogenic amino acids from muscle and glycerol formation via triglyceride hydrolysis, making these substances available to liver tissue for rapid gluconeogenesis. The observation that glycogen accumulates rapidly in skeletal muscle following exercise illustrates a marked difference between liver and muscle in the regulation of glycogen metabolism.

Changes in circulating catecholamines found during exercise are somewhat modified by exercise training. At a given level of exercise intensity and duration, decreased levels of norepinephrine and/or epinephrine have been found in trained individuals (Bloom et al., 1976; Hartley et al., 1972a, 1972b.). The intrepretation of these observations may need to be somewhat modified to account for the apparent increase in receptor site sensitivity to a given plasma level of catacholamines (Pavlik and Frenkl, 1972; Von Euler, 1974). Submaximal endurance training significantly alters the insulin/glucagon response during acute exercise, with the typical decrease in plasma insulin and the increase in plasma glucagon being greatly reduced or eliminated (Galbo et al., 1977)

In addition, skeletal muscle is insensitive to glucagon (Robinson et al., 1971). This fact, operating in concert with the powerful insulin like effect that exercise has on muscle, results in rapid glucose uptake for a considerable period of time after exercise stops (Fell et al., 1982; Ivy and Holloszy, 1981; Terjung et al., 1974; Holloszy and Narahara, 1965). The magnitude of this effect is highlighted by the observation that additional insulin does not change the increment in glucose uptake caused by exercise (Zinman et al., 1979).

Recent findings by Conlee and co-workers (1978) demonstrated that low muscle glycogen is an extremely potent stimulator of muscle glycogen synthesis. This effect overrides the synthesis inhibiting, and glycogenolytic effects of elevated catacholamine levels and/or reduced insulin/glucagon ratios. The mechanism of this action appears to be that glycogen binds glycogen synthetase and protects it from the action of synthetase phosphatase, which is also bound to glycogen. As glycogen is depleted, both enzymes are released, enabling the phosphatase to catalyse the conversion of synthetase D to the I form (Villar-Palasi, 1969). Thus, in rodents (Danforth, 1965b; Conlee et al., 1978) and in man, it has been confirmed (Bergstrom et al., 1971) that there is a good inverse correlation between the percentage of glycogen synthetase in the I form (active) and glycogen concentrations. The suggestion that glycogen levels per se are responsible for accelerated glucose uptake and enhanced glycogenesis is supported by Poland et al. (1980). These investigators demonstrated that fasting for 24 hours had the same effect as exercise in promoting rapid

glycogen resynthesis and even supercompensation.

The concentrations of muscle glycogen obtained in the fasting state and the rate of recovery repletion are generally much less than those seen in the glycogen "supercompensated state" (Terjung et al., 1974; Hultman et al., 1971; Bergstrom et al., 1966;). Nevertheless, glycogen increases significantly during fasting in man (Maehlum et al., 1978; MacDougal et al., 1977; Hermansen and Vaage, 1977) and in rats (Fell et al., 1980; Gaesser and Brooks, 1980; McLean and Holloszy, 1979). Since muscle glycogen is essential for performance of sustained, heavy work (Bergstrom et al., 1967; Pernow and Saltin, 1971), a teleological rational exists in the preferential channeling of gluconeogenic glucose away from liver and into muscle glycogen as a strategy for enhancing the carbohydrate starved animals' capability to survive "fight or flight" situations.

4.4 MUSCLE LACTATE CONTENT

The lactate content of both sedentary and trained control soleus and gastrocnemius muscles agree with previously reported data (Baldwin, Campbell and Cooke, 1977b). There have been numerous studies in both rodents and humans (see Tesch, 1980 for review) which show that lactate accumulates exponentially depending on work intensity and modality (i.e. continuous versus intermittent). The response however is not that well defined. Fell and co-workers (1980) used a very intense exercise protocol requiring rats to run five minute intervals at .70 $m \cdot sec^{-1}$ up a 15% incline followed by a five minute interval at .37 $m \cdot sec^{-1}$, repeated to exhaustion. In both soleus and plantaris there was a

non significant increase in muscle lactate. To examine the relationship between exercise intensity and muscle lactate, Baldwin and associates (1977a) ran rats on a treadmill at power outputs up to 90% of $\dot{V}O_2max$ (.80 $m \cdot sec^{-1}$, up a 17% incline). After 5 minutes of continuous work the lactate content of the soleus muscle increased two fold over controls whereas there was very little change in the lactate content of FOG vastus muscle. The degree of glycogen depletion obtained by this exercise protocol was similar to that observed in the present study.

The data suggest that some of the lactate pool in the gastrocnemius muscle was metabolized during the rest intervals, undoubtedly in competition with the pyruvate being formed via glycolysis. Because the present study used intermittent work the possibility exists that considerable lactate was produced during the exercise bouts but was rapidly removed during the one minute rest periods. Both the SO fibres of the soleus and the FOG fibres of the gastrocnemius possess a large capillary density which would facilitate rapid equilibrium of lactate in various compartments.

It was initially postulated that the exercise protocol selected would be of such an intensity that the animals would fatigue rapidly, and low glycogen/high lactate levels would be induced. In the initial phases of training this appeared to be the case, in that the animals had great difficulty performing five intervals at the criterion power output. During the terminal exercise test the average number of work bouts performed was 29 with two of the animals performing 42 and 45 repeat interval runs. If this work rate was performed

continuously it is likely that it could not be maintained for more than 4 to 5 minutes (Baldwin et al., 1977a).

By using intermittent work as a model, more efficient use is made of stored muscle glycogen or a shift is made towards more oxidative metabolism with a greater contribution from substrates other than glycogen (Essen, 1978). Continuous release of lactate from muscle during interval exercise with subsequent uptake by heart, liver, kidney and non-working muscle is a possibility that should not be ruled out. Since blood lactate was not measured it is impossible to speculate on the magnitude of this factor. It is also possible that there was a rapid resynthesis of glycogen from intramuscular lactate during the rest intervals (Hermanssen and Vaage, 1979), leading to lowered lactate levels and enhanced intramuscular glycogen.

4.5 MUSCLE LACTATE UPTAKE

Using a lactate concentration of 8 mM in the incubation medium a lactate uptake rate of 20 $\mu mol \cdot gram^{-1} \cdot hour^{-1}$ was observed. In studies of perfused rat hindlimbs MacLean and Holloszy (1979) observed that at lactate concentrations greater than 4 mM muscle consistantly removed lactate. Between 6 and 26 mM lactate there was a linear relationship between perfusate lactate concentration and the rate of lactate uptake. Interploation of these data reveals that at lactate concentrations of 8 mM the rate of lactate uptake was 24 $\mu mol \cdot gram^{-1} \cdot hour^{-1}$, in agreement with the present findings.

The linearity of the lactate uptake curve reported by McLane and Holloszy (1979) suggests that the mechanism of

lactate uptake may be quite different from the process involved in lactate transport out of the cell. The often observed concentration gradient from muscle to blood (Tesch, 1980; Sahlin, 1978) has led to postulation of a rate limited carrier translocation mechanism for moving lactate out of the cell (Sahlin, 1978; Hirche et al., 1970). At high intramuscular lactate concentrations a well defined levelling off in lactate release is observed (Karlsson et al., 1972) indicating saturation of the translocation mechanism (Mainwood and Worsley-Brown, 1975). The observations of McLane and Holloszy were not supported in an earlier report by Jorfeldt (1970) indicating a non-linear relationship between lactate uptake and arterial lactate inflow, suggesting a saturation state in the mechanisms responsible for lactate uptake.

4.6 EFFECTS OF in vitro INCUBATION ON MUSCLE GLYCOGEN CONTENT

In vitro incubation of muscle tissue slices in a bicarbonate buffered lactate solution had highly significant effects on the muscle glycogen content of all groups, except in the soleus muscle of animals which were fasted following exhaustive exercise. Pearce and Connett (1980) have observed rapid glycogenolysis in rat soleus incubated for 30 minutes in a straight bicarbonate buffer gassed with 95% oxygen/5% carbon dioxide similar to those seen in the TC and SC groups of the present study.

Due to the theoretical diffusional limitations in incubated muscle slices of 1 millimeter thickness, it may take tissue considerable time to recover from the hypoxic stress of

dissection (Pearce and Connett, 1980). The acceleration of glycolysis in hypoxic hepatic tissue has been clearly shown (Hems and Brosnan, 1970; Woods and Krebs, 1971). There are multiple responses which are a function of oxygen tension. The diffusion limitations that may exist in in vitro incubation studies have not been well considered. In addition, treatments such as slicing the tissue increases metabolic rate (Stainsby and Welch, 1966).

If skeletal muscle in this study responds similarly to hepatic tissue under hypoxic conditions it may be that an inhibition of the dephosphorylation of phosphorylase a by AMP (Stalmens and Hers, 1974) was activating glycogenolysis. Inorganic phosphate and glucose-6-phosphate would contribute to an increase in phosphorylase a and thus enhance the glycogenolytic effect. The incubation system used in the present study may have presented a hypoxic stress to the tissue since the oxygen tensions of the medium were less than those used by Pearce and Connett (1980), although the use of 95% air/5% CO_2 in tissue incubation studies has precedence in the literature. The inclusion of 5% CO_2 in the gas mixture was considered necessary based on the work of Bendall and Taylor (1970) which suggested that this molecule is necessary for the operation of the malic enzyme (see Review of Literature). These observations underscore the need to allow sufficient time for a steady-state metabolic condition to be achieved in incubation studies of this type.

It is however, a presumption that the tissue in these incubations was hypoxic. Metabolizing tissue will make use of the preferred fuel source which in the case of rested muscle is

glycogen. Thus in the supercompensated muscle tissue rapid depletion might be expected. High glycogen levels have been recognized for some time to stimulate glycogenolysis. The mechanism of this action is through an activation of glycogen phosphorylase (Maddaiah and Madsen, 1966) and a inhibition of glycogen synthetase (Hultman, 1971; Danforth, 1965b).

Trained exhausted soleus and gastrocnemius tissue demonstrated a significant repletion rate of muscle glycogen during the one hour *in vitro* incubation. The mean increases in glycogen were 0.184 $mg \cdot gram^{-1}$ wet weight of soleus and 0.206 $mg \cdot gram^{-1}$ wet weight of gastrocnemius tissue. In a similar study McLane and Holloszy (1979) demonstrated a rate of glycogen accumulation of 0.043 $mg \cdot gram^{-1} \cdot hour^{-1}$ in soleus muscle and 0.205 $mg \cdot gram^{-1} \cdot hour^{-1}$ in gastrocnemius muscle of rat hindlimbs perfused 12 mM lactate. The finding of a net rate of glycogen synthesis in soleus which is 4 times greater than that shown by McLane and Holloszy (1979) may be explained on the basis of the low initial glycogen levels in the present study (ie. 0.108 $mg \cdot gram^{-1}$ compared to 0.421 $mg \cdot gram^{-1}$) which in turn activate glycogen synthetase (Conlee *et al.*, 1978; Bergstrom *et al.*, 1971; Danforth, 1965a).

The rate of glycogen synthesis from lactate in the gastrocnemius muscle of the present study is nearly identical to the value reported by McLane and Holloszy (1979) perhaps indicating a maximum rate of lactate to glycogen conversion in the range of 0.200 $mg \cdot gram^{-1}$ wet weight $\cdot hour^{-1}$. These values are generally less than those reported for glycogen repletion in carbohydrate fed animals. The reported rates, corrected for

diurnal variation, are 0.5 mg·gram-1·hour^{-1} in white m. vastus lateralis and 1.4 mg·gram^{-1}·hour^{-1} in m. soleus (Poland et al., 1980; Terjung et al., 1974).

In vitro incubation of exhausted fasted muscle tissue caused no net change in glycogen content from pre incubation values in the soleus muscle. When compared with the results of trained or sedentary control animals it may be implied that glycogen synthesis is keeping pace with, or has made up for the glycogen degradation that may have occured earlier in the incubation. The metabolic features which are unique to this type of glycogen depleted muscle tissue remains unclear, however there appears to be a trend towards lower glycogen degradation and/or increased synthetic rates.

4.7 METABOLISM OF ^{14}C LACTATE

In order to desribe and quantify the fate of lactate in skeletal muscle the appearance of radiolabelled carbons derived from uniformly labelled ^{14}C lactate were determined in carbon dioxide and glycogen of incubated muscle tissue. Exhaustive exercise had a highly significant effect on increasing the incorporation of the radiolabelled tracer into glycogen. When the specific activity of the tracer was corrected for dilution by the endogenous muscle lactate pool and for dilution by lactate in the incubation medium, a slightly different profile emerged. The most obvious feature is that lactate conversion to glycogen in gastrocnemius muscle was approximately 100% greater than soleus muscle. The rate of soleus muscle lactate glycogenesis was two fold greater than previously reported

values (McLean and Holloszy, 1979), whereas the value for gastrocnemius was very similar. The most striking feature of the soleus profile was observed in tissue sampled from exhausted animals. The rate of lactate glycogenesis in this group was not statistically different from gastrocnemius and the rate of lactate conversion to glycogen was much higher than any previously reported. In vivo studies by Brooks and Gaesser (1980) confirmed these findings in continuous and intermittent exercise stimulating glycogen synthesis in skeletal muscle. The observation of rapid glycogen synthesis in incubated soleus muscle was especially interesting based on the activity of the glycogenic enzymes that bypass the pyruvate kinase and phosphofructokinase reactions.

The major metabolic fate of lactate in both control soleus and gastrocnemius muscle in the present study, was oxidation to carbon dioxide. This finding confounds those of Meyerhof (1920), Bendall and Taylor (1970) and Hermansen and Vaage (1979, 1977) which indicated that approximately 25% of the lactate metabolized by skeletal muscle is oxidized to carbon dioxide, the rest being converted to glycogen. The present results indicated that oxidation accounted for between 46 and 86% of the total lactate metabolized. In all groups the greatest fractional oxidation occurred in soleus muscle, which is concomitant with the SO motor unit profile of this muscle. Exhaustive exercise greatly increased the total metabolism of radiolabelled lactate in soleus muscle and reduced the fraction of total lactate which is oxidized in both muscles. Concurrently, the fraction of the total lactate converted to glycogen was increased. Unpublished

data by Brooks (cited by Brooks and Gaesser, 1980) describe a decrease in the recovery of lactate derived carbons as carbon dioxide from 98% at rest to 60% during recovery from intermittent exercise. The present data illustrated a similar phenomena in that oxidation of lactate decreased from 86 to 52% in soleus and from 76 to 46% in gastrocnemius muscles of exhausted (TE) animals.

The data of Brooks and Gaesser (1980) suggest that only 7.9% of the total radiolabelled lactate is incorporated into glycogen during one hour of recovery from exhaustive intermittent exercise. After four hours 10.8% of the total is incorporated into glycogen. The results from the present study demonstrated that 48% and 53% of the total lactate metabolized is incorporated into glycogen in soleus and gastrocnemius muscles respectively within one hour post exercise. The calculations of Brooks and Gasser (1980) were based on the following assumptions:

1. that muscle weight was 45% of the whole body mass in the rat,
2. that the quadriceps muscle was representative of this fraction,
3. that 40% of the total muscle mass was involved in the exercise,

The in vitro design of the present study avoided the necessity for assumptions of this nature.

In an original re-investigation of the whole question of whether skeletal muscle posesses the ability to convert lactate to glycogen Bendall and Taylor (1970) showed that in excess of 80% of the lactate metabolized by incubated frog sartorius

muscle is converted to glycogen, apparently vindicating Meyerhof's original observations (1920, 1925). Support for this theory has also been provided by McLane and Holloszy (1979) who showed that 44% of the total lactate metabolized by a perfused rat hindlimb is incorporated into glygogen. In vivo support for these findings in humans has been demonstrated by Hermansen and Vaage (1979, 1977), who calculated that following maximal intermittent leg exercise, 75% of the lactate removed is recovered as glycogen. Brooks and Gaesser (1980) criticised these results suggesting that the use of venous plethysmography to measure blood flow and the absence of direct determinations (^{14}C tracers) invalidate the conclusions.

The isolation of the radionuclide in glycogen extracted from all experimental groups of both muscle types was surprising in view of the significant glycogen depletion which was observed in both control groups and the steady state observed in the tissue from fasted animals following incubation in lactate. The observation has been made that in the first 30 minutes of incubation glycogenolysis in skeletal muscle is pronounced and rapid (Pearce and Connett, 1980). Thus, the incorporation of the radionuclide in all the experimental groups used in this study may be indicative of a recovery of the tissue samples from the trauma of dissection and a shift towards a glycogenic phase of metabolism. However, this study does not provide definitive evidence for such an occurance.

The greatly increased incorporation of the radionuclide into glycogen in the soleus and gastrocnemius of trained exhausted muscle is evidence in support of glycogen depletion

being a potent stimulator of glycogen synthesis. However, the specific activity of the recovered glycogen and the calculated rates of lactate to glycogen conversion are not sufficient to account for the magnitude of glycogen repletion observed in incubated muscle from exhausted animals. This observation was not unique to this study. McLane and Holloszy (1979) documented an actual measured increase in muscle glycogen of 2.4 $\mu mol \cdot gram^{-1} \cdot min^{-1}$ in the soleus and 11.2 $\mu mol \cdot gram^{-1} \cdot hour^{-1}$ in the plantaris muscles of rats during one hour perfusions with lactate as the only substrate. However, when calculated from ^{14}C lactate incorporation they obtained values of 0.87 and 5.8 $\mu mol \cdot gram^{-1} \cdot hour^{-1}$ for soleus and plantaris muscle respectivley. The authors noted the discrepancy but offered no explanation. A similar observation was made in a study using glycerol as the sole substrate in a rat hindlimb prefusion (Terblanche et al., 1981). These investigators measured a rate of glycogen synthesis of 4.24 and 2.26 $\mu mol \cdot gram^{-1} \cdot hour^{-1}$ in plantaris and soleus muscle respectively. On the basis of radiolabelled glycogen synthesis, however, they could only account for 1.02 $\mu mol \cdot gram^{-1} \cdot hour^{-1}$ of glycogen synthesis in plantaris muscle and 0.75 $\mu mol \cdot gram^{-1} \cdot hour^{-1}$ in soleus. Although these authors claim that glycerol was not an important precursor for the observed increase in muscle glycogen content they could not offer an alternative.

The studies by McLane and Holloszy (1979), by Terblanche and co-workers (1981), and the present study highlight an interesting point. It is apparent that lactate, under certain conditions, is an important glycogenic precursor in skeletal

muscle. However, this does not appear, at least in *in vitro* preparations, to account for the measured rates of glycogen resynthesis. It thus remains to be determined what additional glycogenic precursors may be of importance in this process. Glycerol seems to have limited glycogenic potential based on the absence of activity of glycerol kinase in skeletal muscle (Newsholm and Taylor, 1969). Vaage, Newsholm, Gronnerod and Hermansen (unpublished, cited by Hermansen and Vaage, 1977) measured the increase of the glycolytic intermediates glucose-6-phosphate, malate and alpha glycerolphosphate following intense exercise. Based on the decline in concentration of these metabolites during the recovery period, they calculated that approximately 3 mmol glucosyl U·kgm^{-1} wet weight could be accounted for if these intermediates were synthesized to glycogen. This value is approximately 30% of the actual measured glycogen resynthesis in their experiments.

During prolonged exercise substrate mobilization and hormonal changes are in many ways analogous to the situation that occurs during starvation (Lemon and Nagle, 1981). During prolonged exercise protein may contribute significantly to the body's total metabolic requirement (Refsum and Stromme, 1974) particularly in muscle with low glycogen content (Lemon and Mullen, 1980). These observations suggest a relationship between carbohydrate supply and protein degredation. The ability of skeletal muscle to oxidize protein, particularly the branched chain amoin acids (BCAA) is well recognized (Buse *et al.*, 1975; Dohm *et al.*, 1976). It is expected that during exercise the uptake of BCAA to skeletal muscle would be decreased because of

decreasing insulin (and possibly testosterone). However, an exercise induced increase in growth hormone release could promote amino acid uptake into muscle (Hartley et al., 1972a). In addition, increasing glucagon, catecholamine and cortisol levels would enhance protein degredation (Lemon and Nagle, 1981). It is hypothesized that carbon skeletens from this readily available pool of BCAA may serve as direct intramuscular percursors of glycogen. Further work is needed to quantify the relative importance of other endogenous substrates to skeletal muscle glycogenesis.

Calculations of glycogen synthesis from radiolabelled lactate assume a constant specific activity of the tracer. Previously held views that lactate is freely diffusable are not supported by the current values of Km and Vmax for lactate movement across muscle membranes (Karlsson et al., 1972; Mainwood and Worsley-Brown, 1975). Hultman and co-workers suggested a saturation state may exist in the mechanisms responsible for lactate uptake into skeletal muscle (Jorfeldt, 1970). Thus it may take considerable time for equilibration of the radionuclide with tissue metabolite pools. The present findings confirmed the observation by Connett (1979) that net glycogen content does not reflect labelled lactate incorporation in these types of incubation studies of short duration. These findings are in agreement with the observations of Hermansen and Vaage (1979, 1977) that the majority of the glycogen repletion following exercise arises from endogenous intramuscular precursors. However, if in vitro preparations are provided an initial incubation period for equilibration with intracellular

pools (one hour) incorporation of the labeled carbons is linear for up to 6 hours (Connett, 1979). Thus, the appearance of radiolabelled carbons derived from lactate in the present study reflects the rate of conversion of lactate to glycogen but not the absolute magnitude.

The major assumption made in this study is that there are only two metabolic fates of lactate in skeletal muscle; oxidation to carbon dioxide and conversion to glycogen. Other metabolic fates, such as transamination of pyruvate to alanine (Mole et al., 1973), would reduce the magnitudes of the fractional incorporations reported in this study. However, this appears to be quantitativly non significant and in similar studies, immeasurable (McLane and Holloszy, 1979). Pyruvate is also released from skeletal muscle, and as noted by McLane and Holloszy (1979) can account for up to 20% of the total lactate metabolized by perfused rat hindlimb.

4.8 GLYCOGENIC ENZYME ACTIVITIES

The thermodynamic barriers that block the direct conversion of lactate to glycogen provide not only an energetic but also a conceptual blockade to the possibility of intramuscular glycogenesis. The current evidence of glycogen synthesis from lactate-derived carbons therefore implies the existence of several unique enzyme systems. The question is: "by what pathway is the flux of lactate carbons to glycogen occuring" (Krebs and Woodford, 1965; Bendall and Taylor, 1970).

Because of the absence in skeletal muscle of the mitochondrial protein pyruvate carboxylase (Crabtree et al.,

1972; Krebs and Woodfrord, 1965), Bendall and Taylor (1970) hypothesized the formation of an extramitochondrial intermediate catalysed by the malic enzyme (malate dehydrogenase, $NADP^+$, decarboxylating). Thus glycogenesis is perceived to occur via a pathway independent of mitochondrial dicarboxylic acid pools.

The present findings confirm several reports of significant activity levels of the malic enzyme in mammalian skeletal muscle (McLean and Holloszy, 1979; Opie and Newsholm, 1967). The present values for soleus muscle are within one activity unit of the values reported by McLean and Holloszy. Due to the 4^oC cooler temperature of the assay used in the present study the enzyme activities might be expected to be higher than values reported by McLean and Holloszy. The malic enzyme activity level in the gastrocnemius muscle compares favorably with the data of McLean and Holloszy (1979) for FG and FOG (plantaris) muscle but is aproximately 50% below the values reported for FOG (deep medial gastrocnemius). Since gastrocnemius lacks homogenicity of fibre populations, sampling site may be expected to play a significant role in determining measured activity levels. McLean and Holloszy sampled from deep portions of the medial gastrocnemius where a predominance of FOG motor units are found, whereas the present study used a cross section through the same area where a substantial inclusion of FG motor units would contribute to the sample.

In both hepatic and skeletal muscle glycogenesis phospho(enol)pyruvate carboxykinase catalyses the phosphorylation of cytosolic oxaloacetate to phosphoenolpyruvate. The activities of this enzyme, similar to

those seen in liver and kidney have been demonstrated in a variety of skeletal muscle, including soleus, gastrocnemius and diaphragm (Mclane and Holloszy, 1979; Opie and Newsholm, 1967; Krebs and Woodford, 1965). The phospho(enol)pyruvate carboxykinase activity observed in the soleus muscle was considerably higher than values reported by McLean and Holloszy. PEP carboxykinase activity in gastrocnemius muscle is about 300% higher in both experimental groups. Training had the effect of potentiating the activity of this enzyme.

Further along the glycogenic pathway is the cytosolic enzyme fructose-1,6-diphosphatase, catalysing the dephosphorylation of fructose-1,6-diphosphate. This enzyme too, has been demonstrated in the skeletal muscle of numerous mammalian species (McLean and Holloszy, 1979; Opie and Newsholm, 1967; Krebs and Woodford, 1965). Gastrocnemius fructose-1,6-diphosphatase activity did not change with training and values lie within those given by McLean and Holloszy. The results for soleus muscle are interesting in view of the fact that McLean and Holloszy could not demonstrate activity of fructose-1,6-diphosphatase in this muscle. Hintz et al. (1980), however, have demonstrated the activity of this enzyme in soleus muscle of the rat. Under the assay conditions used by this group the reported values are lower than the present findings and are probably much lower than the actual fructose-1,6-diphosphatase which was measured at 20°C instead of body temperature.

The intense exercise training programme used in this study had surprisingly little effect on the activity levels of the enzymes assayed. The increase in oxidative and, to some degree,

glycolytic potential of various muscle fibres following different training regimens has been well documented. Presumably, the increased demand on the energy transduction systems of muscle during exercise is the stimulus. It was hypothesized in this study that one minute work/rest intervals at the intensity used during training would place considerable load on the systems responsible for lactate removal. In terms of the enzymes considered this was not a valid assumption. One possibility is that the training programme induced considerable aerobic adaptation, and that high muscle lactates were not encountered once the animals had adapted to the running regimen (Essen, 1978). A potential shortcoming of this study may be that by week eight of the training programme the animals could maintain the required power output for ten bouts. The effects on the glycogenic enzyme activities may have been enhanced by continuing the progressive overload nature of the training, rather than having a four week maintainance period during which work output was not increased.

These enzymatic studies further emphasize the uniqueness of exhausted, glycogen depleted muscle. In spite of a fairly consistent glycogenic enzyme profile across sedentary and trained groups, soleus muscle from trained exhausted animals replete glycogen and incorporate radiolabelled carbons from lactate at rates far exceeding all other treatment conditions.

V. SUMMARY

This thesis was prompted by a report by Hermansen and Vaage in 1977 which described what appeared to be a direct intramuscular conversion of lactate to glycogen in the vastus muscles of subjects recovering from maximal exhaustive work. This observation has considerable theoretical appeal to those interested in the field of exercise science in light of the numerous publications criticising such a process on the basis of the thermodynamic nature of the reactions involved. However, a review of the literature of the past century revealed that there is considerable a priori evidence for glycogenesis from lactate in the skeletal muscle of mammals.

It was hypothesized that if such a process was occurring in skeletal muscle, conditions such as high intensity exercise, which place a metabolic load on the biochemical pathways of lactate metabolism, should affect the glycogenic potential of this metabolite. Male Wistar rats were trained to run 10 repeat one minute work/rest intervals on a motor driven treadmill at 1 $m \cdot sec^{-1}$ up an 8% incline. Following 12 weeks of training the animals were assigned to one of three treatment groups; a trained control group (TC), a trained group that performed an acute interval run to exhaustion (TE), and a trained group that performed a similar exercise test but were subsequently placed on a 12 hour fast (TF). These three groups were paired with an age matched sedentary control group (SC).

Twelve weeks of training resulted in what is a well documented increase in the storage of glycogen in both soleus

and gastrocnemius muscles. The acute exercise test revealed that the training programme had been quite successful in that the animals were able to maintain the required power output for a mean of 29 work bouts. This observation was also supported by the fact that muscle lactate did not increase as a result of the exhaustive run. The run to exhaustion produced a highly significant glycogen depletion in both muscles. When the run was superceded by a 12 hour fast, considerable glycogen resynthesis occured. This was a unexpected result since the purpose of this procedure was to obtain glycogen depleted muscle in a metabolically rested state.

Tissue slices of the soleus and gastrocnemius muscles from animals in each group were incubated in an oxygenated, buffered Ringers solution containing 8 mM l-lactate and 1 μCi of uniformly labelled ^{14}C l-lactate. Following one hour of incubation; muscle glycogen synthesis and carbon dioxide production were determined in terms of both specific activity of the radiolabel and the amount of lactate involved in each process. It was observed that during the incubation muscle glycogen content in all groups except TE, decreased rather substantially in both soleus and gastrocnemius muscles. In the acutely exhausted animals, rapid and pronounced muscle glycogen resynthesis occured in both muscles.

When the beta emmision from the extracted muscle glycogen was quantified, the tracer carbons were found to be incorporated in all treatment groups and in both muscles. Gastrocnemius muscle appeared to be quantitativly more active in this process. However, in the glycogen extracted from the acutely exhausted

animals the incorporation of the radiolabel was markedly increased. When the specific activity of the radiolabel was expressed as a rate of lactate conversion to glycogen it became obvious that the gastrocnemius muscle had a much greater potential to perform this function. Acute exhaustive exercise accelerated lactate conversion to glycogen in the soleus muscle such that the rate was not significantly different from gastrocnemius muscle. This enhancement of lactate to glycogen conversion following exhaustive exercise was not observed in the gastrocnemius muscle.

The rate of tracer oxidation to carbon dioxide was significantly greater in the soleus muscle, and 12 weeks of interval training was observed to enhance this process. When the rate of lactate oxidation was calculated from the specific activity of the tracer, acute exhaustive exercise induced a decrease in the rate of lactate oxidation in both muscles.

When the ~~rates~~ of lactate conversion to glycogen and oxidation to carbon dioxide were expressed as percentages of the total lactate metabolized, it was observed that there was a much greater percentage of lactate converted to glycogen in the gastrocnemius muscle of all treatment groups. In addition, acute exhaustive exercise increased this fraction in both soleus muscle and gastrocnemius muscle, with the most dramatic increase being demonstrated in the soleus muscle. The percentage of the total lactate oxidized was greater in the soleus muscle of all treatment groups except the acutely exhausted group. In this group, the acute exhaustive run had the effect of reducing the fraction of lactate oxidized in both the soleus and

gastrocnemius muscles.

The incorporation of radiolabelled lactate carbons into glycogen extracted from both soleus and gastrocnemius muscles suggests the presence of an enzyme system in muscle, generally considered to be exclusive to the gluconeogenic tissues, the liver and the kidney. The present study confirmed the existence of phosphoenolpyruvate carboxykinase, fructose-1,6-diphosphatase, and the cytosolic malic enzyme in both the soleus and gastrocnemius muscle of the rat. With the exception of phospho(enol)pyruvate carboxykinase, the activities of these enzymes did not increase as a result of the training programme. The activities of these enzymes, particularly fructose-1,6-diphosphatase, were generally lower in the soleus muscle. This may be the basis for the greater rate of conversion of lactate to glycogen observed in SC, TC, and TF groups but does not explain the potentiation of this process in the soleus muscle of acutely exhausted animals.

VI. CONCLUSIONS

Within the limitations of *in vitro* rodent experimentation the following conclusions were drawn from this study:

1. Twelve weeks of a progressive treadmill running programme increased the glycogen storage capacity of both soleus and gastrocnemius muscle.
2. Running repeat one minute work/rest intervals at 1 $m \cdot sec^{-1}$ up an 8% grade will result in significant glycogen depletion in both soleus and gastrocnemius muscles, with little increase in tissue lactate.
3. Fasting an exhausted, glycogen depleted rat for 12 hours will not maintain the glycogen depleted state as significant muscle glycogen will be resynthesised.
4. When lactate is the only substrate available to incubated soleus and gastrocnemius muscle tissue lactate carbons will be incorporated into the glycogen stores, although gastrocnemius muscle tissue has a much greater capacity for this process.
5. Glycogen resynthesis from lactate in soleus and gastrocnemius muscles is dramatically enhanced by acute exhaustive exercise performed immediately prior to incubation of muscle tissue.
6. The conversion of lactate to glycogen during one hour incubation accounted for only 40% of the actual measured glycogen content of soleus and gastrocnemius muscle sampled from exhausted animals.
7. The major fate of the lactate metabolised by incubated

soleus and gastrocnemius muscle is oxidation to carbon dioxide. Typically, soleus muscle will oxidize 87% and gastrocnemius 67% of the total metabolised lactate.

8. Following acute exhaustive exercise the rate of lactate oxidation decreased from 87 to 50% in soleus muscle and from 67% to 47% in gastrocnemius muscle.
9. Acute exhaustive exercise shifts the fraction of lactate converted to glycogen from 13% to 48% in soleus and from 23% to 53% in gastrocnemius muscle.
10. Soleus and gastrocnemius muscles of the rat contain measurable quantities of the glycogenic enzymes fructose-1,6-diphosphatase, phosphoenolpyruvate carboxykinase, and the "malic enzyme".
11. The activity levels of PEP carboxykinase and fructose-1,6-diphosphatase were significantly higher in gastrocnemius muscle when compared to soleus muscle. It was suggested that this may account for the greater capacity of this type of tissue to convert lactate to glycogen.
12. Twelve weeks of interval training had no effect on increasing the activity levels of the glycogenic enzymes except for PEP carboxykinase activity in gastrocnemius muscle.

It was concluded from the present study that lactate can serve as an important precursor of glycogen in rat skeletal muscle. Although it appears that this process is quantitatively more important in the gastrocnemius muscle, acute exhaustive exercise and/or the accompanying glycogen depletion substantially increases the rate and extent of this process both

in the gastrocnemius and soleus muscle. The evolutionary advantage of being able rapidly to reconvert the end-product of high intensity work to glycogen directly within the muscle, may be a strategy for enhancing an animal's abilty to survive repeated "fight or flight" situations.

APPENDIX 1. Buffered Incubation Medium

1.6 mM $MgCl_2$

126 mM NaCl

5 mM KCl

3 mM $CaCl_2$

16 mM $NaHCO_3$

0.667 mM Na_2HPO_4

0.172 mM NaH_2PO_4

addition of 8 mM Na^+ l-lactate

APPENDIX 2. Glycogen Assay

REAGENTS

1. 30% Potassium hydroxide solution (KOH), saturated with sodium sulfate (Na_2SO_4).
2. 95% ethanol
3. 5% phenol
4. Standard glycogen solution 25 mg glycogen powder is dissolved in distilled water to 100 ml, and lower standards are made by dilution.

PROCEDURE

1. Muscle samples of 35-50 mg are trimmed of connective tissue, weighed and frozen in isopentane cooled in liquid nitrogen. Muscles are stored frozen at -60°C.
2. 0.5 ml of KOH/Na_2SO_4 is added to the sample making sure the tissue is immersed.
3. Boil 20-30 minutes until the solution is homogenous.
4. Remove from boiling bath, cool briefly (5 min.) in ice.
5. Add 0.7 ml of 95% ethanol to precipitate glycogen.
6. Put samples on ice for 20-30 minutes.
7. Centrifuge at 2000.g for 10 minutes.
8. Carefully aspirate and retain the supernatant leaving precipitated glycogen in the bottom of the tube. (The ethanol-KOH supernatant is retained for lactate analysis).
9. Wash the glycogen pellet with cold 66% ethanol and centrifuge at 2000.g for 10 minutes.
10. Repeat procedure 8 three times, retaining all supernatants for lactate determination.
11. Dissolve the glycogen pellet in 3.0 ml distilled water.

12. Use 1.0 ml of this glycogen-water solution for the assay by pipetting it into a clean test tube in duplicate. At this point pipette 1.0 ml of each of the prepared standards into clean test-tubes in duplicate.
13. Add 1.0 ml of 5% phenol.
14. Add 5.0 ml of 95-98% sulphuric acid rapidly so it does not touch the sides of the test tube.
15. Allow to stand for 10 minutes, shake and place in 25-30°C water bath for 20 minutes.

APPENDIX 3. Lactate Assay

REAGENTS

1. 50 mM 2-amino-2-methyl propanol buffer, pH 9.9
2. 1.5 mM NAD^+
3. 50 mM glutamate
4. 100 µg/ml (20 u/ml) lactate dehydrogenase (beef heart)
5. 100 µg/ml (8 u/ml) glutamic-pyruvic transaminase (pig heart)

PROCEDURE

1. KOH-ethanol supernatants are aspirated and retained following glycogen precipitation with ethanol, neutralized with $HCLO_4$ and decanted from the $KCLO_4$ precipitate.
2. Prepare a solution of 50 mM 2-amino-2-methyl propanol buffer containing 50 mM glutamate (pH 9.9).
3. Pipette 2.8 ml of above solution into reaction cuvette.
4. Dissolve 0.06 g NAD^+ in 1 ml distilled water. Pipette a 50 µl aliquot of this solution into the reaction cuvette.
5. Add 24 units of lactate dehydrogenase in 50 µl to the cuvette.
6. Add 24 units of glutamate-pyruvic transaminase in 50 µl to the cuvette.
7. Mix contents of the reaction cuvette thoroughly.
8. Pipette 50 µl of neutralized KOH-ethanol supernatant into the reaction cuvette. At the same time pipette 50 µl of prepared lactate standard into cuvettes at least in duplicate.
9. Allow reaction to occur for 10-12 minutes.
10. Read at 340 nm.

COMMENTS

1. Because the enzymes are somewhat unstable at this alkaline pH, neither enzyme should be added to the reagent more than 30 minutes before starting the reaction.
2. All glassware used for reagents and analysis should be repeatedly rinsed. It is recommended that gloves be worn during the RINSING PROCESS.

APPENDIX 4. Fructose-1,6-Diphosphatase Assay

Determined on the supernatant fraction of 5% (w/v) muscle homogenate centrifuged at 4°C for 10 minutes at 2000.g.

REAGENTS

A. Homogenizing Medium, pH 7.0 at 4°C

1. 50 mM tris-HCL buffer
2. 154 mM KCL
3. 10 mM mercaptoethanol
4. 1 mM EDTA

B. Assay Medium, pH 7.5 at 25°C

1. 50 mM tris-HCl
2. 20 mM mercaptoethanol
3. 6 mM $MgSO_4$
4. 1 mM EDTA (neutralized)
5. 0.2 mM NADP
6. 0.1 mM Fructose-1,6-diphosphate

PROCEDURE

to 2 ml of assay medium add:

1. 4 µg glucose-6-phosphate dehydrogenase
2. 4 µg phosphoglucose isomerase/2 ml assay medium
3. 0.05 ml of tissue preparation

APPENDIX 5. Phosphoenolpyruvate Carboxykinase Assay

Determined on the supernatant fraction of 5% (w/vol) muscle homogenate prepared in distilled water, and centrifuged at 4 ^{o}C for 10 minutes at 2000.g.

REAGENTS

Assay Medium, pH 7.5 at 25^{o}C

1. 66 mM tris-HCl
2. 1.1 mM $MnCl_2$
3. 1.1 mM phosphoenolpyruvate (tricyclohexylammonium salt)
4. 17 mM $NaHCO_3$ (freshly gassed with 5% CO_2 in O_2)
5. 0.16 mM $NADH_2$
6. 1.54 mM IDP
7. 0.01 ml malate dehydrogenase

PROCEDURE

1. Add 50 µl of crude extract to 3 ml of assay medium.
2. Follow the rate of oxidation of $NADH_2$ at 340 nm.
3. Run a control cuvette omitting $NaHCO_3$ to compensate for the nonspecific oxidation of $NADH_2$.

APPENDIX 6. Malic Enzyme Assay

Determined on the supernatant fraction of a 10% (w/v) muscle homogenate centrifuged at 4°C for 10 min at 2000.g.

REAGENTS

A. Homogenizing Medium, pH 7.4.

1. 175 mM KCL
2. 10 mM glutathione
3. 2 mM EDTA

B. Assay Medium, pH 7.7 at 26°C

1. 54 mM triethanolamine buffer containing:
2. 0.5 M l-malate
3. 3.4 mM $MgCl_2$
4. 0.218 M NADP
5. 0.1 ml of enzyme

PROCEDURE

1. The reaction is followed by measuring the increase in absorption at 340 nm.
2. The reactants are added to the cell while it is in position in the holder. After the addition of all the reactants except enzyme 2 min. is allowed for temperature equilibration.
3. The enzyme is added to start the reaction and all contents are stirred vigorously for 10-15 sec.
4. The first reading is taken 30 sec. after addition of enzyme and every 30 sec. for 4 min., or every 15 sec. for 2 min.

APPENDIX 7. REVIEW OF LITERATURE

The metabolic significance of the three carbon molecule l-lactate, has been the object of considerable scientific investigation since its first reported discovery in the muscle tissue of fatigued game (cited from Hermansen and Vaage, 1979). Since then physiologists and biochemists alike have devoted much time to the study of this metabolite. The lactate molecule maintains an intimate association with processes fundemental to the performance of normal work tasks and those tasks reserved for elite athletic performances.

Lactate production is a strategy adopted by a cell to maintain a mileau intern conducive to drive the energy producing breakdown of glycogen. The production of lactate is not, however, without compromise. Lactate itself is constantly touted as an important factor in the genesis of fatigue and thus a limiter of one's ability to perform muscular work. This review attempts to relate the relevant information regarding the production of lactate, its relation to glycogen metabolism, and its role in fatigue. The mechanism of lactate acid removal, as it relates to glycogen metabolism, is also considered.

LACTATE PRODUCTION, ACCUMULATION AND RELEASE

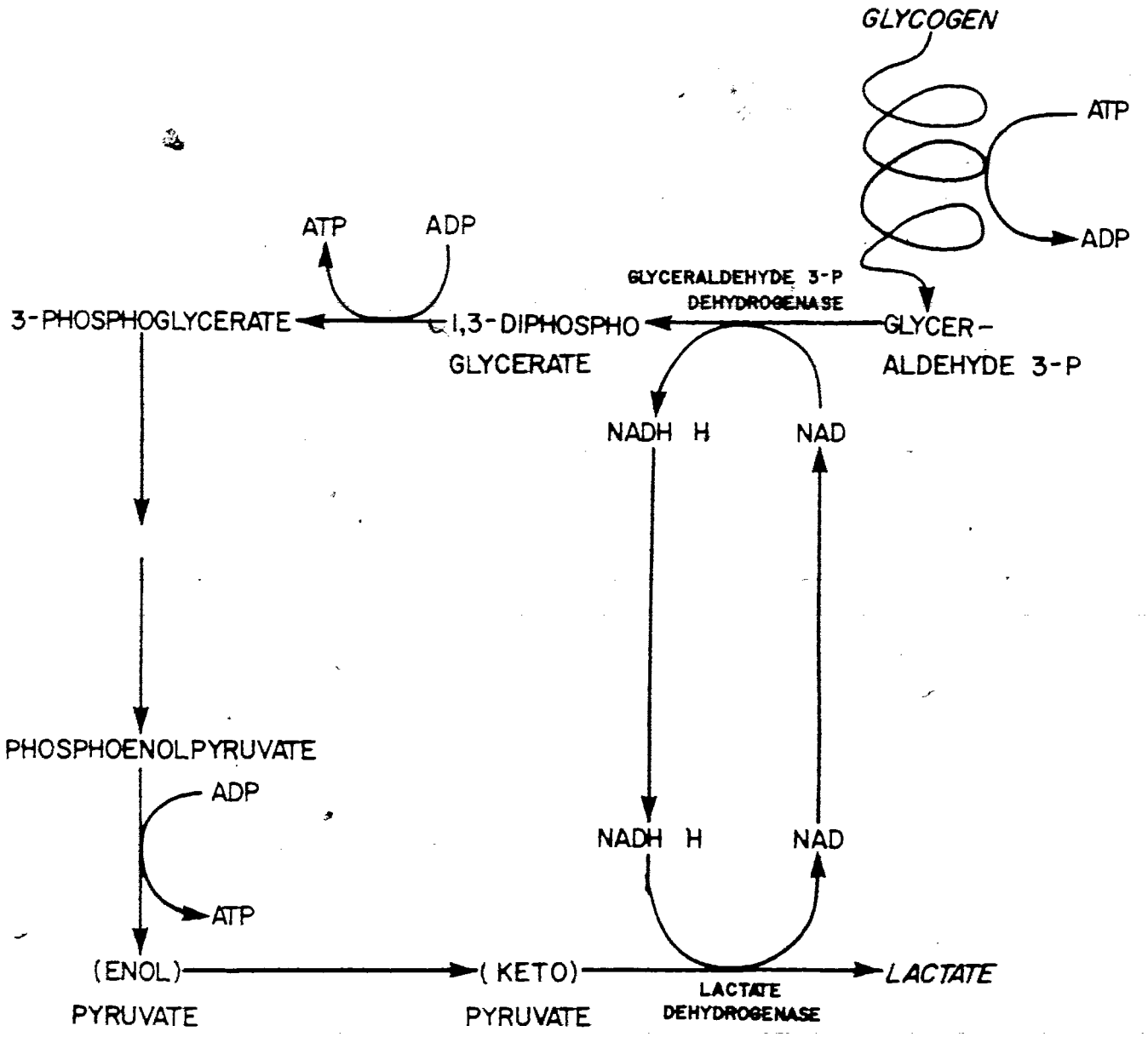
In the glycolytic cascade, regulation of glycolysis by ADP provides an automatic adjustment of carbohydrate metabolism supplying high energy phosphate for metabolic processes, such as muscle contraction. However, an accumulation of ADP cannot accelerate glycolysis to the rate required to match energy consumption in high intensity exercise unless the NADH being

produced is somehow re-oxidized. If the cytoplasmic NAD/NADH ratio is low, the rate of glycolysis is slowed. This implies that work can only proceed at a reduced rate or must cease altogether.

In resting states and in low intensity/long duration exercise, reducing equivalents in the form of NADH are used as substrate for intermitochondrial oxidative phosphorylation. Since NADH cannot diffuse across the mitochondrial membrane a number of hydrogen ion shuttle mechanisms have been postulated (Harper et al., 1976). When faced with an excessive metabolic load as imposed by high intensity exercise, the shuttling of reducing equivalents into the mitochondria may become compromised. This situation would lead to an unfavorable redox state of the muscle cytosol and lower glycolytic activity (Edington et al., 1971; Wendt and Chapman, 1976; Wenger and Reed, 1976). In actual fact the cytosolic NAD/NADH ratio is observed to increase 14 fold (Edington, 1971). The consequence of maintaining this ratio is the formation of lactate from pyruvate. Thus the maintainance of a more oxidized cytoplasm during periods of excessive energy demands infers the formation of lactate. Figure 12 diagrams the formation of lactate through the close coupling of two pyridine nucleotide linked reactions each catalysed by specific dehydrogenases.

During intense exercise of near $\dot{V}O_2max$, the FG motor unit pool is substantially recruited (Edgerton et al., 1973; Gollnick et al., 1973a., 1973c.; Tesch and Karlsson, 1977) resulting in an increased production of lactate (Bergstrom et al., 1971; Saltin et al., 1971). This increased production is credited to

FIGURE 12. THE FORMATION OF LACTATE



the presence and higher activity of the M isozyme of lactate dehydrogenase (LDH) (Tesch et al., 1978) and to the increased activity of the enzyme itself (Tesch and Karlsson, 1977). LDH exists as five isozymes which can be detected and separated by gel electrophoresis. Although all LDH isozymes catalyze the same reaction, they differ in their dependence on substrate, particularly pyruvate. The H^{+} isozyme of LDH, which predominates in slow twitch oxidative fibres (SO) and to a certain extent in the oxidative fibre properties of fast twitch oxidative/glycolytic fibres (FOG), is inhibited by relatively low concentrations of pyruvate. The M isozyme, found in fast twitch glycolytic fibres and to a certain extent in FOG fibres, maintains its activity at high concentrations of pyruvate (Close, 1972). On the basis of these differences it has been suggested that the H form of LDH is inhibited when pyruvate levels rise during periods of rapid glycolysis, thus favoring the removal of pyruvate via other metabolic pathways. These pathways include the oxidative utilization of pyruvate in the mitochondria (Krebs Cycle), the conversion of pyruvate and CO_2 to malate via the malic enzyme (EC 1.1.1.40) and the transamination to alanine via glutamate-pyruvate transaminase (Mole et al., 1973). In FT fibres in which rapid release of energy may be needed, the predominant M-isozyme will maintain catalytic efficiency even at high pyruvate concentrations, thus allowing glycolysis to proceed at a rapid rate, forming lactate as an end product.

Tesch (1980) demonstrated that following short term cycling exercise, individual variation in lactate concentration

correlated positively with the percentage of FT fibres in the muscle. These data confirmed earlier findings that muscle motor unit distribution will in part determine the metabolic quality of the muscle in terms of its potential for lactate formation (Karlsson et al., 1974; Sjodin, 1976). However, recruitment of muscle fibres, as determined via glycogen depletion studies, does not accurately reflect the actual lactate concentration. Tesch et al., (1978) demonstrated a differential accumulation of lactate in FT fibres during the first 30 seconds of exercise with this differential disappearing after one minute of work. This effect has been explained in the past on the basis of preferential recruitment of FT motor units early in rapid dynamic contractions (Burke and Edgerton, 1975), however, it is more likely due to a lower rate of lactate eflux from FT fibres due to poorer capillarization (Andersen, 1975). Using separated fibre fragments from selected motor unit pools, Tesch (1980) confirmed earlier findings that there is higher lactate production in FT motor units as compared to SO motor units, with peak values reaching 53 mmoles·kgm^{-1} wet weight. However, when maximal work (isokinetic knee extensions) was performed for durations exceeding 25 seconds the lactate accumulation, expressed as a FT/ST ratio, decreased from 1.4 to 1. This suggests that a lactate 'flow' is occuring from FT to ST fibres as described by Essen et al. (1975), mediated by membrane bound M-LDH (Sjodin, 1976). This may explain, in part, the lactate content in ST fibres.

Since lactate is an organic acid, its intramuscular accumulation leads to a drop in pH. During dynamic exercise to

exhaustion the pH of m. quadriceps femoris of man has been shown to drop from a mean of 7.08 to 6.60 (Sahlin et al., 1976). Hermanssen and Osnes (1972) showed blood pHs following supramaximal continuous and supramaximal intermittent exercise of 7.42 and 6.8, which correspond to a consistantly lower intramuscular pH of 6.41 formaximal intermittent exercise. These results are consistant with those of Sahlin et al., (1975, 1976).

The concentration of lactate in the blood has classically been used to provide a description of the metabolic state of the muscle. However, present evidence demonstrates clearly that this is inadequate and that the concentration of lactate in the blood simply indicates that the production or cellular release of lactate exceeds the rate of removal, as a dynamic balance exists between the two. Tesch (1980) in a study ofmaximal one legged cycling, showed that after 50maximal contractions muscle lactate exceeded blood lactate eight fold. Furthermore, the highest blood lactate was demonstrated in subjects with the lowest muscle lactate and vice versa. A negative relationship was demonstrated between blood lactate concentration and fatigue, with high blood lactates in subjects resistant to fatigue and low values in subjects more fatigable.

After cessation of exercise peak blood lactate values may not become evident for up to 10 minutes (Bonen and Belcastro, 1977). The marked difference between skeletal muscle and blood lactate concentrations observed during heavy exercise demonstrates a concentration gradient from muscle to blood (Tesch, 1980; Sahlin, 1976; Karlsson, 1971a; Karlsson et al.,

1968). This gradient suggests that the translocation of lactate across the muscle membrane is hindered and implies that lactate transport may be via a carrier mechanism, rather than by simple diffusion (Sahlin, 1976; Hirche et al., 1970). Karlsson et al. (1972) examined the lactate release process from contracting canine m. gracilis and arrived at Km and Vmax values for lactate release similar to the values obtained in the two previous studies. In all of these investigations a well defined levelling off in lactate release was observed at higher tissue lactate concentrations indicating that the translocation mechanism was saturated (Mainwood and Worsley-Brown, 1975). In accordance with animal experiments Jorfeldt et al. (1978) showed a levelling off of lactate release from m. vastus lateralis with increased lactate concentrations that could not be explained by inadequate leg blood flow. The maximal release rate of tissue lactate appears to be of the order of 4-5 mmoles$\cdot$kgm$^{-1}\cdot$min^{-1}. These observations are supported by Jorfeldt's earlier observations of a nonlinear relationship between lactate uptake and arterial lactate inflow. This suggested that a saturation state may also exist in the mechanisms responsible for lactate uptake into exercising muscles (Jorfeldt, 1970).

Sahlin (1976) suggested that the lactate translocation mechanism may be a pH influenced system. In accordance with this suggestion Hirche et al. (1975) found the lactate permeation rate in isolated canine m. gracilis to be increased by $NaHCO_3$ induced metabolic alkalosis (pH 7.5) in combination with low H^+ activity. The opposite results occurred with HCl induced metabolic acidosis (pH 7.0). Thus by lowering pH, muscle lactate

permeability is decreased indicating a change in membrane properties.

LACTATE, pH and FATIGUE

In the performance of physical work it is imperative that the processes generating ATP keep pace with those degrading it, else the intensity of work must decrease or cease altogether. The transient state of fatigue has a a complex etiology. With the advancement of accurate techniques of muscle lactate determination, significant inverse correlations have been shown to exist between muscle tension and lactate during fatigue ($r=-0.99$) and during recovery ($r=-0.92$) (Fitts, 1976). Lactic acid is a relatively strong proton donor ($pk=3.6$) and thus exists intercellularly in the anionic form. The concept that lactic acid and its associated pH affects results in muscle fatigue has considerable theoretical appeal, since evidence exists for several mechanisms that would interfere with contractile function.

Muscle contraction is initiated by a release of calcium ions from sites on the terminal cisternae of the sarcoplasmic reticulum, coupled to the passage of local current flow through the area. Calcium in turn allows the formation of cross bridges and the release of products of ATP hydrolysis. The maximum ATPase activity of actomyosin is decreased by 25% when pH decreases from 7.0 to 6.5 (Portzehl et al., 1969) which is also reflected by an increase in the required amount of Ca^{++} for obtaining maximum activation. Fuchs et al. (1970) demonstrated that a lowering of pH decreases the affinity of troponin for

calcium and destabilizes the Ca^{++}/troponin complex. In additon to the direct affect of H^+ on the rate of ATP hydrolysis, these authors suggested a competitive interaction of H^+ for calcium binding sites on troponin. Recent evidence by Fuchs (1979) and by Stull and Buss (1978) demonstrated that in the presence of magnesium in a calcium buffered medium, H^+ displacement of Ca^{++} from binding sites was not a likely fatigue mechanism. However, support for such a mechanism is provided by the data of Robertson et al. (1978) showing that pH had no effect on Ca^{++} binding to Ca^{++}/Mg^{++} binding sites on troponin; however Ca^{++} binding to Ca^{++} specific sites on troponin was pH dependent over a range from 7.5 to 6.0. Kentish and Nayler (1978) showed that a drop in pH from 7.2 to 6.4 produced an increase in Ca^{++} requirements for half activation of five fold in cardiac and four fold in skeletal muscle. In addition, the affinity of the sarcoplasmic reticulum for Ca^{++} depends specifically on pH. Nakamuru and Schwartz (1972) demonstrated that a drop in pH to 6.5 led to increased protein binding of calcium to SR.

Pannier et al. (1970) have hypothesized that the excitability of skeletal muscle is pH dependent. Brooks and Hutter (1963) found a decrease in Cl^- conductance with a decrease in pH causing, higher threshold potential, slower action potential propogation and slower rise and fall of the action potential. Wenger and Reid (1976) suggested that a decrease in pH may result in a hyperpolarized state of the membrane by affecting the permeability of Na^+ and K^+. The physiological significance of these results remains obscure but it is apparent that the interaction between hydrogen ions and

sarcolemmal functions are intricate and numerous.

The possible affects of pH on energy transduction in the mitochondria has also been investigated. Mitchelson and Hird (1973) showed that oxidative phosphorylation was quite unaffected by extramitochondrial pH in the range of 6.5 to 7.0, whereas severe inhibition was obtained at pH 6.0. Senger (1975), using l-lactate in the concentration range seen after intense exercise, was able to partially uncouple oxidative phosphorylation presumably due to the lysing affects of lactic acid on the cristae of the mitochondrial membrane. Hydrogen ion is also thought to be intimately related to calcium uptake by the mitochondria (Mitchell and Moyle, 1967; Chance, 1965). High intramitochondrial calcium concentration is believed by several investigators to be an important fatigue factor (Bonner et al., 1976; Tate et al., 1979; Tate et al., 1980), since high intermitochondrial calcium is shown to uncouple oxidative phosphorylation (Wrongmann et al., 1973) and decrease isocitrate dehydrogenase activity (Zammitt and Newsholm, 1976). None of these studies are conclusive in their findings but allude to further possible roles of lactate in the fatigue process.

Increased hydrogen ion concentration may also exert an effect at the neuromuscular junction. Bergmans et al. (1976) postulated that repetitive stimulation of an alpha motor neuron progressively decreases the number of acetylcholine quanta released per impulse (del Castillo and Katz, 1954). Landau and Nachshen (1975) suggest that protonation of a site on the presynaptic membrane reduces acetylcholine release and blocks calcium flux into the nerve terminal during the action

potential.

Increased intramuscular lactate concentration may also affect muscular performance via destruction or inhibition of acid labile cell components, particularly the enzymes of energy transduction. It has been shown that phosphofructokinase (PFK), the rate limiting enzyme of glycolysis, is inhibited *in vitro* at low pH (Ui, 1966; Karlsson *et al.*, 1974; Gesser, 1976). At the ATP concentration typically seen in resting muscle (7 mM), PFK is almost completely inactive at pH 6.4 which is close to the measured muscle pH after exhaustive exercise (Sahlin, 1978). PFK inhibition would result in the accumulation of glycolytic intermediates, including glucose-6-phosphate which is known to inhibit hexokinase and phosphorylase *a*, resulting in further decreases in glycolytic rate (Fitts and Holloszy, 1976; Wenger and Reid, 1976). Danforth (1965b), showed that the conversion of phosphorylase *b* to *a* during stimulation of muscle is inhibited at low pH, possibly by inhibition of phosphorylase b kinase and adenyl cyclase (Mawatari *et al.*, 1974; Sahlin, 1978). The slowing of glycolytic rate may be partially offset by the low pH (range 6.1 to 6.5) affect of stimulating AMP deaminase with the resulting ammonia serving to buffer hydrogen (Mutch and Banister, 1982). But by the same mechanism, AMP levels are lowered and glycolysis is slowed. The increased lactate/pyruvate ratios observed following exhaustive exercise will mean pH dependence occurs in all reactions involving NADH/NAD couples. Additionally, it is believed that the activities of the various isozymes of creatine kinase are influenced by pH (Karlsson *et al.*, 1975; Sahlin *et al.*, 1975; Sahlin, 1978). The effects of

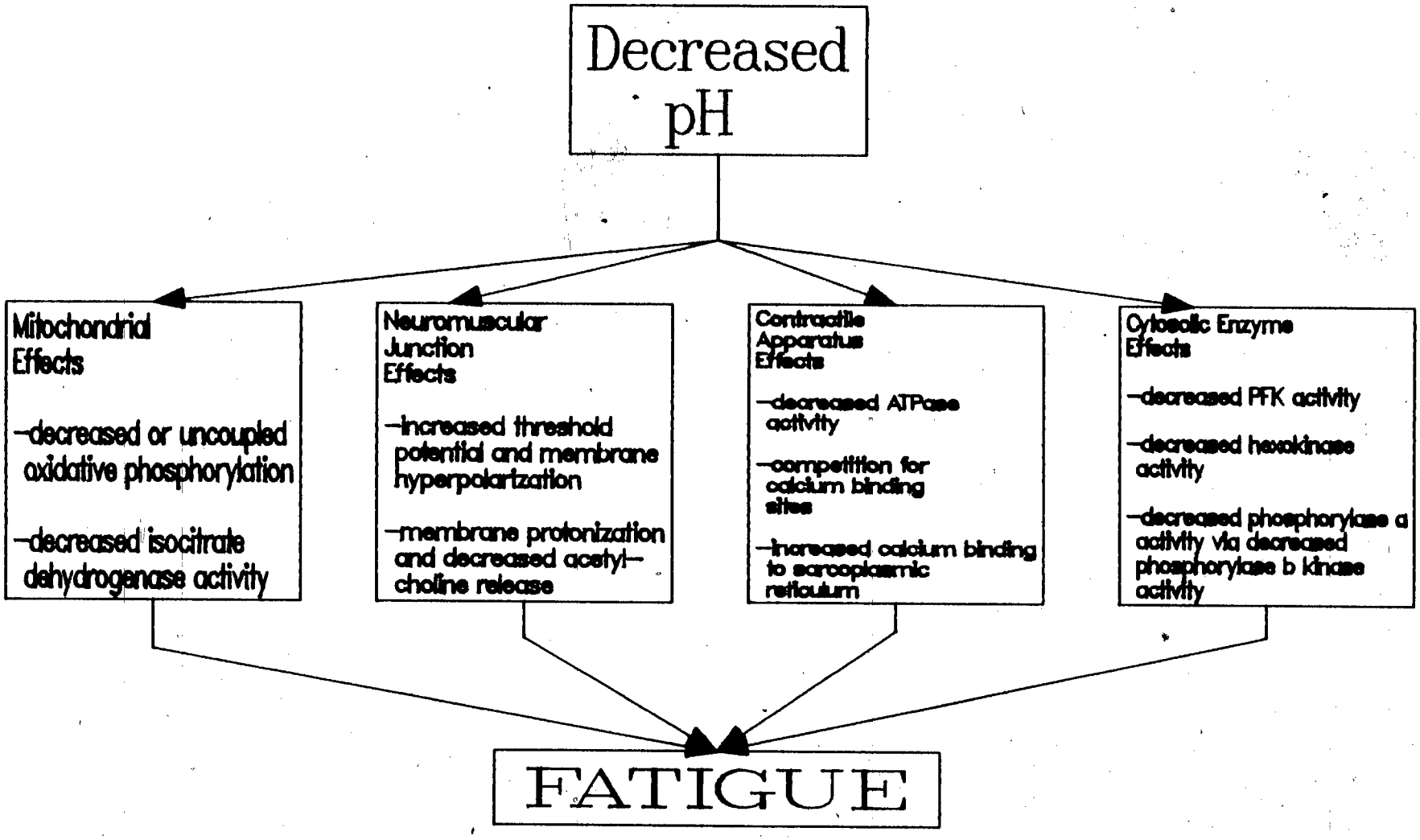
lactic acid and the associated pH changes on muscle contractile activity are summarised in Figure 13.

GLYCOGEN DEPLETION AND MOTOR UNIT RECRUITMENT

Determination of the degree of glycogen depletion is an established histochemical means of evaluating whether a certain type of motor unit has been metabolically active during exercise. Glycogen content determined by the histochemical periodic acid Schiff (PAS) stain is a non-quantative measure with amaximum sensitivity of 80-100 mmoles glucose units$\cdot kgm^{-1}$. Although several authors have pointed out the limitations of this method (Burke and Edgerton, 1975; Essen, 1978), glycogen depletion over a period of time must indicate that the depleted fibres have contibuted to the total tension output and can thus be taken to reflect motor unit recruitment.

Early exercise studies demonstrated an almost linear depletion of muscle glycogen with time in both continuous and intermittent exercise (Hermansen et al., 1967). Together with myosin ATPase stains, PAS stain has proved to be a valuable tool in identifying not only the degree of glycogen depletion but also the class of motor unit recruited during exercise of different intensity and duration. Control of motor units appears to be based on the differences in motor unit size and, accordingly, activation threshold (Henneman, 1977). Fibres with high oxidative capacity (SO) comprise motor units with low activation thresholds and conversely fibres with high glycolytic activity make up motor units with high activation thresholds. Studies of differential glycogen depletion rates support, to

Fig 13. Muscle Fatigue Associated With Decreased pH



some degree, this concept of recruitment based on the size principle.

The phenomenon of preferential depletion of glycogen from muscle fibre types related to exercise protocol has been thoroughly investigated. On the basis of PAS staining, glycogen depletion studies in human skeletal muscle have revealed a pattern related to duration, intensity, maximal tension, and speed of contraction. Baldwin (1973) demonstrated that when exercise is of an intensity that can be maintained for two hours or longer, SO and FOG fibres are depleted of glycogen with little or no involvement of FG fibres. As exhaustion approaches, FG motor units are recruited to a greater extent as shown by decreased glycogen levels. This additional recruitment of FG fibres near exhaustion was not demonstrated by Costill et al. (1973) in a field study of long distance runners. This inconsistency may be explained by the fact that as subjects became exhausted, they walked difficult sections and hills (Reardon, 1975). Biopsies of m. vastus lateralis after submaximal bicycle exercise revealed glycogen depletion occurring in SO fibres prior to FG (Gollnick et al., 1972; Edgerton et al., 1975).

Motor units investigated during varying intensities of exercise, with workloads ranging from 30-150% $\dot{V}O_2max$ (Gollnick, 1973c), revealed a different glycogen depletion pattern. PAS staining demonstrated a dramatic decrease in total muscle glycogen with increasing workload, SO fibres being the first depleted at submaximal workloads with a progressive depletion of high glycolytic FG fibres as work time increased. Further

pronounced depletion of both fast twitch fibre types occurred at workloads close to or above $\dot{V}O_2max$ (Andersen and Sjogaard, 1976; Gollnick, 1974, 1973c.). Piehl (1974a) reported that a preferential depletion of FG fibres was not evident unless a workload greater than 90% of VO_2max was demanded.

Studies of isometric work (Exner, 1973 a,b; Gollnick, 1974) suggest a similar pattern of differential glycogen depletion. Biopsies taken following repeated 40 second isometric contractions calling for less than 20% ofmaximum voluntary contraction (MVC) reveal a selective loss of glycogen in SO fibres with little or no involvement of FG fibre types. At workloads greater than 20% MVC only FG fibres were depleted. Gollnick (1974) concluded that selective glycogen depletion was indicative of a differential recruitment of muscle fibres during isometric exercise of varying intensity and that the maximal tension that the muscle needed to develop was an important factor influencing the activation of different fibre types (Essen, 1978).

In studies using bicycle ergometey (Gollnick, 1973c.) pedalling rate (30-150 rev$\cdot$min^{-1}) did not influence glycogen depletion and was independent of the time/intensity factor. However, in isokinetic exercise (Cybex 11, Lenox Hill) contraction velocity was found to influence muscle fibre recruitment pattern, suggesting a greater involvement of FG fibres at faster velocities (Lesmes et al., 1976).

Inferences to the precise pattern of motor unit recruitment are limited when based only on glycogen depletion data. The pattern of depletion in different muscle fibre types depends not

only on the usage of particular motor units but also on the possible utilization of other energy sources in addition to glycogen. Where investigations have used supramaximal work rates of short duration, it is justifiable to assume that glycogen is the major substrate utilized by the muscle fibre types. However, it is not justified to infer that these FG motor units are recruited contrary to the size principle, that is, preferential to low threshold SO units. Glycogen is probably less precise an index of fibre recruitment after extended periods of submaximal work. With prolonged work, free fatty acids and glucose are taken up by active muscles and the liver can provide significant amounts of glucose during exercise (Baldwin et al., 1973). These fuel sources are all used at different rates depending on the work intensity, the duration of the exercise, and their availability (Essen, 1978). It is important, therefore, that care be exercised when intrepreting studies of muscle fibre usage based upon glycogen depletion patterns.

REGULATION OF MUSCLE GLYCOGEN METABOLISM

Regulation of glycogen metabolism in skeletal muscle is an interplay between humoral and enzymatic controls. In animals there are two reactions of major importance in regulating glycogen metabolism. On the synthetic side is the glycogen synthetase (E.C. 2.4.1.11) reaction and on the glycogenolytic side is the phosphorylase (E.C. 2.4.1.1) reaction. Both of these enzymes have extensive allosteric properties, suggesting that their activities can be readily controlled through metabolic interaction.

The rate of glycogen synthesis, as well as the rate of glycogenolysis, is regulated in response to muscle contraction. Under resting conditions the concentration of two important modulators, AMP and P_i, are sufficiently high that if these were the only operative factors, phosphorylase b would be active and glycogenolysis would occur even in the absence of contraction. This is not the case however, because of the opposing actions of ATP and glucose-6-phosphate which counteract the effect of these activators. At onset of muscle contraction the balance between these various activators is shifted and phosphorylase b becomes active.

When glycogen concentration is reduced by muscular contraction, it's resynthesis is favored by activation of the synthetase. It has been confirmed in rodents (Danforth, 1965a; Conlee et al., 1978) and in man (Bergstrom et al., 1972) that when glycogen level is low, there is a good inverse correlation between percentage glycogen synthetase in the I form and glycogen concentration. The mechanism of this action appears to be that glycogen binds glycogen synthetase and protects it against the action of the phosphatase which is also bound to glycogen; when glycogen concentration decreases, both enzymes are released, enabling glycogen phosphatase to catalyse the conversion of synthetase D to the I form (Villar-Palasi, 1969). When glycogen concentrations have been restored, synthesis of the polymer is slowed due to the inhibition of synthetase phosphatase. The regulation of glycogen levels by glycogen per se may involve an effect on glycogenolysis as well as on glycogenesis. Glycogen is a strong activator of phosphorylase b

kinase (Delange et al., 1968) so that high levels of glycogen would favor phosphorylase a formation and glycogenolysis.

The reactions regulating glycogenolysis are modulated by several important hormones, particularly epinepherine, insulin and the glucocorticoids. The causal effect of epinephrine on glycolysis was established by Cori and Cori (1928). The activation was demonstrated to be a cascade reaction involving the phosphorylation of phosphorylase b kinase and subsequently a cyclic AMP dependent protein kinase which catalyses the reaction. Epinepherine stimulated glycogenolysis is further enhanced by a decreased rate of glycogen synthesis through the conversion of glycogen synthetase I to D which is catalysed by the same cyclic AMP dependent protein kinase.

It has long been recognized that insulin has a specific action in facilitating glycogen apart from its effect on promoting glucose uptake by muscle. Villar-Palasi and associates (1971) demonstrated that insulin activates glycogen synthesis by promoting the formation of synthesis I via a decrease in the activity of the kinase (Shen et al., 1970). How this is actually accomplished is not well understood.

Administration of adrenal corticol hormones to adrenalectomised or normal animals causes an increase in the level of stored muscle glycogen. This is most likely an effect which is secondary to the hyperglycemia resulting from the gulconeogenic action of glucocorticoids on the liver (Schaeffer et al., 1969).

LACTATE REMOVAL AND INTRAMUSCULAR GLYCOGENESIS

A primary controversy exists over the removal of lactate between those who believe that its major fate during recovery is reconversion to glycogen and those who believe this metabolite is virtually 100% oxidized. Secondly, it is regularly debated as to whether it is the liver or skeletal muscle that is responsible for these processes. The metabolism of an organism or muscle tissue after exercise is characterized by a prolonged elevation in oxygen consumption. The classic "excess post exercise oxygen consumption" theory (EPOC) suggests that the primary fate of 80% of the lactate removed during recovery from high intensity exercise is conversion to glycogen with oxidation of the remaining 20% providing the energy for glycogenesis. In opposition to this theory is the interpretation that virtually all the lactate produced in high intensity exercise is oxidized to carbon dioxide and water. The EPOC theory is described as "inadequate" and "simplistic" since only 9-25% of metabolized lactate can be accounted for by glycogenesis or gluconeogenesis (Brooks and Gaesser, 1980).

The major criticisms of the lactic acid theory of oxygen debt comes from Brooks and co-workers (1980), Depocas et al. (1969) and Searl and Cavalieri (1972). Brooks et al. (1973) using ^{14}C labelled lactate infused into exhausted and pair fasted controls, showed no glycogen synthesis from lactate during the post exercise period of elevated oxygen comsumption held by the EPOC theorists to represent repayment of the oxygen debt. On the contrary, they found 84% of the label appearing as carbon dioxide after two hours recovery. More recently Brooks

and Gaesser (1980) observed in radiochromatograms, that following both continuous and intermittent exercise, glycogen synthesis did not coincide with lactate removal or with the phase of oxygen consumption considered to represent EPOC.

The importance of lactate as a source of oxidizable carbon is further enhanced by exercise performed below some critical level of oxygen uptake during recovery from maximal exercise. Hubbard (1974) showed that 35-68% of administered lactate is recovered as carbon dioxide within 30 minutes when exercise at 62-75% of $\dot{V}O_2$max is performed. Essen et al. (1973) and Hermansen and Stensvold (1972) have demonstrated that uptake of lactate by skeletal muscle can occur even at workloads demanding 80-90% of $\dot{V}O_2$max. The strategy of active recovery by athletes to enhance lactate removal has been further demonstrated by Bonen and Balcastro (1977). There is thus ample evidence to support the hypothesis that lactate removal is primarily accomplished by oxidation and that skeletal muscle has the mass and metabolic profile compatible with being a major contributor to this process.

The site or sites for the metabolic removal of lactate following maximal exercise, be it oxidative or glycogenic, is a matter of some contention. The importance of the liver in the elimination of lactate has been investigated in the work of Rowell et al. (1966) and Davis et al. (1970) which demonstrated that 50% of the total lactate formed during high intensity exercise is removed by the liver. In contrast Brooks et al. (1973) and Miniare and Forichon (1973) have suggested a minimal hepatic role accounting for approximately of 9-25% of the

lactate removed. Hermansen et al. (1973) recalculated the data of Rowell and co-workers and showed that the liver was able to remove only about 0.1-0.2 grams of lactate per minute. Previous work by Hermansen and Stensvold (1973) disclosed that aproximately 1.3 grams of lactate per minute were removed during resting recovery from maximal intermittent exercise and up to 24 grams of lactate per minute if light exercise was performed. This suggested that 25 times more lactate was removed during recovery from exercise than can be accounted for by the liver. Therefore, the importance of the liver in the elimination of lactate following exercise may be less than previously thought.

In addition to the liver, skeletal muscle (Jorfeldt, 1970) and other organs including heart and kidney are able to take up and remove lactate (Knuttgen, 1971). Heart and kidney, however, contribute to only about 10% of the removal (Carlsten et al., 1961; Krebs, 1965). Since skeletal muscle represents the largest tissue mass of the body, constituting 40-50% of the body weight of man (Anders et al., 1956) and having the appropriate metabolic profile it would be a likely candidate for the site of lactate removal.

There is compelling evidence to suggest that EPOC is a valid theory. The original proposal that lactate is removed by skeletal muscle via a conversion to glycogen came from the work of Meyerhof (1920). It was observed that isolated frog sartorius muscle was able to remove lactic acid formed during fatigue when muscle was allowed to recover in oxygen, but only 20-25% of the removed lactate could be accounted for by oxidation to CO_2 and water. Meyerhof, Lohman, and Meier (1925) reconfirmed Meyerhof's

original hypothesis by reporting significant gains in the glycogen content of skeletal muscle of frog hindlimbs perfused with 0.12% lactate. Hill (1925) examined the fate of lactate in terms of its heat of combustion. The only possible conclusion was that for every five moles of lactate removed in the recovery process only one was oxidized, again accrediting oxidative removal to 20% of the total lactate metabolized. In a comparison of the rate of glycogen synthesis in intact and eviscerated animals Eggleton and Evans (1930) demonstrated a gain in muscle glycogen of eviscerated animals suggesting direct intramuscular conversion of lactic acid.

Meyerhof (1925) showed that if isolated skeletal muscle was allowed to recover in oxygen, glycogen was synthsized at the same time as lactate disappeared with 20% oxidized, and the other 80% converted to glycogen. Eggleton and Evans (1930) were able to confirm these findings whereas Sacks and Sacks (1935) could not demonstrate glycogen synthesis from lactate following contractile activity in mammalian muscle. In the 1960's radioactive tracer studies performed by Bar and Blancher (1965) and Gourly and Suh (1969) showed that the labelled carbon atom of 1-^{14}C lactate was incorporated into glycogen in both isolated rat diaphragm and isolated sartorius muscle.

Many textbooks of biochemistry suggest that lactate must first escape from the muscle into the general circulation where it is carried to the liver and converted to glucose which is again released to the circulation and ultimately taken up and stored as glycogen by skeletal muscle. This idea of a complete cycle of the glucose molecule within the body comes from the

work of Himwich et al. (1930) and Cori and Cori (1929) in which decerebrate dogs were used to compare the concentrations of lactate and glucose in arterial blood with that of the venous blood draining various organs. It was concluded that the main source of the lactate is skeletal muscle with the liver being the organ chiefly concerned with its removal. Since all organs, except liver, remove glucose from the blood, a complete carbohydrate cycle between liver and muscle is envisioned.

With the availability of improved biochemical techniques Bendall and Taylor (1970), attempted to repeat Meyerhof's original experiments. The evidence strongly supported Meyerhof's original hypothesis that, of the total lactate disappearing from frog skeletal muscle during aerobic recovery, only one sixth can be accounted for by oxidation, the rest appears in the form of intramuscular glycogen. Hermansen and Vaage (1977) investigated intramuscular glycogenesis in humans recovering frommaximal intermittent leg exercise. These investigators measured muscle glycogen and lactate changes and a-v lactate, glucose and alanine changes in human subjects during the first 30 minutes of recovery. The findings supported Meyerhof's original postulate in that only 10% of the lactate recovered could be accounted for by an efflux from leg muscles into the circulation. Of the remaining 90% of the lactate metabolized within the muscle tissue less than 25% appeared to be oxidized to carbon dioxide and water. The remaining 75% had to be metabolized via another route, of which conversion to glycogen seemed the most likely. Brooks and Gaesser (1980) have criticised these results suggesting that the use of venous plethysmography to measure

blood flow and the absence of direct tracer determinations invalidate the conclusions.

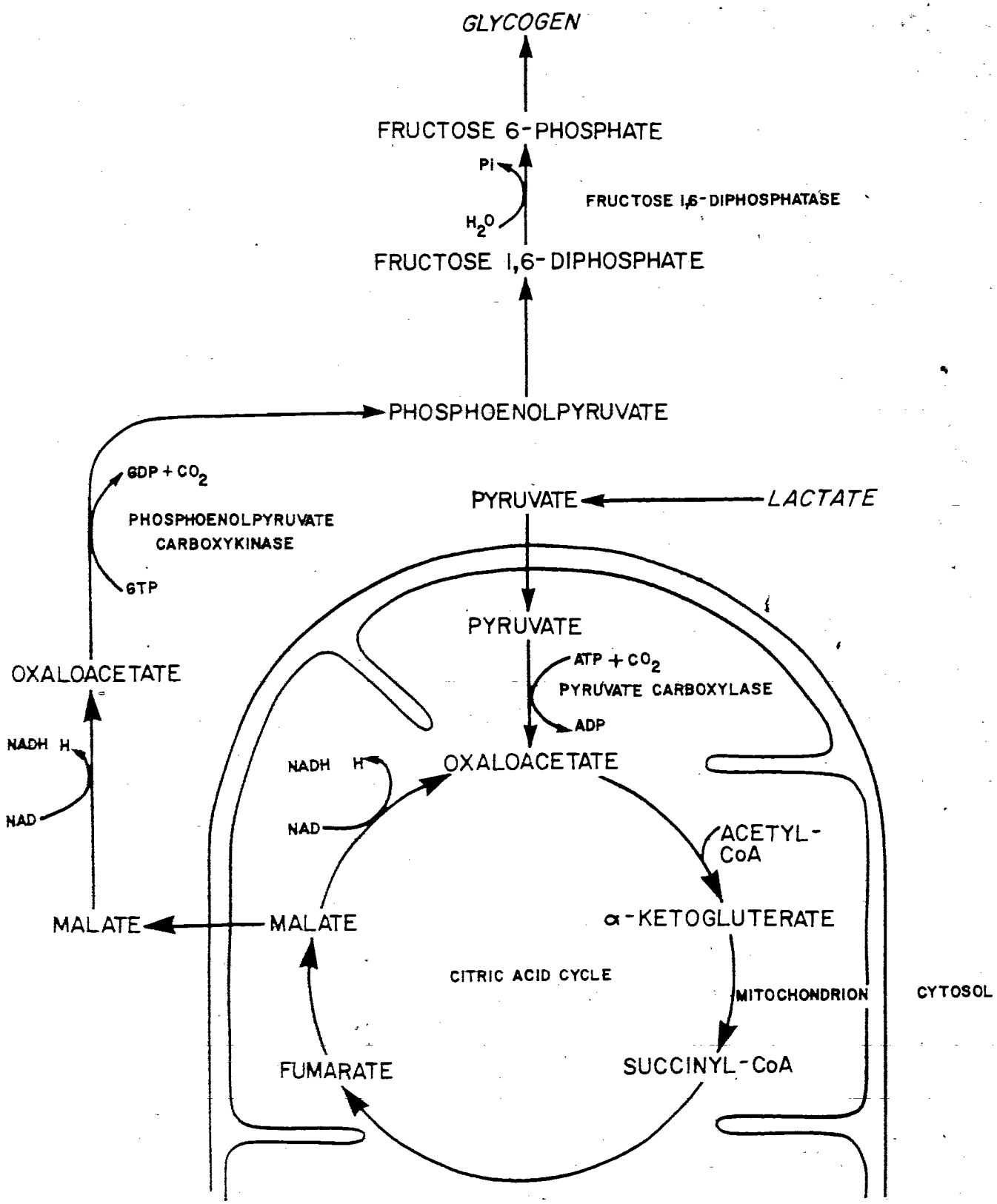
In a recent investigation of muscle glycogenesis McLane and Holloszy (1979) perfused isolated rat hindlimbs with 12 mM lactate and 3.3 $\mu Ci \cdot mmole^{-1}$ of U-^{14}C lactate. They observed a rapid increase in glycogen in fast-twitch red and fast-twitch white types of muscle with up to 44% of the infused lactate appearing as glycogen. Brooks and Gaesser (1980) conceeded that a significant incorporation of ^{14}C lactate into skeletal muscle glycogen was observed in rats recovering from continuous or intermittent exercise. The time course of this incorporation suggested direct lactate to glycogen conversion within the muscle. The data of Connett (1979) showed in isolated frog sartorius muscle that incorporation of labeled lactate into glycogen was five orders of magnitude greater than into carbon dioxide.

ENZYMES OF GLYCOGENESIS

Many studies criticize Meyerhof's original hypothesis based solely on the unfavorable energetics of reverse glycolysis (Krebs, 1965; Krebs and Woodford, 1965). The overall process under standard conditions for the reduction of lactate to one mole of glucose has a delta G^{O} of +48,000 $cal \cdot mole^{-1}$. Therefore, in order for this reaction to occur it must be driven by a greater energy input. The problem, as several authors have asserted (Krebs and Woodford, 1965; Bendall and Taylor, 1970), is the pathway by which the flow of lactate carbons to glycogen is occurs.

It is accepted that glycogenesis involves some reactions of glycolysis in reverse and some additional reactions which overcome free energy barriers preventing direct reversal of glycolysis. The two thermodynamic barriers which exist for the direct conversion of lactate to glycogen are between pyruvate and phosphoenolpyruvate, and between fructose-1,6-diphosphate and fructose-6-phosphate. These thermodynamic barriers are effectively sidestepped by reactions involving the cytosolic enzymes fructose-1,6-diphosphatase and phospho(enol)pyruvate carboxykinase. Both of these enzymes have been demonstrated to possess a range of activities in a variety of skeletal muscle such as soleus, gastrocnemius, and diaphragm, from such species as man, dog, cat, rabbit, and rat (Krebs and Woodford, 1965: Opie and Newsholm, 1967: McLane and Holloszy, 1979). However, the third key enzyme, the mitochondrial protein pyruvate carboxylase, as it exists in the glycogenic tissues, liver and kidney, has not been demonstrated in skeletal muscle (Bucher et al., 1965; Krebs and Woodford, 1965; Crabtree et al., 1972; Opie and Newsholm, 1967; Keech and Utter, 1963). As is pointed out by major critics of muscle glycogenesis (Krebs and Woodford, 1965), a pathway involving the Krebs cycle as seen in liver and kidney cannot operate in muscle (Fig. 14). Supportive evidence is provided by the observation that in both isolated muscle and whole animals, there is no randomization of the label from 2-^{14}C pyruvate which would occur in a pathway interacting with mitochondrial dicarboxylic acid pools (Krebs and Woodford, 1965) as in liver and kidney (see Connett, 1979). This hypothesis is supported in the data of Connett (1979) which showed in isolated

FIGURE 14. THE PATHWAY OF LIVER GLYCOGENESIS



frog sartorius that the incorporation rate of ^{14}C from 1-^{14}C acetate into glycogen is more than two orders of magnitude less than the incorporation from U-^{14}C lactate, while the $^{14}CO_2$ production rates were identical. The conclusion is that there is very little flux from Krebs cycle intermediates to glycogen in muscle, and lactate incorporation must proceed via a pathway independent from the Krebs cycle.

To account for these data Bendall and Taylor (1970), suggested the formation of an extramitochondrial intermediate using the "malic enzyme" (l-malate dehydrogenase decarboxylating, $NADP^+$). This pathway would not require a large increase in pyruvate concentration and a decrease in phosphoenolpyruvate to function. This is consistant with the observations of Connett (1979) that the pyruvate kinase reaction is far from equilibrium and that there was no fall in phosphoenolpyruvate and rise in pyruvate in response to elevated lactate levels that stimulate glycogen synthesis. Therefore, the activation of glycogenesis at a point beyond pyruvate kinase which would result in reversal of flow through this enzyme seems unlikely. However, McLane and Holloszy (1979) calculated that pyruvate kinase reversal could yield approximately 46 μmol of phosphoenolpyruvate·gram^{-1} of mixed muscle·hour^{-1} at 37°C.

A pathway operating through the malic enzyme would demonstrate some of the features of liver glycogenesis such as inhibition of PEP carboxykinase by 3-mercaptopicolinate (Ferre, et al., 1977: Kostos, et al., 1975). Connett (1979) makes an interesting suggestion based on the fact that reversal of the malic enzyme requires $NADPH+H^+$. The production of cytosolic NADH

will occur via lactate dehydrogenase which in turn should result in increased NADPH via transhydrogenase reactions. Thus the strong stimulation of malic enzyme and glycogenesis by elevated lactate concentrations may be due to this metabolite as a source of reducing equivalents.

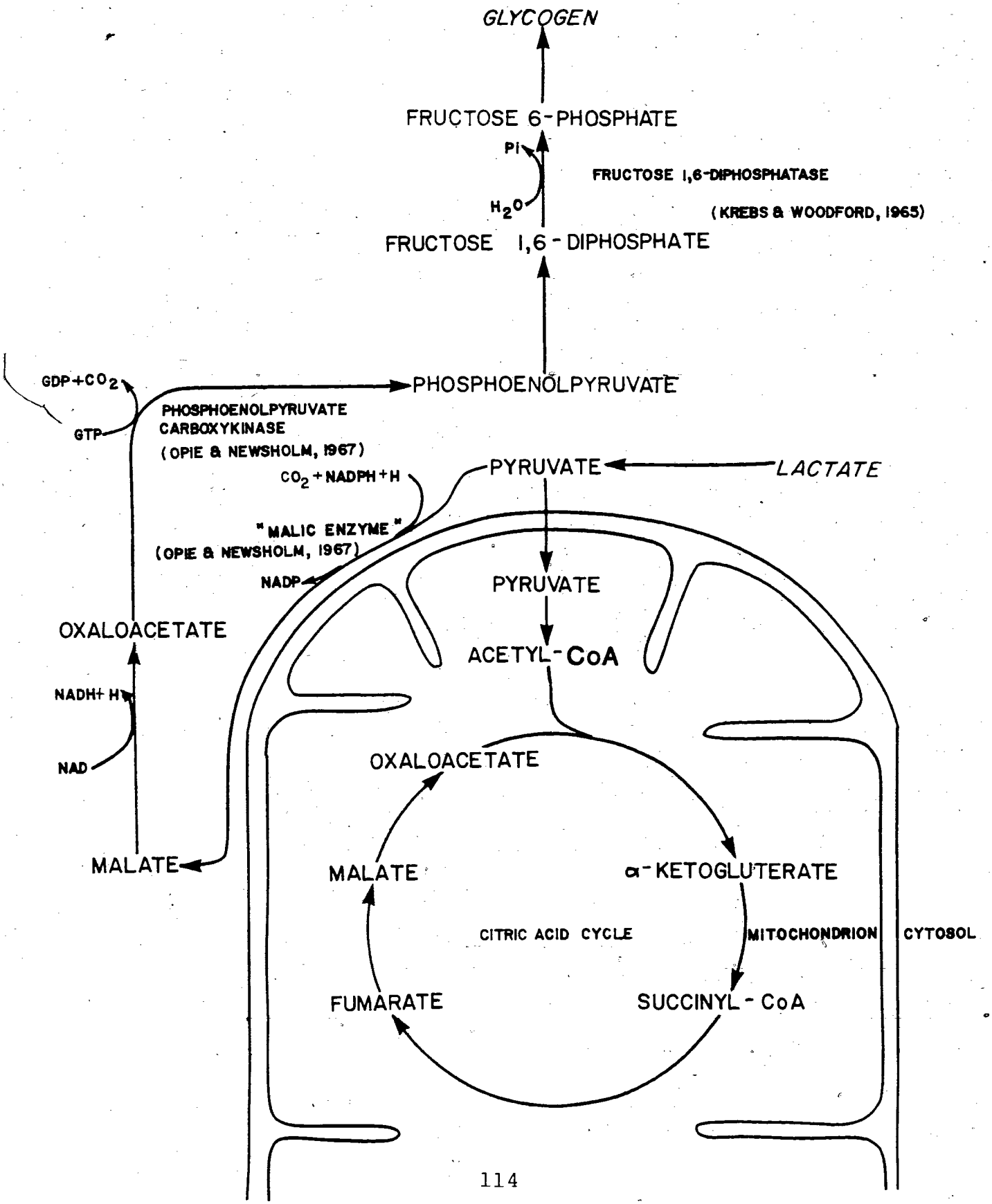
The malic enzyme was demonstrated by Opie and Newsholm (1967) in skeletal muscle of rabbits, frogs, pigeons and hens and its kinetic features further characterized by Frenkel (1972) in bovine cardiac muscle. The kinetic characteristics of this enzyme and the sensitivity of the pathway to bicarbonate (Bendall and Taylor, 1970) and to the phospho(enol)pyruvate carboxykinase inhibitor 3-mercaptopicolinate (Connett, 1979) are consistant with a an extramitochondrial such as the one in Figure 15. If the malic enzyme operates, the overall reaction from lactate to glycogen as shown in Figure 15 can be formulated as follows:

$$2\text{lactate} + 5\text{ATP} + (\text{glucose}) + H_2O \longrightarrow 5\text{ADP} + 5\text{P} + 3H^+ + (\text{glucose})$$

$$\Delta G^{\circ} = -5.4 \text{ kcal x mole-1 of lactate}$$

Thus, 2.5 mole of ATP is used/mole of lactate converted to glycogen and 17 mole is produced/mole oxidized: or for every mole of lactate disappearing (2.5/17) or .147 mole is oxidized and 0.853 mole is converted to glycogen (Bendall and Taylor, 1970).

FIGURE 15. A PROPOSED PATHWAY OF LIVER GLYCOGENESIS



It appears that the theoretical arguments for skeletal muscle glycogenesis have validity and tend to vindicate the observations of lactate conversion to glycogen. However, the physiological importance of such a pathway for lactate metabolism operating in muscle has not been well established. It remains to be seen if further research will absolutely confirm or refute the observations of Meyerhof (1920), Hill (1924), Bendall and Taylor (1970), Hermanssen and Vaage (1977), and others who claim that in excess of 75% of the lactate formed during high intensity exercise is converted to glycogen directly within skeletal muscle.

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