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# Buffer capacity of human skeletal muscle; relationships to fiber composition and anaerobic performance

by

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## Abstract

Twenty male volunteers, comprising four distinct sub-samples (S=800m runners; R=varsity oarsmen; M=marathon runners; UT=untrained controls), participated in this study. They were made aware of the potential risks involved and informed consent was obtained. Anthropometric (hydrostatic weighing), physical characteristic and pulmonary function (Collins Respirometer) were assessed by standard techniques. Maximal oxygen consumption was determined on a progressive treadmill run (0.22 $m.s^{-1}$ every minute; initial speed 2.22 $m.s^{-1}$) to fatigue. Respiratory gases were monitered every 15 seconds (Beckman Metabolic Measurement Cart) with the four highest consecutive oxygen uptake values being averaged for determination of maximal oxygen uptake. Anaerobic performance (AST) was assessed as the time to fatigue a the constant workload treadmill run at 3.52 $m.s^{-1}$, 20 percent incline. Post exercise blood lactate levels (HLa) were determined as an additional variable in assessment of anaerobic capacity. The M were significantly older than the other 3 groups while no significant differences existed between the trained groups for maximal oxygen uptake values. The S and R demonstrated significantly elevated AST ($p<.01$) and post-AST HLa ($p<.05$) levels above the M, whose values were similiar to the UT. This enhanced anaerobic performance could not be attributed to physical characteristic, pulmonary function or aerobic capacity differences of the trained athletes. Post-AST HLa displayed a significant relationship to anaerobic performance ($r=.90$). An enhanced lactate efflux mechanism was shown by the

trained groups, which was not altered by training specificity. Muscle biopsies obtained at rest from the vastus lateralis muscle, were examined for fiber composition, pH, histidine and carnosine levels and buffer capacity (B). B was found to be elevated in the anaerobicly trained groups($p<.01$) demonstrating a significant relationship to AST ($r=.51$) and fast-twitch fiber percentage (FT%;$r=.51$), which implied a relationship to muscle glycolytic capacity. Within the S and R, carnosine levels were found to be significantly elevated ($p<.01$), illustrating a significant correlation to B ($r=.64$) and FT% ($r=.46$), which emphasized the importance of carnosine as a physiological buffer and its possible relationship to the glycolytic capacity of the tissue. No differences in histidine levels or resting intramuscular pH were demonstrated with training specificity. These results suggest that the enhanced anaerobic performance could partially be attributed to elevated B and carnosine levels demonstrated within skeletal muscle subjected to anaerobic training. This may be due to the tissues enhanced capacities to seqestor the protons which accumulate during anaerobic glycolysis.

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## List of Symbols

| | |
|---|---|
| ATP | adenosine triphosphate |
| NADH | reduced nicotinamide adenine dinucleotide |
| Sl | Slyke, standard unit for B (mmol.pH .l IC H O) |
| PFK | phosphofructokinase |
| G6P | glucose-6-phosphate |
| LDH | lactate dehydrogenase |
| CP | creatine phosphate |
| FT | fast-twitch (type II) skeletal muscle fibers |
| ST | slow-twitch (type I) skeletal muscle fibers |
| $pH_i$ | intracellular pH |
| IAA | iodoacetic acid |
| $HCO_3^-$ | bicarbonate |
| F6P | fructose-6-phosphate |
| B | buffer capacity |
| $P_i$ | phosphate |
| HIS | histidine |
| HLa | blood lactate |
| S | sprinters |
| R | rowers |
| M | marathon runners |
| UT | untrained controls |
| $VO_2$max | maximal oxygen uptake |
| AST | anaerobic speed test |
| DMO | 5,5 dimethyl-2,4-oxazolidedione |

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# Acknowledgements

To all my volunteers who through their enthusiastic participation, made this investigation possible, 1 express my gratitude. 1 would sincerely like to thank my committee members: Drs. Peter Hochachka, Bill Ovalle, Ken Coutts and especially Don Mckenzie, who through their knowledge and guidance, made this investigation a rewarding experiance. Special appreciation is extended to Dr. Tom Mommsen and Susan Shinn for their expertise and patience in teaching me the necessary techniques. To all those individuals who helped me collect my data and to Miss Diane Barnett for her support, I extend my thanks.

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## Fields of Study

Major field: Exercise physiology

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## Introduction

Sprint trained athletes demonstrate a remarkable ability to perform high intensity, short duration work, with the energy requirements being met principally by anaerobic glycolysis. Most anaerobic studies have concentrated on changes in substrate levels or enzyme activities and metabolite concentrations which could be capable of generating ATP, and maintaining redox balance (Hochachka 1980). Alterations in substrate levels (Knuttgen and Saltin 1972; Gollnick and Hermansen 1973), glycolytic enzymes (Baldwin et al., 1972; Gollnick et al., 1972; Hickson et al., 1975; Costill et al., 1976) and fiber composition (Thorstenson 1976; Costill et al., 1976,1979; Roberts et al., 1981) associated with anaerobic training have been insufficient to account for the enhanced anaerobic performances of sprint trained athletes.

Anaerobic glycolysis results in the rapid production of ATP which is associated with elevated muscle and blood lactate levels, which have been implicated in reduced performances (Karlsson et al., 1975; Klausen et al., 1972). Proton accumulation associated with the elevated lactate levels results in pH decrements within muscle and blood (Roos and Boron 1981; Sahlin 1978). Reduced rates of glycolysis (Toews et al., 1970; Sutten et al., 1981; Roos and Boron 1981), correlations between pH and fatigue (Fitts and Holloszy 1976; Stevens 1980), inverse relationships between force generation and proton concentrations (Dawson et al., 1978; Fabiato and Fabiato 1978) and proton inhibition of the excitation-contraction coupling mechanisns

(Nocker et al., 1964; Katz 1970) have been demonstrated when the pH decrement was of sufficient magnitude. Thus it is important to buffer the protons which accumulate, thereby altering the rate of pH decrease, which subsequently may adversly effect anaerobic performance.

The ability of a tissue to resist changes in pH upon addition of a strong acid or base, has been termed by Van Slyke (1922) as its buffer capacity(B), reflecting the tissue's ability to sequester either protons or hydroxide ions. Numerous investigations by a variety of techniques have attempted to identify the B of various tissues (Roos and Boron 1981). Though variations in B exist between the methods, it appears that skeletal muscle contributes significantly to overall pH homeostasis of the organism (Clancy and Brown 1966; Siesjo and Messeter 1971; Heisler and Piiper 1971; Lai et al., 1973). Recently, skeletal muscle was examined in relation to glycolytic capacity within a number of terresterial mammals and fish species by the the homogenate technique. Corresponding elevated B and glycolytic capacity values values were obtained (Castellini and Somero 1981). The determination of B by the homogenate technique consists of simply the physico-chemical buffering component, which comprises the buffering within a cell merely as a consequence of proton association with bases (Roos and Boron 1981). Burton (1978) and Somero (1981) have identified the major buffering components of skeletal muscle to be the imidazole- containing compounds: free histidine, histidine-containing dipeptides and protein-bound histidine residues. The

dipeptide carnosine (B-alanylhistidine) has been found to occur in greater concentrations predominantly within muscles classified as white as opposed red (Tamaki et al., 1976). Within human skeletal muscle carnosine concentrations demonstrate a wide variation (Christman 1976; Bergstrom et al., 1978). Carnosine's buffering ability has been suggested to contribute up to 40 percent of the total buffering within pre- and post rigor skeletal muscle (Bate-Smith 1938; Davey 1960b). Therefore the purpose of the present investigation was to examine the inter-relationships between B, pH and fiber composition of human resting vastus lateralis muscle in relation to training specificity. Since the imidazole-containing compounds, carnosine and histidine, have been suggested to contribute significantly to buffering of in vitro preparations (Somero 1981; Burton 1978); their relationships to B, fiber composition and training specificity were examined as possible factors influencing anaerobic performance.

Methodology

Twenty male volunteers served as subjects in this study. They were made aware of the potential risks and informed consent was obtained. Four equal groups of five subjects, consisting of sprinters (S=800m runners), rowers (R=varsity oarsmen), marathon runners (M) and untrained controls (UT) participated in this investigation. The sprinters regularly ran the 800m distance in less than one minute 55 seconds which would make them a highly anaerobically trained group. The marathon runners had to have been actively engaged in endurance training (>40 miles per week for previous 6 months). As well they must have completed a marathon run (26 miles 385 yards) in 2:30 to 2:50 (hours:minutes). The untrained controls only participated in recreational activity. Anthropometric and physical characteristic data were recorded on each subject; percentage body fat was determined by hydrostatic weighing. Standard spirometry was performed at rest (Collins Respirometer).

The subjects performed a continuous treadmill test consisting of a ten minute warm-up at 1.56 $m.s^{-1}$, immediately followed by the test with a starting velocity of 2.22 $m.s^{-1}$, which was increased by 0.22 $m.s^{-1}$ each minute until fatigue. Expired gases were continuously sampled and analyzed (Beckman Metabolic Measurement Cart); measurements were tabulated by a data acquisition system (Hewlett Packard 3052A), which determined respiratory gas exchange variables every 15 seconds. Maximal oxygen consumption was determined by averaging the four highest consecutive 15 second oxygen uptake values.

Anaerobic performance was assessed by the Anaerobic Speed Test (AST) of Cunningham and Faulkner (1969) employing time, in seconds to fatigue as the performance index. The subjects performed an elevated treadmill run consisting of a 30 second warm-up at 2.66 $m.s^{-1}$, 10 degree incline immediately followed by the test at 3.52 $m.s^{-1}$, 20 degree incline until fatigue. Resting and two minute post-exercise blood samples were obtained by venous puncture for determination of blood lactate levels. Analysis of blood lactates (HLa) was via the enzymatic conversion of lactate to pyruvate in the presence of LDH and NAD (Hohorst 1962).

Needle biopsies were obtained at rest from the vastus lateralis muscle by the technique of Bergstrom et al., (1962) within one week of the exercise tests. The subjects had been informed not to have participated in any physical activity prior to the biopsy. The sampling site was 20 cm. above the lateral femoral-tibial joint line. Samples being utilized for histochemical analysis were oriented under a dissecting microscope and mounted in gum tragacanth compound. The samples were then frozen in isopentane cooled to the temperature of liquid nitrogen. Serial sections 10u thick were cut in a cryostat after warming to -20°C. The sections were mounted on cover slips and equilibrated at room temperature. Samples for biochemical determinations were immediately immersed in liquid nitrogen.

Skeletal muscle fibers were stained for myosin ATPase at different pre-incubation pH's (4.3, 4.6, 9.4) and for NADH

Tetrazolium Reductase(Dubowitz and Brooke 1973). Serial sections were obtained for positive identification of fiber types. Fibers were classified on the basis of their staining intensity for myosin ATPase at pH 4.6. A 0.01 sq. cm. cross-sectional area of muscle tissue was employed for determination of percent fast-twitch (FT) or slow-twitch (ST) per sample. Mean fiber type diameters were calculated by projection of the slides on to a screen (300x magnification) with 10 fibers of each fiber type being measured. Magnification was checked by the use of a 1um micrometer projected on the screen.

The determination of resting muscle pH was by the method of Sahlin et al., (1976) involving homogenization of the sample at 25°C in 10 volumes of a salt solution containing 145 mmol.l KCl, 10 mmol.l NaCl and 5 mmol.l iodoacetic acid (IAA). Homogenate pH measurements were made at 38°C with a microelectrode (MI 410, Microelectrodes Inc.) following a 10 minute pre-incubation at 38° C. Inhibition of glycolysis was achieved by the addition of IAA.

The remaining volume was deproteinized with the addition of 3 percent solid sulfosalisylic acid and centrifuged for analysis of buffer capacity. Buffer capacity was determined by a modification of the method of Davey (1960b). Supernatant extracts (100ul) were adjusted to pH 7.00±0.05 with 0.1 N NaOH. The 100ul aliquots were titrated to pH 6.00±0.05 with 0.01N HCl. Reliability was ascertained by re-titration of the extracts following pH readjustment to 7.00±0.05. Buffer capacity was determined as the number of moles per gram tissue (w/w) of $H^+$ required to change the pH one unit over the pH range 7.0 to 6.0.

The remaining supernatant demonstrating a pH of less than 2.2 was used for free amino acid determination. Free amino acid levels were determined on an amino acid autoanalyzer (Beckman 118C) using a single column lithium hydroxide buffer system. AA-20 resin (Beckman) was used on a 510 mm column with a diameter of 6 mm employing direct application of the sample. Standards were run at the beginning and end of each new ninhydrin solution. The amino acid concentrations were determined by manually integrating the area under the curve. By this method histidine immediately preceded carnosine in retention time. To determine the relative contribution of carnosine to total buffer capacity, different concentrations of carnosine (Sigma L-carnosine reagent grade) in 100ul aliquots were titrated and re-titrated between pH 7.00±0.05 and 6.00±0.05 with 0.01 N HCl.

Univariate comparisons of groups were performed on the physical characteristic, pulmonary function, histochemical and amino acid data. Analysis of variance was utilized to evaluate possible intergroup differences. The Scheffe test of significance was performed, on the variables demonstrating significant omnibus F ratios, to identify where group differences existed. Multivariate comparisons of groups with pre-planned orthogonal contrasts were performed on the physiological, post HLa, percent FT and B variables to identify possible intergroup differences. Regression equations were calculated for the variables demonstrating significant correlations with B, as well as those variables demonstrating relationships to carnosine concentrations.

Results

Anthropometric, physical characteristic and pulmonary function data are presented in Table 1. Significant differences existed between the groups for the variables weight and percent body fat ($p<.05$) due to the untrained group. The marathon runners were significantly ($p<.05$) older than the other three groups. No significant differences existed between the groups for pulmonary function. The low intra-variabilities of the trained groups with respect to their pulmonary function and anthropometric data suggest that the sub-samples were relatively homogeneous.

The physiological assessment and histochemical analysis results are contained in Table 2. The inclusion of the untrained group resulted in significantly different ($p<.05$) maximal oxygen uptake values but no differences existed between the trained groups. While no significant differences existed between the M and UT groups in AST performance ($p=.084$), significant differences did exist between groups S and R ($p<.01$). Both the S and R groups demonstrated a significant increase ($p<.01$) in AST times with respect to each of the other groups. Post-AST HLa values were significantly different for the S and R groups ($p<.01$) and with respect to each of the other groups. No post-AST HLa differences existed between the M and UT groups. Anaerobic performance was highly correlated with post-AST HLa values ($r=.90$;Figure 1). The ratio of AST/Post-AST HLa versus Post-AST HLa revealed a superior ratio for the trained groups as compared to the UT group but, no differences between the trained

Table 1. Physical characteristic, anthropometric and pulmonary function profiles (Mean ± SD).

| Group | Age (yrs) | Height (cm) | Weight (kg) | Percent Body Fat (%) | Forced Vital Capacity (l) | Forced Expiratory Volume (l) | $\frac{FEV_{1.0}}{FVC}$ (%) |
|---|---|---|---|---|---|---|---|
| Sprinters | 20.6 ±2.3 | 180.6 ±4.9 | 68.6 ±3.4 | 6.5 ±3.4 | 6.17 ±0.79 | 4.79 ±0.22 | 79 ±8 |
| Rowers | 20.6 ±1.8 | 177.2 ±3.4 | 70.0 ±2.8 | 7.2 ±3.6 | 5.58 ±0.57 | 4.79 ±0.67 | 84 ±8 |
| Marathoners | 37.8 a ±9.3 | 176.5 ±4.2 | 69.0 ±4.4 | 10.6 ±4.0 | 5.26 ±0.63 | 4.22 ±0.59 | 81 ±5 |
| Untrained | 22.6 ±0.9 | 182.4 ±4.3 | 81.7 a ±5.1 | 21.1 a ±4.9 | 5.89 ±0.86 | 4.34 ±0.75 | 74 ±5 |

a $p < 0.05$ significantly different from other 3 groups

Table 2. Physiological assessment and histological analysis of the vastus lateralis muscle (Mean ± SD.)

| Group | Maximal Oxygen Uptake ($ml.kg^{-1}.min^{-1}$) | Anaerobic Speed Test (sec) | Pre Blood Lactate ($mmol.l^{-1}$) | Post Blood Lactate ($mmol.l^{-1}$) | Fast Twitch Percentage (%) | Fast Twitch Mean Diameter (um) | Slow Twitch Mean Diameter (um) |
|---|---|---|---|---|---|---|---|
| Sprinters | 63.2 ±3.1 | 115 c ±18 | 1.1 ±0.3 | 21.9 c ±1.5 | 56.6 ±7.0 | 94.7 ±26.2 | 90.0 ±22.8 |
| Rowers | 62.4 ±1.7 | 76 b ±9 | 1.0 ±0.2 | 13.9 b ±0.9 | 50.4 ±12.3 | 96.0 ±14.4 | 95.0 ±18.7 |
| Marathoners | 60.1 ±4.2 | 53 ±15 | 1.0 ±0.2 | 10.1 ±3.1 | 33.0 a ±12.2 | 74.2 ±9.5 | 79.5 ±16.4 |
| Untrained | 46.9 a ±3.3 | 38 ±9 | 0.8 ±0.2 | 10.1 ±2.6 | 50.6 ±9.9 | 79.1 ±12.2 | 75.0 ±15.2 |

a $p < 0.05$ significantly different from other 3 groups

b $p < 0.01$ significantly > M and UT groups

c $p < 0.01$ significantly > all other groups

Figure 1. Anaerobic performance versus post-AST blood lactate levels.

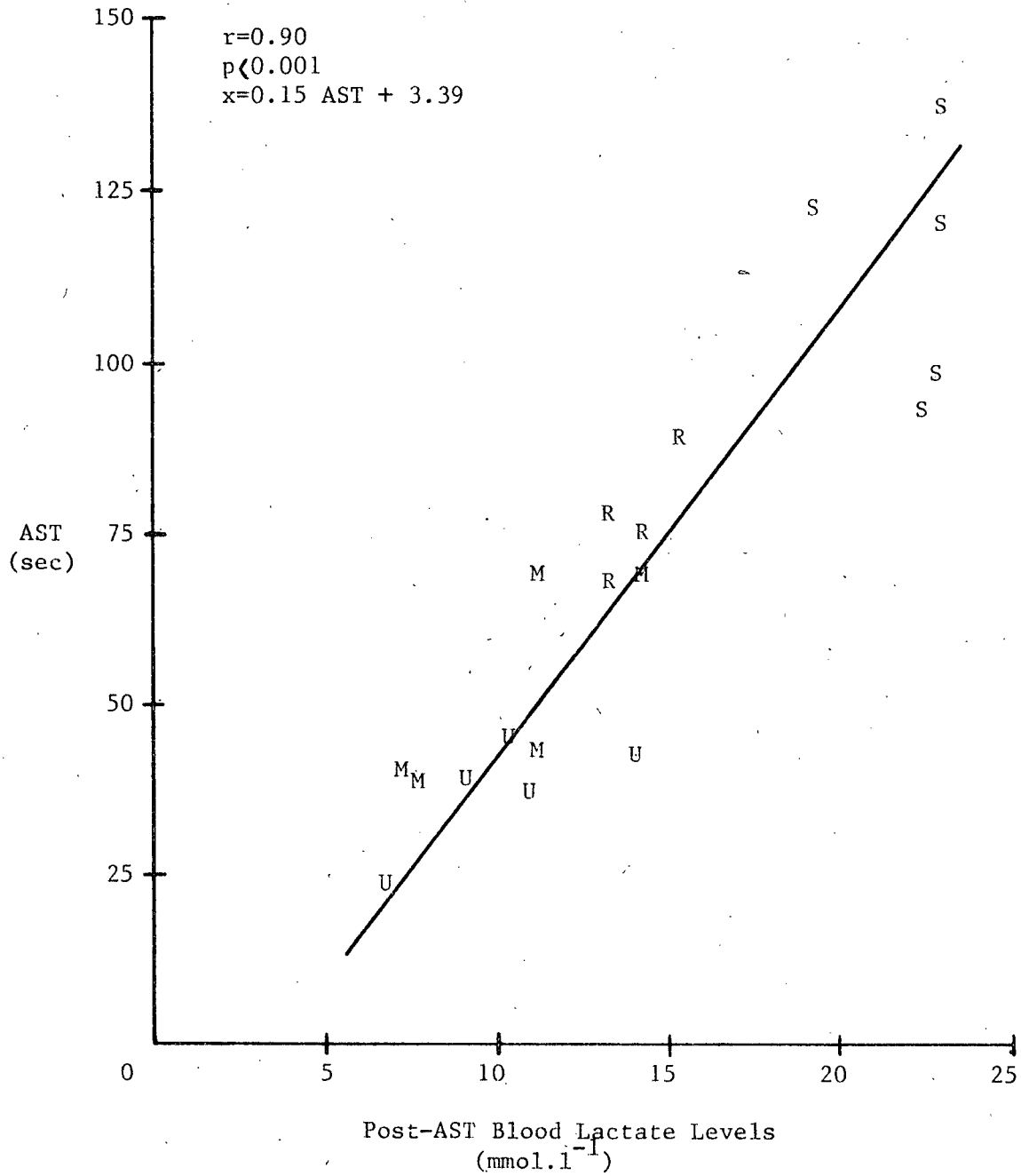
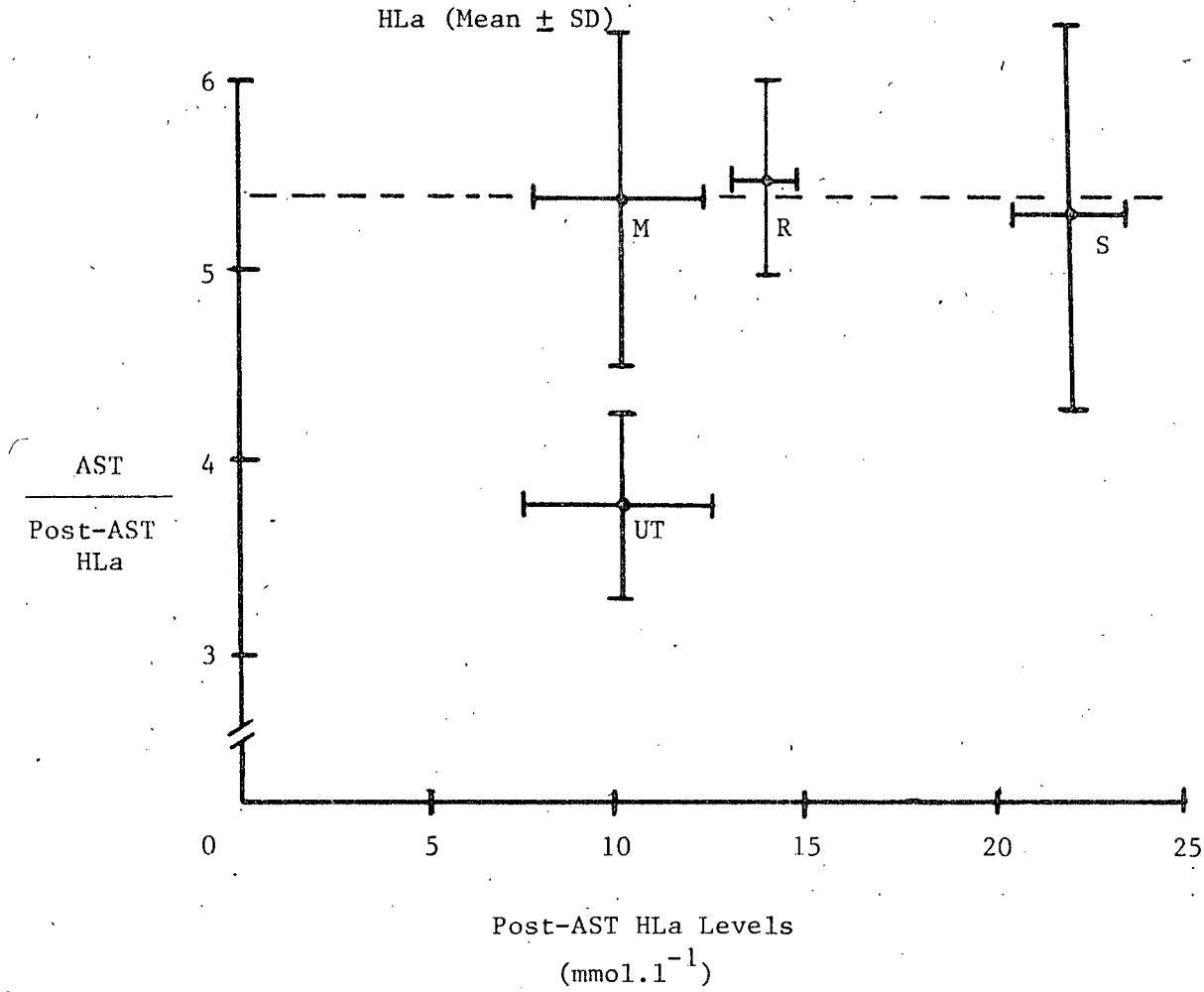


Figure 2. Ratio AST/Post-AST HLa versus Post-AST HLa (Mean ± SD)



groups existed (Figure 2).

Significant differences in fiber composition existed between the groups ($p<.05$). This could be attributed to the low FT fiber percentage of the M group. Though the differences in fiber diameters were not significant ($p>.05$), when FT fiber diameters were expressed relative to ST fiber diameters the S appeared to demonstrate enlarged FT fibers and the M appeared to display enlarged ST fibers.

Resting intramuscular pH and B values are contained in Table 3. No significant differences existed for resting pH ($p>.05$). B revealed no significant differences between the S and R or between the M and UT groups but significant differences ($p<.01$) did exist between these sub-samples. The B of the S and R group were almost 50 percent greater than the B of the M or UT groups.

Multivariate analysis revealed that the S group was significantly different ($p<.01$) from the R group and this was due to significant differences ($p<.01$) in AST performance and post HLa. The M group was significantly different from the UT group in respect to fiber composition ($p<.02$), VO max ($p<.01$), percent body fat ($p<.01$), and age ($p<.01$). Comparison of the S and R groups versus the M group revealed significant differences to exist for AST performance ($p<.01$), post HLa values ($p<.01$), percent FT ($p<.025$) and B($p<.01$).

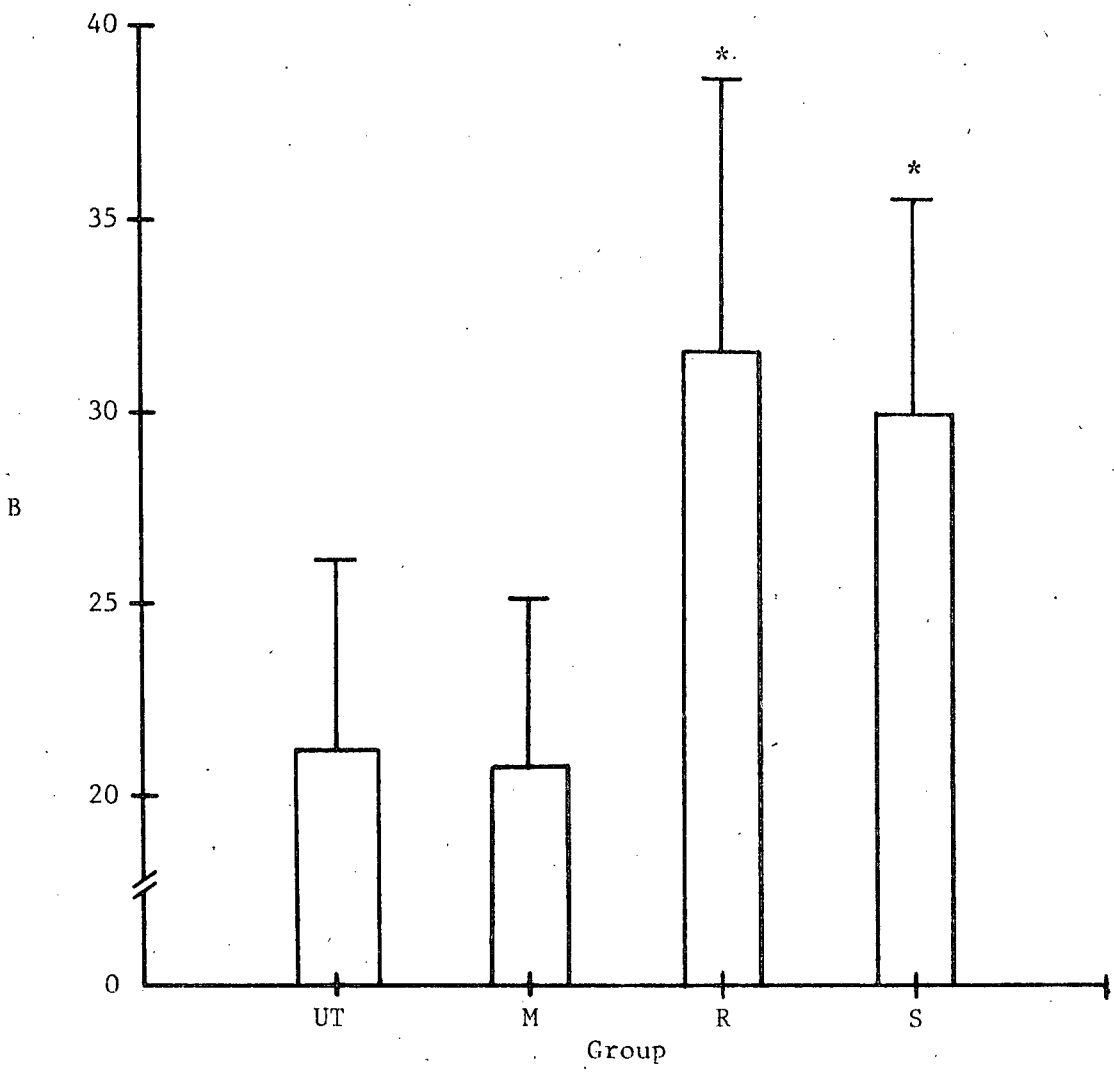
Muscle carnosine and histidine concentrations are also contained in Table 3. Between group comparisons revealed no significant differences in histidine levels but carnosine levels

Table 3. Buffer capacity, pH, histidine and carnosine levels of the resting vastus lateralis muscle (Mean ± SD).

| Group | Buffer Capacity ($umol.g^{-1}.pH^{-1}$) | pH | Histidine Levels ($umol.g^{-1}$) | Carnosine Levels ($umol.g^{-1}$) |
|---|---|---|---|---|
| Sprinters | 30.03 a ±5.6 | 6.99 ±0.13 | 0.64 ±0.06 | 4.93 a ±0.76 |
| Rowers | 31.74 a ±7.2 | 6.97 ±0.11 | 0.71 ±0.10 | 5.04 a ±0.72 |
| Marathoners | 20.83 ±4.4 | 7.11 ±0.11 | 0.63 ±0.14 | 2.80 ±0.74 |
| Untrained | 21.25 ±5.0 | 6.91 ±0.17 | 0.89 ±0.29 | 3.75 ±0.86 |

a $p < 0.01$ significantly > M and UT groups

Figure 3. Buffer capacity (umol.$g^{-1}$w/w.$pH^{-1}$) versus training specificity (Mean ± SD).




* $p < 0.01$ significantly > M and UT groups

Figure 4. Buffer capacity (umol.$g^{-1}$.$pH^{-1}$) versus carnosine concentration (umol.$g^{-1}$.$pH^{-1}$) determined by titration with 0.01N HCl.

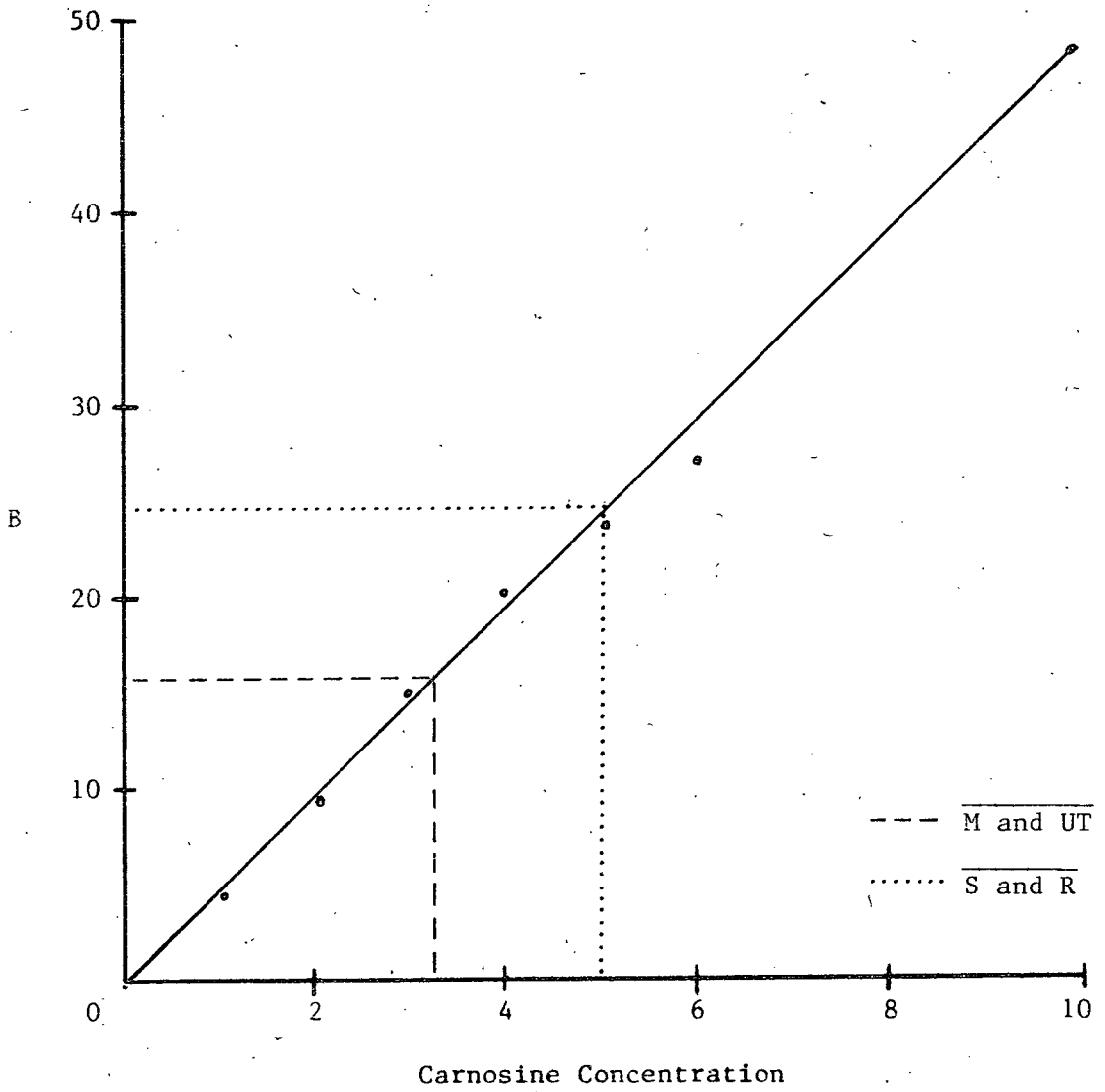


Table 4. Correlation matrix for the biochemical, histochemical and anaerobic performance variables.

| | | | |
|---|---|---|---|
| Carn | .64** | | |
| AST | .51* | .61** | |
| FT% | .51* | .46* | .37 |
| | B | Carn | AST |

* $p < 0.05$

** $p < 0.01$

varied with training specificity as only the S and R demonstrated significant differences ($p<.01$) from the UT group. No significant differences in carnosine levels existed between the M and UT groups. Empirical titration of carnosine (Figure 4) revealed that the increased B of the sprint trained athletes (Figure 3) could be significantly accounted for by the intramuscular carnosine levels.

Correlations are contained in Table 4. B was elevated in the S and R groups (Figures 3). Significant relationships were demonstrated between anaerobic performance and B ($p<.05$;Table 4). A further relationship existed between FT% and B ($r=.51$). Carnosine levels were found to demonstrate significant ($p<.01$) relationships to B and FT%.

Discussion

The major findings of the present investigation are: first, that the anaerobically trained groups exhibited an enhanced anaerobic performance which was significantly related to B; second, that B displayed a significant relationship to fast-twitch fiber percentage and third, that carnosine levels were significantly related to B, demonstrating elevated levels within the anaerobicly trained groups. Although these are descriptive data and it is not possible to state that the biochemical parameters B and carnosine levels, are a function of anaerobic training, it is tempting to speculate that adaptation may occur on the basis of repetitive anaerobic work. Skeletal muscle consists of two distinct fiber types (fast-twitch and slow-twitch), each fiber type possessing different contractile and metabolic properties (Gollnick and Hermansen 1973). Fast-twitch fibers demonstrate a high capacity for anaerobic glycolysis (Lowry et al., 1978; Essen et al., 1975). The enhanced B which was associated with an elevated fast-twitch fiber percentage, suggested that B may be related to the glycolytic capacity of skeletal muscle.

Man performing short duration, high intensity work has a requirement to maintain redox balance, while fulfilling the need for continued muscle energy metabolism, predominantly based upon intramuscular substrates and a capacity to buffer the inhibitory action of the proton accumulation. Hermansen et al., (1971) has reported blood lactate values of up to 32 mM for short duration work. Proton accumulation associated with the elevated lactate

levels alters the acid-base balance of the cytosol resulting in pH decrements within muscle and blood (Sahlin et al., 1978; Hultman and Sahlin 1980; Roos and Boron 1981). The importance of pH to the regulation of anaerobic performance has been demonstrated by Sutton et al., (1981) who reported pH decrements to be associated with decreased anaerobic performances. Relationships between pH and fatigue (Fitts and Holloszy 1976; Stevens 1980), proton accumulation and force generation (Dawson et al., 1977), reduced rates of glycolysis with pH decrements (Toews et al., 1970; Sutton et al., 1981; Roos and Boron 1981) and decreased maximal tension development (Fabiato and Fabiato 1978), possibly due to proton competition with $Ca^{++}$ for the binding sites (Katz 1970) have suggested the importance of buffering the protons which accumulate during anaerobic glycolysis. The present results imply a biochemical phenomenon within anaerobically trained athletes, which would fostor an enhanced capacity for a muscle to function under anaerobic conditions. When the anaerobic glycolytic machinery is functioning, the high buffering capacities of the skeletal muscle may neutralize the accumulated protons. Thus the rate at which pH would normally decrease would be reduced due to the enhanced proton sequestering capacities. It is important to emphasize that the glycolytic enzyme activities examined pre- and post sprint training have reported with minor exceptions no significant differences (Gollnick et al., 1972; Holloszy et al., 1973; Hickson et al., 1975; Costill et al., 1976). Training specificity appears to be associated with a fiber type

predominance and a relationship to fiber type hypertrophy (Gollnick et al., 1972, 1973; Costill et al., 1976; Sahlin et al., 1976; Andersen and Hennriksen 1977). In agreement with the previous investigations, sprinters were found to have an elevated fast-twitch fiber composition while the marathon runners demonstrated a slow-twitch fiber predominance but no significant differences existed between the sprinters, rowers or untrained controls. The interconversion of skeletal muscle fast-twitch sub-group fibers does appear to occur as a result of training (Janssen and Kaijser 1977) but no interconversion of fast-twitch and slow-twitch fibers has been identified with training (Gollnick et al., 1982). Fiber hypertrophy appeared to demonstrate a relationship to training specificity but no differences in fiber diameters existed between the sprinters and rowers. Thus though fiber composition appears to be related to anaerobic capacity, it alone could not account for the enhanced anaerobic performance of the sprinters. Therefore it appears that the enhanced anaerobic capacity associated with the anaerobically trained athletes is more a function of skeletal muscle capacity to buffer the protons in association with fiber composition, than to a change in glycolytic enzyme activities.

The present investigation revealed no significant differences between the trained groups with respect to anthropometric, pulmonary function and maximal oxygen uptake data suggesting the subsamples were relatively homogeneous. The maximal oxygen uptakes demonstrated for each of the groups were comparable to the values reported for similar subjects (Carey et

al., 1974; Hagerman and Mickelson 1980; Roberts et al., 1980; Costill et al., (1976). Although the marathon runners were highly trained competetive athletes, there was no significant difference between this group and the UT controls in measures of anaerobic performance. Anaerobic performance was found to be highly related to training specificity with the run times of the marathon runners and untrained subjects being comparable to the pre-trained test values of Cunningham and Faulkner (1969). Thus anaerobic performance appears to be a function of training specificity and the differences observed could not be attributed to the physical characteristic, pulmonary function, aerobic conditioning or fiber composition.

Post anaerobic performance blood lactate values were significantly elevated in all the groups, demonstrating maximal values which are comparable to those of Karlsson (1971) for short term, intensive exercise bouts of elite athletes. The removal of muscle lactate was previously assumed to be a simple process of diffusion down concentration gradients (Hirche et al., 1971; Jorfeldt 1978) but, recently a carrier mediated, pH dependent lactate transfer mechanism has been identified in a few tissues (Barac-Nieto et al., 1978; Dubinsky and Racker 1978; Spencer and Lehninger 1976; Johnson et al., 1980; Monson et al., 1981). Koch et al., (1981) examining mouse diaphragm muscle have suggested that at least three-quarters of the lactate transfer was carrier mediated. Since protons are effluxed with lactate anions (Mainwood and Brown 1975), an enhanced lactate transfer mechanism associated with sprint training may facilitate the

neutralization of the protons within the cytosol. Post blood lactate values demonstrated a highly significant correlation with anaerobic performance which was even stronger for the trained subjects. Training appears to enhance the lactate transport mechanism producing a similiar rate of lactate release for the trained groups, regardless of training specificity (Figure 2). Thus the greater anaerobic performances of the trained athletes may partially be attributed to this enhanced lactate release, which would facilitate a reduced acidification of the cytosol.

Muscle pH determinations by microelectrode were first performed by Furusawa and Kerridge in 1927 on cat skeletal, cardiac and uterine muscle. More recently a series of experiments examining pH were conducted on human quadricep muscle homogenates obtained pre- and post-exercise (Hermansen and Osnes 1972; Sahlin, Harris and Hultman 1975; Sahlin et al., 1976). A muscle pH of 6.50 to 6.60 was identified as a critical value where fatigue caused cessation of the exercise bout (Sahlin etal., 1978). Decreased muscle pH has been identified to have an inhibitory effect on the contractile mechanism (Nocker 1964; Campion 1974; Katz 1970; Fabiato and Fabiato 1978) and the glycolytic regulatory enzymes (Danforth 1965; Trivedi and Danforth 1966; Toews et al., 1970; Sutton et al., 1981; Roos and Boron 1981). Resting intramuscular pH was found to exhibit no significant differences across the groups, demonstrating a pH comparable to those reported in the literature. Thus achievement of an enhanced anaerobic performance due to a larger

intramuscular pH gradient (pre - post exercise pH) due to elevated initial pH levels associated with training specificity was unfounded.

Heisler and Piiper (1971) stated that skeletal muscle must be subject to large variations in acid production. Thus the ability of a tissue to buffer the protons associated with anaerobic glycolytic energy production may affect performance. The superior buffer capacities demonstrated by the sprinters and rowers suggest that skeletal muscle buffer capacity may be a function of training specificity. Castellini and Somero (1981) found in both mammals and fishes, high buffering capacities in the locomotory muscles of species exhibiting pronounced abilities for burst locomotion. The buffer capacity values found in the present investigation appear to be comparable to the results of Davey (1960b) on deproteinized pre-rigor rabbit psoas muscle. The values are somewhat lower than those reported by Castellini and Somero (1981) for terresterial mammals. This discrepancy may be accounted for by the lack of deproteinization in the Castellini and Somero investigation. Sahlin et al., (1978) calculated proteins to account for an additional 22 percent of buffering in resting samples. The contribution of proteins to total buffer capacity was determined by Bate-Smith (1938), Hultman and Sahlin (1980) to be approxiamently 50 percent of the physico-chemical buffering. Reeves and Malan (1976) found the contribution of proteins to buffering in frog skeletal muscle to be between 40 and 50 percent. Therefore proteins constitute a significant buffering component within

skeletal muscle. Buffer capacity determined by the homogenate technique differs from intact preparations due to the transmembrane fluxes of $H^+$ and/or $HCO_3^-$, which do not occur in a closed system such as the muscle homogenate technique (Heisler and Piiper 1971, 1972; Brown 1971). In agreement with Castellini and Somero (1981) buffer capacity was found to be related to the muscles anaerobic glycolytic potential as evidenced by the relationship to fast-twitch percentage. Buffer capacity was also found to be related to anaerobic performance which suggests that the inherent ability of skeletal muscle buffers to function as proton sequesters in the physiological pH range may augment performance.

Buffer capacity determined by the homogenate technique involves essentially the non-bicarbonate buffering in the cytosol, due principally to phosphate compounds and imidazole-containing compounds (Burton 1978; Somero 1981). Imidazole-containing compounds consist of protein-bound histidyl residues, histidine-containing dipeptides (eg. carnosine and anserine) and free histidine (Somero 1981). Free histidine occurs in much lower concentrations than the other two types of imidazole-containing compounds (Burton 1978). Davey (1960b) suggested that the histidine-containing dipeptides could contribute as much as 40 percent of the total buffering of pre- and post rigor muscle. Castellini and Somero (1981) suggested that the differences in buffer capacities, of the fish species observed, could be due to differences in total protein buffering or free histidine content. Within human skeletal muscle a further possibility to

account for the different buffer capacities may be variations in the histidine-containing dipeptide concentrations. Large variations in carnosine content of the human vastus lateralis muscle have been reported (Christman 1976).

Free histidine concentrations were found to be comparable to those reported by Bergstrom et al., (1974) exhibiting no significant differences between groups. Thus alterations in histidine content can not account for the differences observed in buffer capacities. Carnosine levels were similar to those reported by Zachmann et al., (1966), Christman (1976) and Bergstrom et al., (1978), displaying superior values for the anaerobically trained groups. Thus carnosine levels may possibly be elevated by anaerobic training. In agreement with animal investigations (Zapp and Wilson 1938; Tamaki et al., 1976) carnosine levels demonstrated a relationship to muscle glycolytic capacity. The present investigation revealed that carnosine could contribute significantly to total muscle buffering, accounting for 41 percent of the variance in B. Empirical titrations of carnosine predicted a superior B of approxiamently 9 umoles.$g^{-1}$.$pH^{-1}$ for the sprint trained athletes. Therefore carnosine levels could be responsible for the observed differences in B (approxiamently 10 umoles.$g^{-1}$.$pH^{-1}$). This further emphasizes the the importance of carnosine as a physiological buffer within a muscle homogenate. Therefore the present investigation suggested that carnosine by virtue of its ability to act as a buffer in the physiological pH range could contribute significantly to the observed differences in total

buffer capacity.

In conclusion, buffer capacity was elevated in the anaerobically trained groups and appears to be related to the anaerobic glycolytic potential of muscle. In short duration, high intensity work, the requirement for elevated buffer functions, results from the proton accumulation associated with the maintenance of redox balance, while continuing to produce energy metabolically. Thus the ability of skeletal muscle to sequester protons and therefore buffer the protons which accumulated, may enhance anaerobic capacity. Carnosine by virtue of its significant contribution to buffer capacity may possibly contribute to the enhanced anaerobic performance of sprint trained athletes. Future investigations must attempt to quantify the differences in B and the contrbution of carnosine to buffering within human skeletal muscle which could be attributed to training regime. Furthermore the B of in vivo human skeletal muscle and the buffering constituents contribution to buffering during dynamic exercise must be determined.

## Summary of Findings

1. Anaerobic performance as assessed by the anaerobic speed test appears to be highly training specific such that sprint trained athletes demonstrate significantly elevated performances.

2. Enhanced anaerobic performances were significantly related to elevated skeletal muscle homogenate buffer capacites. This suggests that the ability of skeletal muscle to sequester the protons which accumulated during anaerobic glycolytic energy production may augment anaerobic performance. Anaerobic training may possibly be a necessary pre-requisite for enhanced buffer capacities.

3. The relationship between fast-twitch fiber percentage and buffer capacity suggested that enhanced buffer capacities may be related to elevated glycolytic capacities of skeletal muscle.

4. Intramuscular carnosine levels were elevated only within the anaerobicly trained groups, demonstrating significant correlations with buffer capacity. Carnosine contributed significantly to B, possibly due to its ability to act as a buffer in the physiological pH range.

5. Within animal investigations, carnosine has been found

predominantly within fast-twitch fibers. The relationship between carnosine levels and fast-twitch fiber percentage in the present investigation, suggests a link between carnosine and the glycolytic potential of the muscle.

6. Though variances in histidine levels have been suggested as a possible contributor to differences in buffer capacities across many species, the present investigation, revealed no differences in histidine concentrations with training specificity.

7. Elevated blood lactate levels were significantly related to anaerobic performance, demonstrating an enhanced lactate transport mechanism within trained athletes, which was not altered by training specificity.

8. Training specificity elicited no differences in resting intramuscular pH, which eliminated the possibility of an enhanced anaerobic performance due to an increased pH gradient (rest-post exercise), assuming a constant pH at which fatigue forced termination of the exercise.

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# APPENDICES

## Review of Literature

### A. Acid-Base Status and Performance.

Sprint trained athletes, demonstrate a remarkable ability to perform high intensity, short duration work, with the energy requirements being met principally by anaerobic glycolysis. This metabolic pathway results in the rapid production of ATP, the energy source for muscular contraction, with an associated muscle and blood lactate level increase, which has been demonstrated to inhibit athletic performance (Klausen et al., 1972; Karlsson et al., 1975). Proton accumulation associated with the elevated lactate levels alters the acid-base balance of tissue resulting in pH decrements within muscle and blood (Hochachka and Mommsen 1982; Sahlin 1978; Roos and Boron 1981). Reduced rates of glycolysis (Toews et al., 1970; Sutton et al., 1981; Roos and Boron 1981), correlations between pH and fatigue (Fitts and Holloszy 1976; Stevens 1980) and inverse relationships between force generation of isolated muscle preparations and $H^+$ concentration (Dawson et al., 1978) have been demonstrated when pH drops to too great a degree.

Anaerobic glycolysis which is utilized when energy expenditure is too high to be met through the aerobic combustion of fuels, results in the ultimate production of 2 moles of $H^+$ and 2 lactate anions with the concominant production of ATP per glucosyl unit. Glycolysis and ATP hydrolysis occur during anaerobic energy production demonstrating opposing pH dependencies of $H^+$ production. Thus the relative contribution of either glycolysis or ATP hydrolysis to the 2 moles of $H^+$ produced

per glucosyl unit is pH dependent (Hochachka and Mommsen 1982).

Lactic acid was first identified in muscle by J.J. Berzelius (1807) with the relationship between lactate formation and glucose degradation being initially described by C. Bernard (1877). That lactate was produced within muscle during exercise was first reported by Fletcher and Hopkins (1906), Hill (1926) and Meyerhof (1930). It is now generally accepted that lactic acid accumulates when there is an imbalance in the rate of energy required and that which can be achieved by pyruvate oxidation within the mitochondria. Hochachka and Storey (1975) stated that lactate accumulation was the best indicator of anaerobiosis. Within athletes performing continuous intense short duration exercise, lactate values of 32mM have been reported (Hermansen et al., 1971). Thus the performance of short duration, high intensity exercise places enormous demands on anaerobic glycolysis for energy production and on the mechanisms for handling the accumulation of end products.

1. Metabolism. The biochemical parameters necessary to allow humans to perform at increased rates or during periods of hypoxia can be categorized. First, energy production in the form of ATP synthesis must be performed at a fast rate. Second, redox balance must be maintained so that intermediate metabolites and co-factors do not accumulate in their reduced forms (Casstellini 1981). Third, mechanisms for the buffering of the accumulated protons must be adequate.

Under normal conditions the need for a high energy yield exceeds the need for a high energy rate. Fat demonstrating a

respiratry quotient of 0.7 (Mathews and Fox 1976) and yielding 36 ATP per mole of substrate (Lehninger 1980) is predominantly utilized under these conditions. This pathway bypasses glycolysis entering the Krebs Cycle from Acetyl CoA. Within the oxidative phosphorylation pathway there is an absolute requirement for oxygen at the cytochrome oxidase level. Anaerobic glycolysis produces only 2 or 3 ATP per mole of substrate depending upon whether glucose or glycogen is used. Pyruvate acts as a substrate for both aerobic and anaerobic metabolism with the purpose of its reduction being that of oxidizing NADH to maintain redox balance. When the rate of energy demand is high pyruvate is converted to lactate, NADH is oxidized, redox balance is maintained and ATP production per glucosyl unit is low (Newsholme and Start 1973).

2. Processes of Lactate Disposal and Proton Release. The removal of lactate has generally been assumed to be a simple process of diffusion down concentration gradients (Hirche et al., 1971; Jorfeldt, 1978; Mainwood and Brown 1975). Recently it has become apparent that lactate transfer within a few tissues is carrier mediated. Within the proximal tubule of the kidney it appears to be $Na^+$ transport linked (Barac-Nieto et al., 1978) while in several tumour lines (Spencer and Lehninger 1976; Johnson et al., 1980) red blood cells, (Dubinsky and Racker 1978) and liver (Monson et al., 1981), the carrier mediated transfer of lactate is pH dependent appearing to be an antiport system, lactate anions exchanging for $OH^-$ ions (Hochachka and Mommsen 1982). Within mouse diaphragm muscle Koch et al., (1981)

suggested that at least three quarters of the lactate transfer was carrier mediated. Whatever the mechanism of transfer, lactate has many disposal sites. It can be stored within the producing muscle (Karlsson 1971), or diluted in blood and other body fluids (Hirche et al., 1971; Jorfeldt 1978; Mainwood and Brown 1975). From blood, lactate may be taken up by liver, heart muscle, kidney, brain (Belcastro and Bonen 1975), active (Jorfeldt 1970) and inactive (Poortmans 1978) skeletal muscle. The ultimate fate is reconversion to pyruvate where it is either utilized within the Krebs Cycle or converted into glycogen or glucose (Poortmans 1978). Hermansen and Vaage (1977) found the alanine cycle to be of minor significance in the disappearance of lactate from skeletal muscle.

Protons associated with lactate formation accumulate within muscle and are released to blood and extracellular fluids. It has been reported that the rate of $H^+$ release was dependent upon external bicarbonate concentration (Mainwood and Brown 1975). Benade and Heisler (1978) reported that $H^+$ release initially exceeded lactate release while Hermansen and Osnes (1972) found the H release to occur to a much greater extent than would be expected from their lactate efflux data. The identification of a maximal rate of lactate release by Jorfeldt et al., (1978) may account for this finding.

Anaerobic glycolysis resulting in the production of lactate satisfies the requirements necessary for high intensity, short duration work: first, ATP is generated; second, redox balance is optimized such that the reduction of pyruvate to lactate

provides the co-factor NAD necessary to maintain glycolytic functioning and third, mechanisms for the removal of the inhibiting end products exist. The limitation of anaerobic glycolysis occurs in the second and third areas. The accumulation and removal of end products can not occur indefinitely in a tissue since at high energy demands , the NADH /NAD ratio is increased, altering redox balance and resulting in a proton accumulation, ultimately lowering pH to values too low for glycolysis to continue.

3. Enzymatic Control of Anaerobic Glycolysis. Carbohydrate metabolism from glucose to pyruvate involves nine separate chemical reactions which are catalyzed by specific enzymes. Enzymes can be classified as either regulatory or non-regulatory depending upon their distance from thermodynamic equilibrium. Non-regulatory enzymes involve thermodynamically equivalent reactions which can proceed essentially in either direction. Regulatory enzymes situated far from thermodynamic equilibrium have a large free energy change and proceed essentially in one direction. Regulatory enzymes usually adjust the rate of carbon flow by responding sensitively to changes in metabolite modulators, co-factors, co-enzymes and substrates. Non-regulatory enzymes function to transport carbon along the chain as fast as possible (Hochachka 1980). Several regulatory enzymes have been identified in anaerobic glycolysis.

The enzymes hexokinase (HK), phosphorylase, phosphofructokinase (PFK) and pyruvate kinase (PK) are all situated far from equilibrium and act as regulatory enzymes. The

enzyme HK catalyzes the reaction glucose to glucose-6-phosphate (G6P) therefore being responsible for the pathways ability to utilize blood glucose. Phosphorylase exists in both an active and inactive form being modulated by both contractile and hormonal influences. PFK catalyzes an intermediate step in the pathway with its activity being modified by many factors: adenylate coupling, energy charge of the cell and substrate levels. PK enhances the production of pyruvate which can be metabolized in a variety of ways (Newsholme and Start 1973).

The enzyme lactate dehydrogenase (LDH) catalyzes the pyruvate to lactate reaction and is strongly poised thermodynamically in the lactate direction (Everse and Kaplan 1973). Within skeletal muscle the M4-LDH isoenzyme form predominates. This isoenzyme demonstrates a high Km for pyruvate and NADH while being insensitive to pyruvate or lactate inhibition and has a low affinity for lactate (Hochachka 1980). Thus M4-LDH acts essentially as a pyruvate reductase whose activity is essential to maintain redox balance.

4. Exercise Physiology. Specifically in anaerobic work the initial source of energy for intense muscular contraction comes from creatine-phosphate (CP) and ATP stores which are approximately 16 and 4 $mmol.kg^{-1}$ wet muscle (Karlsson et al., 1971; Knuttgen and Saltin 1972). In humans, these sources can only provide enough energy for the first ten to thirty seconds of exercise after which anaerobic glycolysis provides the necessary energy required for continued muscular contraction. Thus for improved anaerobic performances trained athletes must

have either: elevated levels of stored CP, ATP and/or glycogen, increased activity of the rate limiting regulatory enzymes, and/or an enhanced mechanism for buffering the inhibiting effects of accumulated anaerobic end products.

During high intensity, short duration work, glycogen levels are never depleted before fatigue causes ceasation of the exercise (Gollnick and Hermansen 1973). Changes in intracellular stores of ATP and CP following training are of such low magnitude as not to significantly enhance performance on anaerobic workloads (Knuttgen and Saltin 1972). Thus enhanced performance as a result of increased stored substrate levels does not seem feasible.

The advent of the needle biopsy technique (Bergstrom et al., 1962) and the subsequent identification of two distinct muscle fiber types demonstrating distinguishing enzymatic and contractile properties provided the impetus for much of the investigation surrounding enhanced anaerobic performance. Fibers were originally classified as either fast-twitch or slow-twitch based on their contractile properties and staining intensities for oxidative enzyme activity (Dubowitz and Brooke 1973). Many of the problems associated with skeletal muscle fiber composition can be attributed to the nomenclature utilized for histochemical classification. Briefly for non-human skeletal muscle, fibers are presently being classified on the basis of their reactions to a contractile characteristic (myosin ATPase) and to their oxidative capacity (NADH-TR). Utilizing these stains 3 fiber types have been identified: fast-twitch high

glycolytic (FG), fast-twitch high glycolytic-high oxidative (FOG) and slow-twitch oxidative (SO) (Houston 1978).

With human skeletal muscle most investigations have utilized a two fiber classification based on the histochemical myofibrillar ATPase reaction at pH 9.4 (Houston 1978). The light staining fibers were classified as type I and the dark staining fibers as type II. These fibers represent slow and fast contraction velocities respectively. An alternate classification of slow-twitch (ST) and fast-twitch (FT) has been used extensively (Gollnick et al., 1972; Costill et al., 1976). With the improvement in staining techniques further subdivisions of fast-twitch (type II) fibers have been identified on the basis of their staining intensities for myosin ATPase at different pre-incubation pH's. Thus the type II (FT) fibers have further been classified as types IIA, IIB, IIC. Utilizing a two fiber classification scheme for human skeletal muscle slow-twitch refers to type I and fast-twitch corresponds to type II which includes all the type II subdivisions. Fiber composition of sprint (Edstrom and Ekblom 1972; Thorstenson 1975, 1976; Costill et al., 1976, 1979; Prince et al., 1976; Thomson et al., 1979, Roberts et al., 1981) and endurance (Costill et al., 1971, 1976, 1979; Gollnick et al., 1973, 1974; Eriksson et al., 1973; Prince et al., 1976, 1977; Hennrikson 1977; Jansen and Kaijser 1977; Saltin et al., 1977; Essen et al., 1978; Lithill et al., 1979) trained athletes have been investigated revealing a higher proportion of fast-twitch fibers within sprint athletes and a higher proportion of slow-twitch fibers within endurance

athletes. The interconversion of fast-twitch to slow-twitch and vice versa does not appear to occur as a result of training though the relative glycolytic or oxidative capacities within each fiber type are augmented (Gollnick 1982). Hypertrophy of fibers appears to be selectively chosen as to the type of training performed (Gollnick et al., 1972, 1973; Costill et al., 1976; Prince et al., 1976; Saltin et al. 1976, Andersen and Hennriksen 1977). Increased size and number of fast-twitch fibers within the sprint trained population can not totally account for their enhanced performances.

Fast-twitch fibers have a much higher glycolytic potential than slow-twitch (Lowry et al., 1978; Essen et al., 1975). Enzyme activities (LDH, PFK, phosphorylase, HK) within athletes and animals following sprint training have demonstrated with minor exceptions no significant differences (Baldwin et al., 1972; Gollnick et al., 1972; Holloszy et al., 1971; Hickson et al., 1975). Costill et al., (1976) examined different track athletes and found sprint trained athletes to have elevated LDH and phosphorylase activities as compared to endurance trained athletes. Therefore though some enhancement of glycolytic enzyme activity appears to accompany sprint training, the quantitative changes are of insufficient magnitude to solely account for the differences demonstrated in anaerobic performance.

Accumulation of anaerobic end products has been associated with the fatigue process probably through the proton action on intracellular pH. Studies on exercising man have found intramuscular pH to be as low as 6.4 to 6.6 during a short

intensive exercise bout (Osnes and Hermansen 1972; Sahlin et al., 1976). Associated with the pH decrement are reduced rates of glycolysis (Toews et al., 1970; Sutton et al., 1981; Roos and Boron 1981) and correlations with fatigue (Fitts and Holloszy 1976; Stevens 1980). Thus anaerobic performance may be enhanced by reducing the rate of $pH_i$ decrement which accompanies the proton accumulation during high intensity, short duration work.

B. Intracellular pH.

The pH value of a solution is a measure of the relative chemical potentials of the protons in that solution (Waddell 1971). Several techniques exist for the determination of intracellular pH (pHi): homogenate, distribution of weak acids and bases (DMO), calorimetry and fluorometry, microelectrode and 31P nuclear magnetic resonance spectroscopy. Each method has its respective advantages and disadvantages displaying large variations in intracellular pH determination of human skeletal muscle (Roos and Boron 1981).

Measurement of pH on a homogenate was first employed by Michaelis and Davidoff (1912) on red blood cells. The homogenate technique possess several problems: lactic acid and $CO_2$ production continue after cellular destruction leading to a fall in $pH_i$ (Waddell and Bates 1969), mixing of extra- and intracellular fluids can lead to pH changes if the solutions are of dissimilar pH, the dilution of the extra- and intracellular buffers (Bates 1973), and disruption of intracellular organelles with their respective internal pH (Cohen and Iles 1975).

Furusawa and Kerridge (1927) examined cat skeletal, cardiac and uterine muscle, eliminated the $CO_2$ and lactate problems, by immediate submersion of the sample in liquid air, with subsequent mincing and pH determination by glass microelectrode at 0°C. A series of studies were conducted examining both resting and post-exercise intracellular pH determinations on human quadricep muscle homogenates (Hermansen and Osnes 1972; Sahlin, Harris and Hultman 1975; Sahlin 1976). Reported resting pH values were 6.92±.10 (Hermansen and Osnes 1972) and 7.08±.03 (Sahlin, 1976) respectively. The inclusion of iodoacetic acid (IAA) in the preparation prevented the continuous decrease in pH during the measurement. Sahlin (1976) estimated that the mixing of the intra- with the extracellular compartments would increase the resting intracellular pH of the samples by about 0.03 units. A pH of 6.8 to 7.1 has been obtained in most animal studies on skeletal muscle by a large number of techniques (Furusawa and Kerridge 1927; Millar, Tyson and Relman 1963; Hault et al., 1974; Waddell and Bates 1969; Aickin and Thomas 1977).

1. Total muscle pH in relation to exercise. Furusawa and Kerridge (1927) studying electrically stimulated cat gastrocnemius muscle found pH to decrease from 7.04 at rest to 6.26 at fatigue. The results from more recent studies are in agreement with these figures. Steinhagen et al., (1976) examined interstitial pH of dog working gastrocnemius muscle with implanted glass minielectrodes and found a proton concentration gradient to always exist between interstitial fluid and venous blood. Interstitial pH within muscle has shown an initial

alkalinization followed by an increased acidification to occur during contraction (Gebert and Sydney 1973; Steinhagen et al., 1976). The time course of pH changes are in agreement with the metabolic changes within muscle (Danforth et al., 1965).

The intracellular pH of rat thigh muscle measured by Rooth (1966) using the DMO method was found to decrease only from 6.64 at rest to 6.57 after exhaustive exercise. Similarly, Hermansen (1969) examined one subject running intermittently for 20 minutes, with pH determined by the DMO technique. He found the pH to decrease from only 6.88 at rest to 6.73 after exercise. Reliable pH values determined by the DMO method require at least one hour of equilibration time between the intra- and extracellular compartments (Waddell and Butler 1959). This condition was not met in either the study by Rooth (1966) or Hermansen (1969).

In a study on maximum bicycle exercise of short duration, total muscle pH of the musculus quadreceps femoris was determined by the homogenate technique. Muscle pH was found to decrease from 6.92 at rest to 6.41 after exhaustive exercise (Hermansen and Osnes 1972). Muscle samples of the musculus quadriceps femoris were obtained prior and post isometric exercise to fatigue with muscle pH determinations being made by the homogenate technique (Sahlin, Harris and Hultman, 1975). Muscle pH was found to decrease from 7.09 at rest to 6.56 at fatigue. In a subsequent investigation employing dynamic exercise , muscle pH was found to decrease from 7.08 at rest to 6.60 at exhaustion (Sahlin etal., 1976). It thus appears that

human quadricep total muscle pH determined by the homogenate technique is roughly 7.0 and that intense muscular exercise to fatique results in a reduction in intramuscular pH to approximatly 6.5.

2. pH changes and muscular work of skeletal muscle. The effects of acid-base changes on skeletal muscle were first demonstrated by Creese (1950) on isolated repetitively stimulated rat diaphragm muscle. The transients which occurred in twitch tension could be ascribed to the pH changes resulting from removal and readmission of $CO_2$. Foulks and Perry (1977) found extracellular pH changes from 5 to 9 at constant $PCO_2$ to affect twitch tension of frog muscle very little. Stimulating frog muscle to fatigue, Mainwood and Brown (1975) found twitch tension decreased to 20 percent of control. In an earlier investigation, Mainwood et al., (1972) found external $HCO_3^-$ to modulate intracellular proton balance and to limit lactate efflux. Increasing extracellular pH, thus raising internal pH, increased lactate efflux leading to nearly 100 percent recovery of tension in fatigued muscle. In experiments where pH was monitored by 31P-nuclear magnetic resonance Dawson et al., (1978) found inverse relationships between isolated frog muscle preparations force generation capabilities and proton concentration. Stevens (1980) found isolated frog sartorius muscle preparations to demonstrate correlations between pH and fatigue. Similar relationships were demonstrated by Fitts and Holloszy (1976) on frog muscle preparations between lactate levels and fatigue.

3. Mechanisms of Action. It has been suggested by Nocker (1964) that decreased pH may affect the membrane permeability to $Na^+$ and $K^+$ resulting in a hyperpolarized state. This effect may be even more important in fast-twitch fibers than slow-twitch fibers since they have a lower resting membrane potential (Campion 1974). Muscle contraction would be impaired since membrane permeability is vital to the elicitation of an action potential. A decreased active cross bridge formation due to proton competition with $Ca^{++}$ for the actomyosin binding sites, may reduce work capacity (Katz 1970). Fabiato and Fabiato (1978) examined the effects of pH on the myofilaments and sarcoplasmic reticulum of skinned frog skeletal muscle cells. They found skeletal muscle increased its release of $Ca^{++}$ at moderate acidification in an attempt to compensate for the decreased sensitivity of the myofilaments to $Ca^{++}$. Therefore they identified the only effect of pH variation within skeletal muscle, was the decreased maximum tension development capability during acidosis.

Intracellular pH has many interactions with metabolic transformations. Ionizable groups of active sites on enzymes, may affect the enzymes conformation and thus its substrate binding and catalytic properties through their state of ionization. Specific groups on substrates or cofactors through their degree of ionization may affect their ability to bind to the enzyme. The direct uptake or release of protons or $CO_2$ by the metabolic transformations themselves may produce pH changes (Roos and Boron 1981).

Within the glycolytic pathway the conversion of inactive

phosphorylase b into active phosphorylase a is pH sensitive. Danforth (1965) examined intact frog muscle and demonstrated a lag period before appearance of phosphorylase a in response to muscle stimulation as $CO_2$ concentration was raised. In vitro preparations similarly depressed phosphorylase b to a conversion as pH decreased.

Trivedi and Danforth (1966) identified a marked pH sensitivity of PFK, the enzyme that phosphorylates fructose-6-phosphate (F6P). Utilizing an in vitro preparation, a 10 to 20 fold reduction in enzyme activity occurred when pH was reduced by 0.1 units. The actual pH range that produced the markedly decreased enzyme activity was dependent upon F6P concentration. The increased concentrations of G6P as a result of elevated F6P activity tends to inhibit reactions higher up in the glycolytic pathway (Trivedi and Danforth 1966). The concentrations of muscle glycolytic intermediates determined at exhaustion, were utilized to determine at which point in the glycolytic pathway acidosis produced its inhibitory action (Toews et al., 1970). PFK was identified as the enzyme whose action was inhibited by acidosis. The enzyme actions of PFK and pyruvate kinase (PK) are linked by adenylate coupling, thus inhibiting the production of pyruvate. Decreases in pH lead to large changes in ATP and CP sensitivity as well as large increases in affinity for both substrates (Hochachka 1980).

Sutton et al., (1981) examined the effect of pH on muscle glycolysis during exercise, concluding that in agreement with the in vitro studies, elevated proton concentrations eventually

inhibit glycolysis reducing the supply of ATP necessary for continued muscular contraction. These actions appear to be elicited by pH dependent inhibition of the contractile process and of the glycolytic regulatory enzymes phosphorylase and phosphofructokinase. Thus the importance of neutralizing the proton accumulation and limiting the decrement in pH associated with muscular work may influence anaerobic performance.

C. Buffer Capacity

Most anaerobic studies have concentrated on changes in substrate levels or enzyme activity and metabolite concentrations which could be capable of generating ATP and maintaining redox balance (Hochachka 1980). Few investigations have centred on the capacity of intracellular fluids to buffer the acidic end products of anaerobic glycolysis. Heisler and Piiper (1971) state that skeletal muscle by virtue of its high mass, its abrupt and large changes in metabolic activity, and its high anaerobic capacity, show that it must be subject to large local variations in acid production. Therefore they suggest that skeletal muscle must be the most important determinant of overall buffering ability of the organism. Although B of skeletal muscle had been recognized as an important factor in pH regulation, its involvement to pH homeostasis within trained athletes has not been investigated.

Homeostasis of pH relies on the cells ability to extrude $H^+$ and/or accumulate $HCO_3^-$ or $OH^-$ (Roos and Boron 1981). It is generally accepted that an acid is a proton doner and a base is

a hydrogen acceptor, while a buffer is something that resists change. A pH buffer is a substance, or mixture of substances, that permit solutions to resist large changes in pH upon the addition of small amounts of $H^+$ or $OH^-$ ions (Segal 1976). Buffer capacity refers to the ability of a buffer to resist changes in pH. B can be defined as the number of moles per liter of $H^+$ or $OH^-$ required to cause a given change in pH of 1 unit. Essentially B is the reciprocal of the slope of the titration curve at any point (Segal 1976). Larsen and Burnell (1978) stated that B was a function of buffer concentration and the proton sequestering capabilities of the buffers in the specific pH range.

In 1908 Henderson and Washburn working separately reported that a weak acid exerted its maximum buffering when its dissociation constant equalled the $H^+$ concentration. Koppel and Spiro (1914) were the first to demonstrate that total buffer action was the sum of the individual buffer actions. They demonstrated that the maximal buffer action of all monovalent weak acids at equivalent total concentrations were the same. Michaelis in 1922 modified the definition of buffering power to $B = dB/dpH$. Van Slyke (1922) defined buffering power in the same way as Michaelis. This definition involves the so called self buffering of water, which only becomes apparent at extreme pH values and is negligible in the physiological pH range (Roos and Boron 1981). This definition of buffering power given by Michaelis and Van Slyke is now readily accepted.

1. Skeletal Muscle Buffering Capacity. Several methods employing the addition of acid or base loads while monitoring pH changes by a variety of means have been utilized for the determination of B within skeletal muscle. Titration of cellular homogenates was first used by Furusawa and Kerridge (1927) on cat skeletal, cardiac and smooth muscle at 0°C over the pH range 6.4 to 7.4. Dilution did not substantially alter the pH. Titration of intracellular fluid in situ by the injection of acid or alkali or by exposure to a weak acid or base was another technique employed. Titration of intact cells impaled with a pH sensitive microelectrode is a third technique which has been used.

Intracellular buffer capacity of skeletal muscle has been determined in a variety of species and a summary of the results are contained in table 5. Values range from 40 to 100($mmol.pH^{-1}.l^{-1}$ IC $H_2O$) and appear to be related to the muscles capacity for high glycolytic function (Castellini and Somero 1981). Heisler and Piiper (1971, 1972) demonstrated that these values differ from the B of intact preparations and that these discrepancies are due to the transmembrane fluxes of $H^+$ and/or $HCO_3^-$ in intact muscle. Protons appear to be transported into skeletal muscle and out of heart and brain tissue during severe respiratory acidosis (Clancy and Brown 1966; Siesjo and Messeter 1971; Lai et al., 1973). Thus the large buffering ability of muscle appears to be utilized for the protection of more critical tissues. Hultman and Sahlin (1980) examining acid base balance during exercise calculated an apparent B of 73.5 ($mmol.pH^{-1}.l^{-1}$ $H_2O$) based on proton release calculated from lactate production and

Table 5. Average non-bicarbonate buffer values for skeletal muscle determined by homogenate titration with HCl or NaOH ($mmol.kg^{-1}.1\ H_2O$ IC).

| Reference | Tissue | Buffer Capacity |
|---|---|---|
| Furusawa and Kerridge 1927 | Cat gastrocnemius | 43 |
| Bate-Smith 1938 | several in rigor | 74-97 |
| Eckel et al. 1959 | rat | 61 |
| Davey 1960b | mouse soleus pre-rigor | 40 |
| Larsen and Burnell 1978 | | 66 |
| Castellini and Somero 1981 | terresterial mammals | 85 |

the change in pH with exercise. A similar value of 68.5(Sl) was earlier calculated by Sahlin (1978) for B by the various buffering constituents during dynamic exercise when intramuscular pH decreases from 7.0 to 6.4.

2. Skeletal Muscle Buffering Constituents. Siesjo and Messeter (1971) have classified the major buffering constituents into three components: first, physico-chemical buffering; second, consumption or production of non-volatile acids and third, transmembrane fluxes of $H^+$ and $HCO_3^-$. B of in vitro preparations consists of simply the physico-chemical buffering component which comprises the buffering within a cell merely as a consequence of $H^+$ association with bases (Roos and Boron 1981). Burton (1978) suggested that the histidine related compounds and inorganic phosphate ($P_i$) were the major buffering components of skeletal muscle. Titration of muscle homogenates provides a closed system whereby the HCO buffering mechanism can be neglected (Larsen and Burnell 1978). Sahlin (1978) suggested that HCO could contribute as much as 15 to 18 percent of total B in vivo during exhaustive exercise.

Davey (1960b) suggested that at least 90 percent of the B on deproteinized homogenates of pre- and post rigor skeletal muscle could be accounted for by ATP, inorganic phosphate, carnosine, anserine and unidentified phosphate. ATP has an appropriate pKa (7.0) but, occurs complex bound to Mg and proteins, rendering its buffering power negligible (Burton 1978; Hultman and Sahlin 1980). ADP, demonstrating a pKa of 6.7, occurs in too low concentrations to contribute to buffering

(Dawson et al., 1977; Burton 1978; Hultman and Sahlin 1980). Burton (1978) suggested that $P_i$ contributed relatively little to buffering until muscle contraction is activated and CP is hydrolyzed. Somero (1981) suggested that free $P_i$ was the second most important buffer in biological fluids. It appeared that $P_i$ was poorly suited for stabilizing pH as its pk was insensitive to temperature. Imidazole type buffers, especially anserine, carnosine and ophidine which occur predominantly in highly glycolytic tissue, have been suggested to dominate buffering of biological fluids due to their ability to maintain their B with elevated temperatures (Somero 1981). This buffer type consists of free histidine, the histidine dipeptides and protein bound histidine residues. Proteins are recognized as a major buffer within skeletal muscle (Bate-Smith 1938; Woodbury 1965; Sahlin 1978). Woodbury (1965) calculated the buffer value of muscle protein based on histidine content to be about 15 Sl while Bate-Smith (1938) examining titrations of muscle extracts with and without proteins found protein contribution values of 17 to 37 Sl which corresponds to approxiamently 40 to 50 percent of B. Hultman and Sahlin (1980) calculated proteins to contibute up to 50 percent of the total physico-chemical buffering. The contribution of the free amino acids to B in the physiological pH range is limited to those demonstrating a pKa for their ionizable R groups in that range (Hultman and Sahlin 1980). Somero (1981) found the free Histidine contribution to total B to be of minor importance, while Sahlin (1976) calculated the free histidine contribution to buffering to be only 0.1 Sl.

Since amino acids demonstrating these pK characteristics occur in such minor concentrations within skeletal muscle, their relative contribution to total B is negligible.

Differences in buffer capacity across fish species were attributed to variations in protein content or free histidine concentrations (Castellini and Somero 1981). Within humans the concentration of free histidine is substantially less than that of the histidine-containing dipeptides (Bergstrom et al., 1974; Rennie et al., 1981). Thus the possibility exists that within human skeletal muscle, alterations in content of the histidine-containing dipeptides may account for the variances observed in buffer capacity. Therefore the relative role of the dipeptides carnosine and anserine in relation to buffer capacity of human skeletal muscle must be investigated.

3. Carnosine and Anserine. The dipeptides anserine (B-alanyl-N$^{\pi}$-methylhistidine) and carnosine (B-alanylhistidine) are found in the skeletal muscles of many species of animals (Crush et al., 1970; Christman 1976). Carnosine was first discovered by Gulevich in 1900. The biosynthesis of anserine and carnosine in rat skeletal muscle has been demonstrated by Aonuma et al., (1969, 1970) to involve the conversion of anserine to carnosine preceded by synthesis of anserine from B-alanine and N$^{\pi}$-methylhistidine. Carnosine and its methylated analogues play some physiological role in the specialized tissues where they are found but, no unified explanation of their role exists. Anserine and carnosine were demonstrated to act as buffers to neutralize the acidosis which occurred during anaerobic

glycolysis (Shertsner 1958; Davey 1960a,b; Quershi and Wood 1962; Meshkova 1965). The pK characteristics of both carnosine (pk = 6.83) and anserine (pk = 7.04) were identified by Bate-Smith (1938) and Eggleton and Eggleton (1938) who suggested that they were ideally suited for the role of buffers in the physiological pH range. Bate-Smith (1938) and Davey (1960a) suggested that as much as 40 percent of the total buffering of pre- and post rigor muscle could be attributed to the action of these dipeptides. Severin (1963) found that frog muscle immersed in a solution containing carnosine could contract longer and with greater amplitude. Meshkova (1965) could not account for the increased glycolytic activity demonstrated when carnosine was added to the medium solely to its buffer action which suggests that carnosine may augment glycolysis by more than one mechanism.

The dipeptides were found to occur otogenetically at the onset of muscle function (Skvortsova 1953). In a series of investigations, Severin (1962, 1963, 1966) found the concentration of carnosine to be greatest at nerve endings and that the dipeptide increased the work ability of exhausted frog muscle. Bowen in 1965 demonstrated carnosine and histidine to be powerful potentiators of ATP induced muscular contraction of rabbit psoas. The dipeptides were later identified as myosin ATPase activators (Avena and Brown 1969; Parker and Ring 1970). Boldyrev in a series of investigations (1971a,b, 1978; Lopina and Boldyrev 1974) suggested that the specific activating effect of carnosine was on the sarcolemma $Na^{+}, k^{+}$ - ATPase. Ikeda et al.,

(1979) found the activity of fructose 1,6 bisphosphatase to be stimulated by carnosine and anserine. This enzyme is involved in the substrate cycle between fructose 6 phosphate (F6P) and fructose 1,6 diphosphate which involves continuous hydrolysis of ATP. This cycle is necessary in muscle whose energy utilization varies widely in order to augment the rate of F6P phosphorylation and changes in AMP concentration (Newsholme and Start 1973).

Brown (1981) found carnosine and anserine to be located within the skeletal muscle of rat exhibiting active oxidative metabolism and/or glycolysis. Brown suggested that the dipeptide's role may be intracellular transport of copper for activation of cytochrome oxidase at the end of the electron transport chain and in regulation of anaerobic glycolysis. It was hypothesized that carnosine could reverse the inhibition of glycolysis within skeletal muscle by chelating copper.

Though many physiological roles for the dipeptides have been proposed and a single function is unlikely, the only role universally accepted is that of a that of physiological buffer (Boldyrev 1978). More recent investigations on pH regulation and B, have discussed the relative contribution of the imidazole containing dipeptides (Burton 1978; Hultman and Sahlin 1980; Somero 1981). It appears though that these dipeptides have many functional roles ultimately linked to regulation of aerobic and anaerobic metabolism.

It has been demonstrated that carnosine and anserine levels within many species are higher in skeletal muscle denoted as

being white as opposed red under normal conditions (Christman 1976; Tamaki et al., 1976), while in humans anserine levels appear to be either nonexistant or insignificant (Christman 1976). Tamaki et al., (1976) reported that denervation resulted in decreased carnosine levels and increased carnosinase activity. Determinations made following an acute bout of swimming exercise or electrical stimulation resulted in no significant change in rat carnosine levels (Eggleton and Eggleton 1933). Hunter (1924, 1925) demonstrated reduced carnosine levels upon starvation and elevated levels upon a protein diet in rats. Christman (1976) reported an inverse relationship between age and carnosine levels in human skeletal muscles, carnosine levels decreasing as age increased. Values in $mmol.l^{-1}$ $H_2O$ IC ranged from 1.5 in 60+ year old individuals (n=2) to 7.2 in the 15 to 19 year old age range (n=5), for the limited number of samples investigated (Christman 1976). A mean intracellular carnosine value of 6.15 ($mmol.l^{-1}$ $H_2O$) was reported by Bergstrom et al., (1978) for normal healthy adults.

Carnosine occurs in large concentrations within human skeletal muscle and has been found to occur primarily within skeletal muscle denoted as being white of a variety of species. Thus due to its high concentration, optimal pK characteristics and possible predominance within fast glycolytic fibers, carnosine may play a significant role in buffering the protons which accumulate during intense muscular exercise.

D. Summary. Lactic acid accumulation within muscle and blood appears to be one of the factors involved in the complicated process of fatigue as it relates to performance (Bagby et al., 1978, Klausen et al. 1972, Karlsson et al., 1975). Fast twitch (FT) fibers demonstrate the highest degree of fatiguability and have higher concentrations of the M-LDH isozyme (Sjoden 1976) suggesting that fiber composition of skeletal muscle may be related to performance (Essen and Haggmark 1975; Jordfelt 1970). Ivy et al., (1980) found that both the proportion of slow twitch fibers and the muscle respiratory capacity play a role in determination of lactate thresholds. Trained subjects demonstrate superior capabilities than untrained subjects to tolerate high blood lactate levels. It has been suggested that intracellular buffering capacity may play a role in the regulation of intracellular pH. During a high intensity, short duration workload, the B of tissue may be of importance to reduce the accumulation of protons which would ultimately decrease pH resulting in a decrement in performance. The major buffering components within human skeletal muscle, if comparable to animal tissue, are inorganic phosphate, protein bound histidine residues and the dipeptide carnosine. Carnosine levels within animal tissues appear to be highly related to the glycolytic capacity of skeletal muscle and may play a role in anaerobic performance. Buffering in vitro consists merely of proton association with bases. There appears to be a specific intramuscular pH at which fatigue forced cessation of the exercise (Sahlin et al., 1975, 1976; Hermansen and Osnes 1972).

An elevated resting pH may prolong one's ability to perform by increasing the amount of protons which must accumulate before the critical pH level is attained. Thus the capacity of skeletal muscle to buffer the resultant pH decrement, associated with the proton accumulation which accompanies high intensity, short duration work, may enhance anaerobic performance, possibly through alterations in buffer capacity and carnosine with training specificity.

Appendix A. Repeated buffer capacity determinations ($umol.g^{-1}.pH^{-1}$).

| Group | Subject | Buffer Capacity Titration 1 | Titration 2 |
|---|---|---|---|
| Sprinters | WF | 31.98 | 32.29 |
| | TG | 24.27 | 22.67 |
| | KB | 32.30 | 31.98 |
| | BS | 39.64 | 35.61 |
| | SH | 25.67 | |
| Rowers | GB | 32.68 | 31.93 |
| | KW | 26.95 | |
| | GS | 26.91 | 27.32 |
| | AH | 28.90 | 29.41 |
| | SB | 45.45 | 42.86 |
| Marathoners | BP | 15.84 | 15.72 |
| | BB | 17.72 | 17.02 |
| | JC | 25.30 | 22.88 |
| | DS | 20.92 | 20.43 |
| | NW | 27.23 | 25.25 |
| Untrained | BA | 17.20 | |
| | RW | 14.56 | 15.18 |
| | RW | 24.60 | 25.00 |
| | BF | 26.46 | 27.14 |
| | KM | 23.02 | 23.26 |

Reliability r=0.99

Appendix B. Blood lactate levels pre- and post-anaerobic performance ($mmol.l^{-1}$).

| Group | Subject | Blood Lactate Concentration Pre-AST | Post-AST |
|---|---|---|---|
| Sprinters | WF | 1.4 | 22.7 |
| | IG | 1.1 | 22.8 |
| | KB | 1.4 | 22.6 |
| | BS | 0.8 | 22.3 |
| | SH | 0.9 | 19.3 |
| Rowers | GB | 1.4 | 13.3 |
| | KW | 0.9 | 13.9 |
| | GS | 1.0 | 14.1 |
| | AH | 0.8 | 13.0 |
| | SB | 1.0 | 15.2 |
| Marathoners | BP | 0.8 | 11.1 |
| | BB | 1.3 | 10.8 |
| | JC | 0.9 | 7.2 |
| | DS | 1.3 | 6.9 |
| | NW | 0.9 | 14.5 |
| Untrained | BA | 0.6 | 9.0 |
| | RW | 0.7 | 6.5 |
| | RW | 0.9 | 13.6 |
| | BF | 1.0 | 10.8 |
| | KM | 0.6 | 10.4 |

Appendix C. Buffer capacity conversions

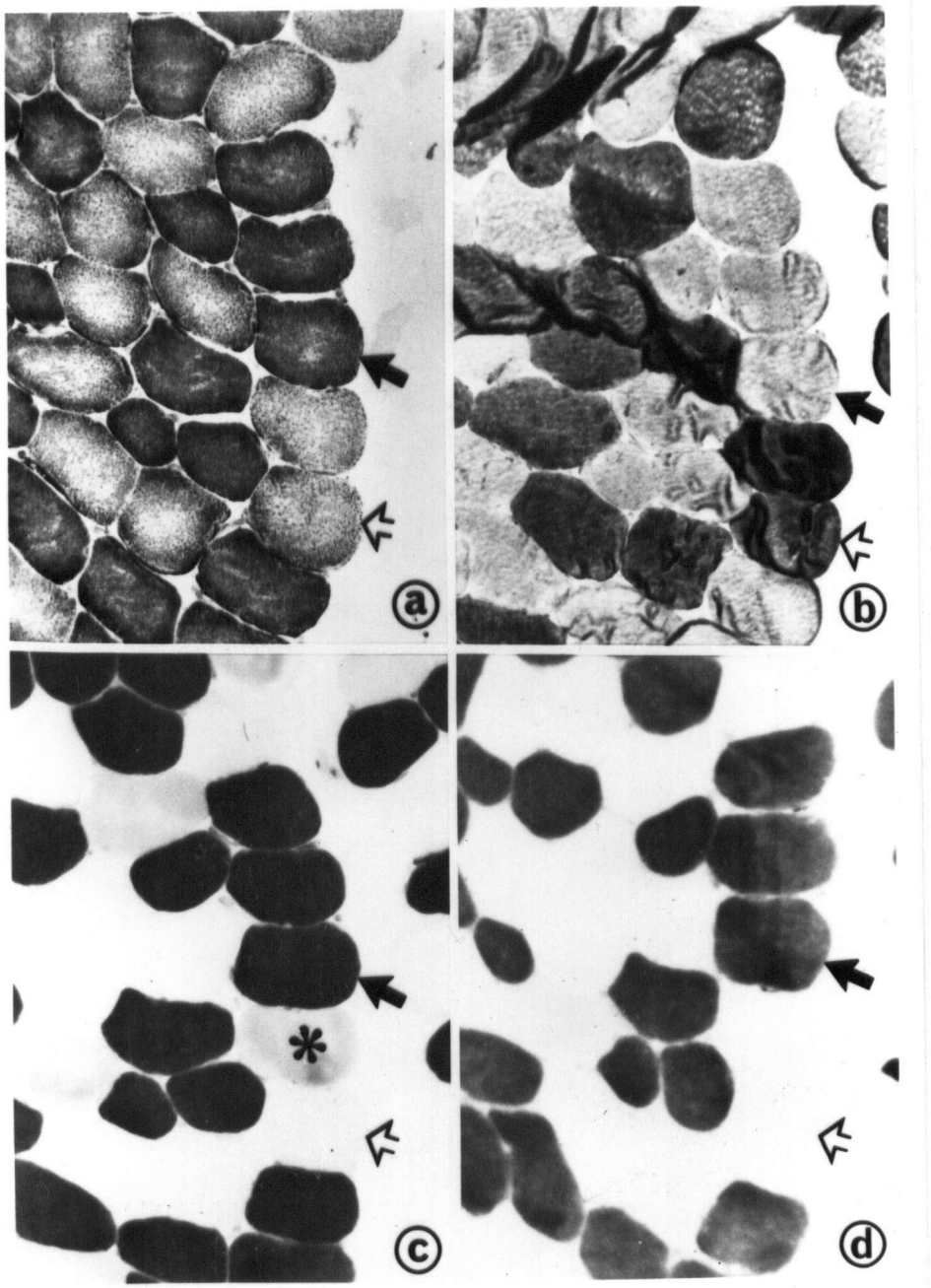
1 $mmol.pH^{-1}.Kg^{-1}$ = 1.29 $mmol.pH^{-1}.l^{-1}$ tissue water

1.29 $mmol.pH^{-1}.l^{-1}$ tissue water = 1.548 $mmol.pH^{-1}.l^{-1}$ IC $H_2O$

| Group | $umoles.pH^{-1}.g^{-1}$ | $mmol.pH^{-1}.l^{-1}$ IC $H_2O$ |
|---|---|---|
| Sprinters | 30.03 + 5.6 | 46.49 + 8.7 |
| Rowers | 31.74 + 7.2 | 49.13 + 11.2 |
| Marathoners | 20.83 + 4.4 | 32.25 + 6.8 |
| Untrained | 21.25 + 5.0 | 32.90 + 7.7 |

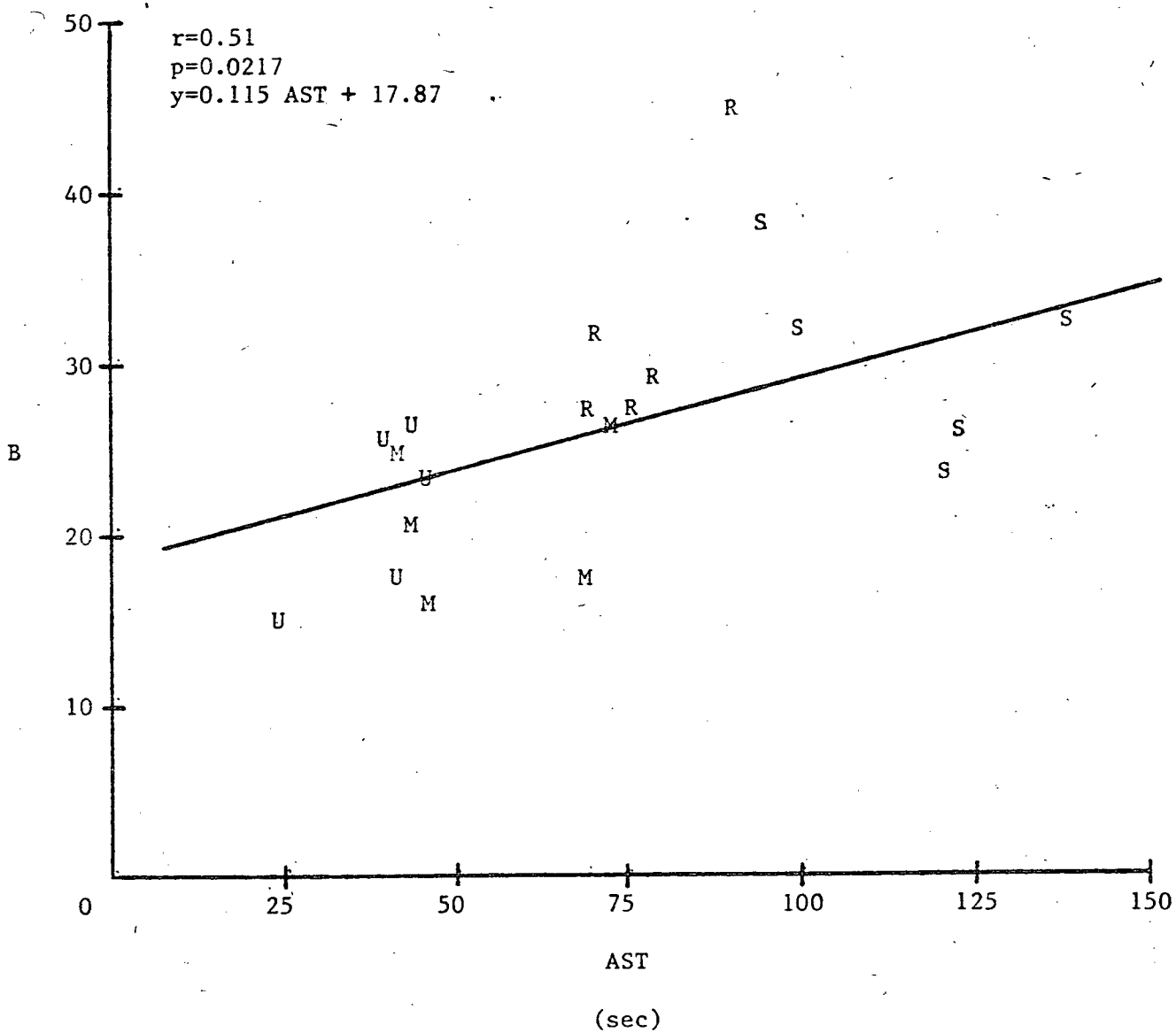
Appendix D. Serial sections of UT vastus lateralis muscle stained for Myosin ATPase and NADH-TR

| | Fiber Type | Staining Intensity |
|---|---|---|
| a NADH-TR | Type I | dark |
| | Type II | light |
| b Myosin ATPase pH 9.4 | Type I | light |
| | Type II | dark |
| c Myosin ATPase pH 4.6 | Type I | dark |
| | Tpye $II_A$ | light |
| | Type $II_B$ | moderate |
| d Myosin ATPase pH 4.3 | Type I | dark |
| | Tpye II | light |

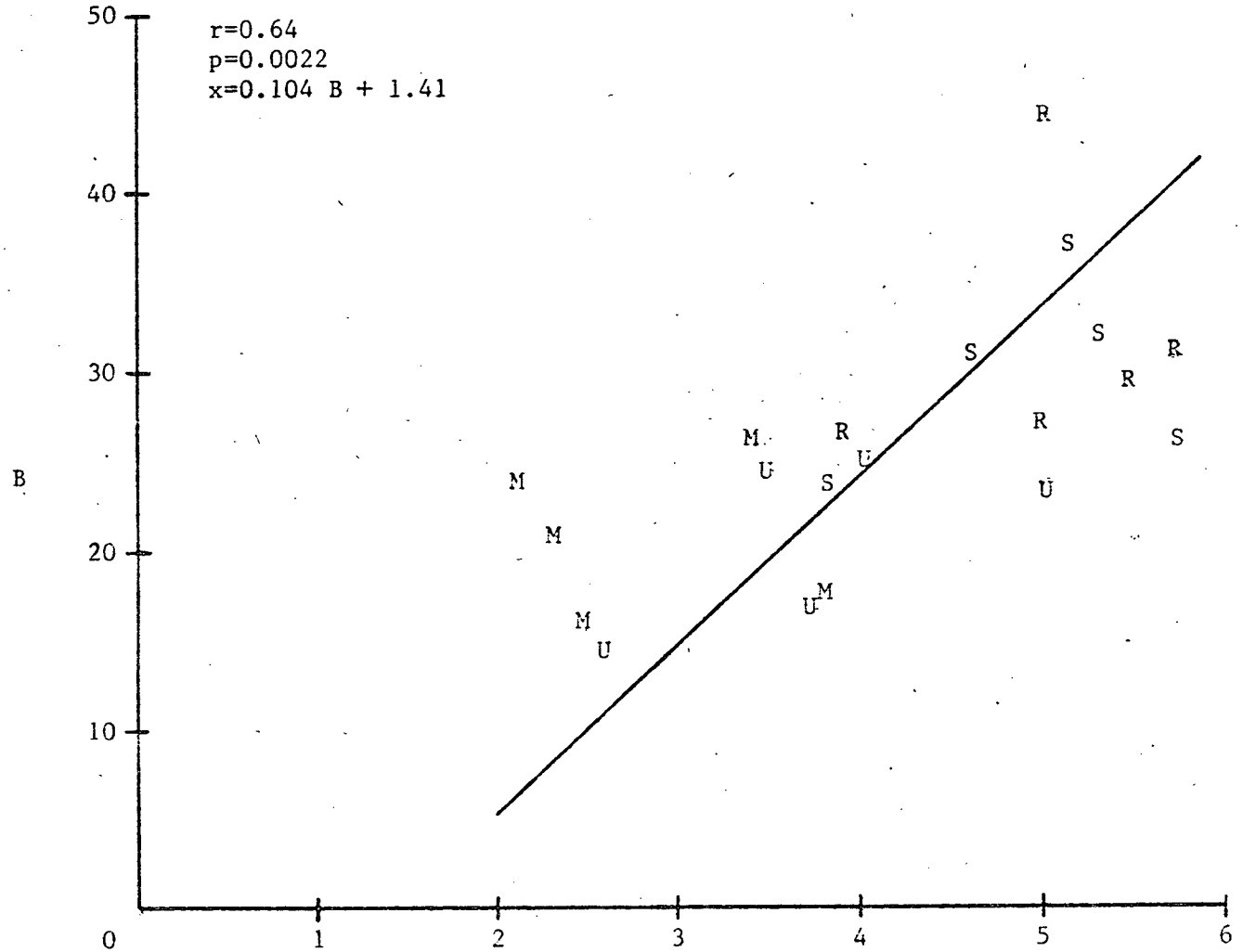

a
b
c
d

# Appendix E. Regression analyses

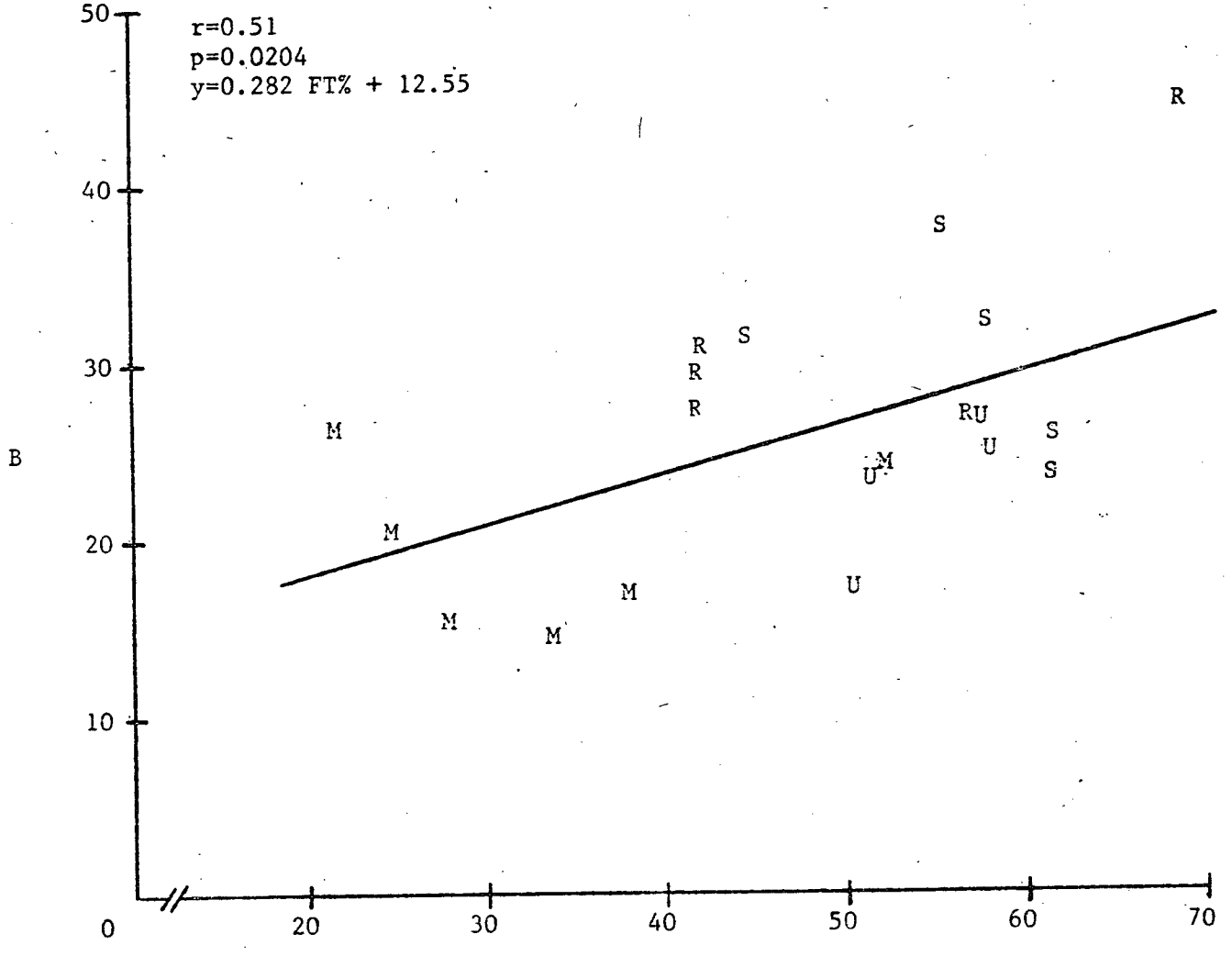
Buffer capacity (umol.$\bar{g}^{1}\bar{w}$/w.$p\bar{H}^{1}$) versus anaerobic performance.



Buffer capacity (umol.$g_w^{-1}$/w.$pH^{-1}$) versus carnosine concentration.



Buffer capacity (umol.$\bar{g}\bar{w}$/w.p$\bar{H}^{1}$) versus fast-twitch fiber percentage.



Fast-twitch fiber percentage versus carnosine concentration ($umol.g^{-1}$).