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Creatine Kinase Equilibrium and Lactate Content Compared with Muscle pH in Tissue Samples Obtained after Isometric Exercise

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Muscle biopsies taken from the musculus quadriceps femoris of man were analysed for pH, ATP, ADP, AMP, creatine phosphate, creatine, lactate and pyruvate. Biopsies were taken at rest, after circulatory occlusion and after isometric contraction. Muscle pH decreased from 7.09 at rest to 6.56 after isometric exercise to fatigue. Decrease in muscle pH was linearly related to accumulation of lactate plus pyruvate. An increase of 22μmol of lactate plus pyruvate per g of muscle resulted in a fall of 0.5 pH unit. The apparent equilibrium constant of the creatine kinase reaction (apparent K_{CK}) increased after isometric contraction and a linear relationship between log(apparent K_{CK}) and muscle pH was obtained. The low content of creatine phosphate in muscle after contraction as analysed from needle-biopsy samples is believed to be a consequence of an altered equilibrium state of the creatine kinase reaction. This in turn is attributed mainly to a change in intracellular pH.

It has long been known that lactate is produced and H^+ released in the muscle cells during hard exercise. The resultant acidification will exert a profound influence on metabolism. By affecting the charge of certain ionizable groups on enzymes and substrates involved, the rates of enzymic reactions will also be affected. In those reactions where H^+ is produced or consumed a decrease in intracellular pH will change the steady-state concentrations of the metabolites involved.

A close relationship between lactate and creatine phosphate contents of muscle after contraction has been found (R. C. Harris, E. Hultman & K. Sahlin, unpublished work). The relationship was believed to be a consequence of decreased intracellular pH, which is known to influence the equilibrium of the reaction catalysed by creatine kinase (Noda *et al.*, 1954) (ATP–creatine *N*-phosphotransferase, EC 2.7.3.2):

$$H^+ + \text{creatine phosphate} + \text{ADP} \rightleftarrows \text{creatine} + \text{ATP}$$

The equilibrium constant for this reaction K can (in simplified form) be expressed as:

$$K = \frac{[\text{creatine}][\text{ATP}]}{[\text{creatine phosphate}][\text{ADP}][H^+]} \quad (1)$$

This expression can be transformed to:

$$\text{pH} = -\log \frac{[\text{creatine}][\text{ATP}]}{[\text{creatine phosphate}][\text{ADP}]} + \log K \quad (2)$$

It was suggested by Rose (1968) that the creatine kinase equilibrium could be used for the estimation of the intracellular pH and this was also adopted by Siesjö and co-workers in a study of rat brain metabolism during arterial hypoxemia (MacMillan & Siesjö, 1972) and hypercapnia (Siesjö *et al.*, 1972). The values obtained for intracellular pH were in close agreement with those obtained simultaneously by other methods. From this it was concluded that the creatine kinase equilibrium, as determined from the molar proportions for the total tissue contents of creatine phosphate, creatine, ATP and ADP, under these circumstances could be used for the determination of intracellular pH.

In the present study similar measurements of the creatine kinase equilibrium and values for the lactate content in human muscle samples taken after isometric exercise were compared with muscle pH. The pH values were obtained from direct measurements in muscle homogenates with a micro electrode.

Experimental

Conditions for muscle biopsy

The present work is part of a project which has been approved by the Ethical Committee of Karolinska Institute, Stockholm, Sweden. This is an obligatory condition which must be fulfilled for grants to be obtained from the Swedish Medical Research Council.

Seven healthy male subjects from whom prior informed consent had been obtained participated in this study. Subjects, aged between 20 and 27 years, performed isometric contractions with the knee

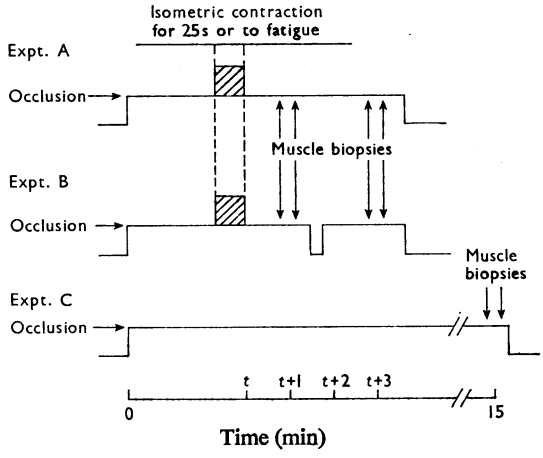

Fig. 1. *Experimental schedules*

Symbol t denotes the time at which the contraction was terminated; $t+1$ min and $t+3$ min the times at which muscle biopsies in type A and type B experiments were taken.

extensors, chiefly the musculus quadriceps femoris, in a chair of the type described by Tornvall (1963).

Training of the subjects and determination of their maximum voluntary contraction force held for 2s was performed at least 2 days before the experiment. The different types of experiments performed are explained in Fig. 1. In Expts. A and B, blood circulation to the leg was occluded 2min before contraction as described by Harris *et al.* (1975) by means of a tourniquet placed around the upper thigh and inflated to 240mmHg. Subjects contracted with $68.3 \pm 1.3\%$ of their maximum voluntary contraction force for either 25 ± 5s or to fatigue, i.e. 45 ± 10s (all values are means $\pm$ S.D. for $n = 14$, 5 and 9 respectively).

Two muscle biopsies (one intended for analysis of metabolites, the other for determination of muscle pH) were taken 55–80s after termination of contraction. In one series of experiments (A) two more biopsies were taken from the same leg 155–200s after contraction, whereafter the circulation was restored. In the other series (Expt. B) blood flow was restored at 90s after termination of contraction but occluded again at 115s. Thereafter two more biopsies were taken 170–200s after contraction.

In a third experiment (C) the circulation was occluded for 15min and at the end of this period two biopsies were taken. Subjects did not perform an isometric contraction.

All subjects participated in two experiments (one with each leg), either Expt. A or/and Expt. B. Four of the subjects participated 5–6 months after these experiments in Expt. C and in addition, biopsies were taken at rest from two of the subjects.

Methods

Muscle samples were taken from the lateral portion of the m. quadriceps femoris by the needle-biopsy technique (Bergström, 1962). After freezing the samples as fast as possible by plunging the biopsy needle into liquid freon maintained at its melting point (−150°C) (Harris *et al.*, 1974) they were stored in liquid N_2 until further treatment.

Muscle samples intended for metabolite determinations were freeze-dried, extracted and analysed for ATP, ADP, AMP, lactate, creatine phosphate and creatine as earlier described (Harris *et al.*, 1974). In most cases (when enough material was available) the mean value of duplicate measurements on the same extract was obtained.

All biochemicals and enzymes were obtained from Boehringer Mannheim G.m.b.H., 68 Mannheim 31, W. Germany, and Sigma Chemical Co., St. Louis, Mo., U.S.A.

The method used for pH determination is principally the same as that described by Hermansen & Osnes (1972). Muscle samples were weighed frozen (27–60mg) and homogenized at 0°C with 5ml per g wet weight of a solution containing 145mM-KCl and 10mM-NaCl in a motor-driven glass–Teflon Potter–Elvehjem-type homogenizer. pH measurements of the homogenates were performed at 38°C with an Astrup micro equipment (Radiometer electrodes, type G297/G2 and K497, connected to a Radiometer digital acid–base analyser, type PHM72), requiring a minimum sample volume of 20μl. pH was followed with a connected recorder during 30s and the value obtained after extrapolation back to zero time was used.

Several measurements could be performed on the same homogenate and all were completed within 15min after the start of homogenization. Generally the first measurement (unaccountably) gave a lower value than the others, and pH of the homogenate was obtained by excluding this and taking the mean of the others.

Calculations

Values for the apparent equilibrium constants for the creatine kinase reaction and the adenylate kinase (ATP–AMP phosphotransferase, EC 2.7.4.3) reaction have been derived from the molar ratios for the relevant metabolites estimated on the freeze-dried samples:

$$\text{Apparent } K_{CK} = \frac{[\text{creatine}][\text{ATP}]}{[\text{creatine phosphate}][\text{ADP}]}$$

$$\text{Apparent } K_{AK} = \frac{[\text{ATP}][\text{AMP}]}{[\text{ADP}]^2}$$

Table 1. *Metabolite contents (mmol/kg dry wt. of muscle) and pH of muscle at rest after circulatory occlusion and after isometric contraction at* 68% *of the maximum voluntary contraction force*

The time after the completion of contraction at which biopsy samples were taken is indicated in parentheses. Circulatory occlusion began 2min before the start of contraction and was continued, unless otherwise stated, until all muscle biopsies had been taken. Values are means with S.D. or range in parentheses.

Conditions	*n*	Creatine phosphate	Creatine	ATP	ADP	AMP	Lactate	Pyruvate	pH
Rest	2	86.1	48.3	25.8	3.15	0.16	3.08	0.30	7.09
No occlusion		(85.0, 87.3)	(42.0, 54.7)	(25.2, 26.3)	(3.10, 3.19)	(0.12, 0.19)	(3.00, 3.15)	(0.23, 0.36)	(7.08, 7.10)
Circulatory occlusion for 15min	4	61.5 (7.6)	71.0 (4.1)	26.4 (0.9)	3.23 (0.07)	0.15 (0.02)	5.68 ($n=3$) (4.34–6.42)	0.66 (0.33)	7.07 (0.06)
Contraction 25s (1min)	6	27.0 (4.5)	106.0 (14.9)	24.4 (1.8)	3.46 (0.26)	0.16 (0.03)	51.2 (11.1)	0.95 (0.51)	6.75 (0.05)
Contraction 25s (3min)	3	18.4 (13.0–24.7)	107.1 (101.8–115.8)	21.3 (17.9–23.3)	2.95 (2.44–3.49)	0.14 (0.08–0.18)	58.2 (55.3–63.8)	0.86 (0.73–1.01)	6.73 (6.66–6.79)
Contraction to fatigue (1min)	8	7.2 (2.2)	111.3 (18.1)	20.7 (3.5)	3.52 (0.62)	0.19 (0.05)	91.3 (17.5)	1.56 (0.64)	6.56 (0.07)
Contraction to fatigue (3min)	2	4.3 (3.1, 5.5)	114.3 (96.8, 131.8)	18.5 (15.0, 21.9)	3.88 (3.76, 4.00)	0.27 (0.23, 0.30)	106.5 (102.1, 110.8)	0.60 (0.32, 0.87)	6.56 (6.54, 6.58)
Contraction 25s and release of pressure (3min)	3	33.3 (27.1–38.3)	88.9 (73.6–108.6)	23.8 (21.2–26.7)	2.98 (2.67–3.35)	0.11 (0.09–0.13)	47.3 (37.7–52.2)	0.57 (0.40–0.68)	6.83 (6.81–6.84)
Contraction to fatigue and release of pressure (3min)	6	22.5 (8.0)	93.6 (16.3)	21.3 (2.9)	3.07 (0.31)	0.16 (0.09)	83.4 (12.1)	0.48 (0.24)	6.69 (0.10)

Table 2. *Apparent equilibrium constants for the adenylate kinase reaction and the creatine kinase reaction*

Values for molar ratios and apparent K values are calculated from the individual data of which the means are presented in Table 1. Values are means with S.D. or range in parentheses.

Condition	n	Creatine/ creatine phosphate	ATP/ADP	Apparent K_{CK}	log (apparent K_{CK})	Apparent K_{AK}
Rest	2	0.56	8.19	4.62	0.66	0.40
No occlusion		(0.48, 0.64)	(7.90, 8.48)	(3.79, 5.45)	(0.58, 0.74)	(0.33, 0.47)
Circulatory occlusion for 15 min	4	1.17	8.17	9.59	0.98	0.39
		(0.19)	(0.10)	(1.64)	(0.07)	(0.05)
Contraction 25 s (1 min)	6	4.07	7.07	28.2	1.44	0.33
		(1.03)	(0.65)	(5.3)	(0.09)	(0.09)
Contraction 25 s (3 min)	3	6.22	7.26	44.7	1.64	0.33
		(4.20–7.82)	(6.49–7.94)	(33.4–57.5)	(1.52–1.76)	(0.24–0.43)
Contraction to fatigue (1 min)	8	16.6	5.98	94.8	1.97	0.31
		(5.3)	(1.19)	(17.4)	(0.08)	(0.06)
Contraction to fatigue (3 min)	2	27.4	4.73	127	2.10	0.32
		(23.9, 31.0)	(3.98, 5.48)	(123, 131)	(2.09, 2.12)	(0.32, 0.32)
Contraction 25 s and release of pressure (3 min)	3	2.78	8.00	22.2	1.33	0.29
		(2.21–4.01)	(7.94–8.09)	(17.6–31.8)	(1.24–1.50)	(0.27–0.31)
Contraction to fatigue and release of pressure (3 min)	6	4.71	6.95	31.8	1.48	0.34
		(1.93)	(0.85)	(9.9)	(0.17)	(0.17)

With the use of these terms, in effect, it has been assumed that there is no cellular compartmentation and that the enzymes involved are sufficiently active to maintain these reactions close to equilibrium (cf. the Discussion section).

Results

It has been discussed that values on muscle creatine phosphate and creatine content as obtained from the analysis of biopsy samples are unexpectedly low and high respectively (McGilvery, 1975). This could be a consequence of the sampling technique, since the time between insertion of the needle and freezing of the sample is about 5 s. Nevertheless, the ratio of creatine to creatine phosphate in this study (0.56) was not higher than values reported for rat muscle where rapid freezing techniques were used, namely 0.53 (Hohorst *et al.*, 1962) and 0.82 (Stock *et al.*, 1973). Further, the variance of values between two different muscle biopsies taken from the same leg is low (Harris *et al.*, 1974), which would be unlikely if a rapid breakdown of creatine phosphate occurred during sampling. We therefore conclude that the values on creatine and creatine phosphate as analysed from needle-biopsy samples do represent the contents *in vivo*.

The metabolite contents and pH of the muscle biopsies taken at rest, after circulatory occlusion and after isometric contraction at 68% of maximum voluntary contraction force for 25 s or to fatigue are shown in Table 1.

The accumulation of lactate, decrease in creatine phosphate, and corresponding increase in creatine are all similar to results obtained by Bergström *et al.* (1971*b*), and this is a distinctive feature of anaerobic energy utilization. Muscle pH was decreased after contraction. Acidity increased with duration of work, reaching a mean value of pH 6.56 at fatigue. It should be noted that no recovery in either creatine phosphate or muscle pH occurred during the 2 min when circulation was occluded (work for 25 s or to fatigue, Expt. A). Instead, the results indicate a further decrease in creatine phosphate. However, when the pressure of the tourniquet was released for 20–25 s, permitting local blood flow, there was a significant return towards normality in the muscle content of creatine phosphate ($P<0.01$), creatine ($P<0.01$), lactate ($P<0.05$) and muscle pH ($P<0.01$). Statistical significances were tested with the Student's t test for paired values and included both values obtained after contraction for 25 s ($n=3$) and to fatigue ($n=6$).

The metabolite data used for the calculation of mean values in Table 1 have been used in Table 2 to calculate the apparent creatine kinase equilibrium constant and its constituent molar ratios creatine/creatine phosphate and ATP/ADP.

The apparent K_{CK} and the ratio creatine/creatine phosphate were considerably increased after contraction, whereas the ratio ATP/ADP was slightly decreased. It is evident that the ratio creatine/creatine phosphate, because of its higher variation, is the major factor responsible for the change in apparent K_{CK}, whereas ATP/ADP acts rather as a negative modulator at high creatine/creatine phosphate ratios.

As can be seen from Table 2 the apparent equilibrium constant of the adenylate kinase reaction did not change in a regular manner. When values for the apparent K_{AK} were collected into two groups accord-

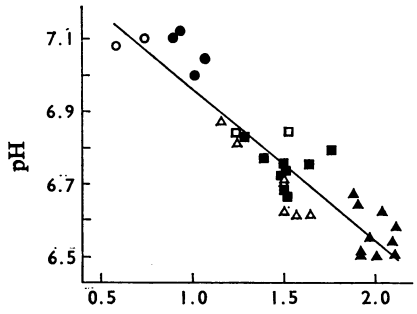

Fig. 2. *Relationship between pH and log ([creatine][ATP]/[creatine phosphate][ADP]) in muscle biopsies obtained at rest* (○), *after 15 min circulatory occlusion* (●), *after isometric contraction at 68% of the maximum voluntary contraction force sustained for 25 s* (■, □) *or to fatigue* (▲, △)

□, △, Values where sampling was preceded by 20–25 s blood flow, and ■, ▲, where no blood was allowed to flow after contraction and preceding sampling.

$$pH = -0.42 \log \left(\frac{[creatine][ATP]}{[creatine\ phosphate][ADP]} \right) + 7.38;$$
$$r = 0.92;\ n = 34$$

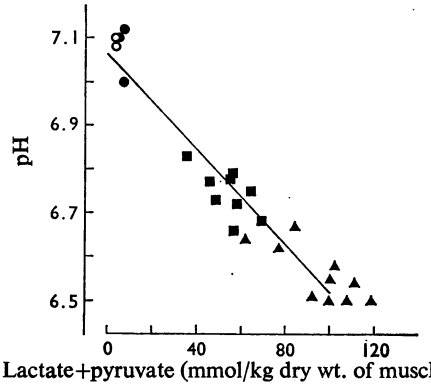

Fig. 3. *Relationship between pH and content of lactate+pyruvate in muscle biopsies obtained at rest* (○), *after 15 min circulatory occlusion* (●), *after isometric contraction at 68% of the maximum voluntary contraction force sustained for 25 s* (■) *or to fatigue* (▲)

$pH = -0.00532$ (lactate+pyruvate)$+7.06$; $r = 0.96$; $n = 24$

ing to simultaneously measured muscle pH, similar values were obtained: pH > 6.70, apparent $K_{AK} = 0.32 \pm 0.08$ (±S.D., $n = 18$); pH ≤ 6.70, apparent $K_{AK} = 0.34 \pm 0.10$ (±S.D., $n = 16$).

In Fig. 2 measurements of muscle pH have been compared with corresponding values of log (apparent K_{CK}). It should be noted that the two sets of data were obtained from two different biopsies taken from two different sites (about 2–5 cm in between). Even so log (apparent K_{CK}) was closely correlated to muscle pH ($r = 0.92$). The equation for the calculated linear-regression line [$pH = -0.42 \log$ (apparent K_{CK})$+7.38$] exhibits a slope factor different from that expected from eqn. (2).

When the local blood supply to the muscle is occluded by means of a tourniquet or by isometric work with a load exceeding 40% of the maximum voluntary contraction force (Edwards *et al.*, 1972) the muscle exists in an isolated state. Accumulation of lactate and pyruvate will result in an equimolar amount of H^+ being released. When muscle pH and lactate plus pyruvate contents were compared (Fig. 3) a linear relationship was obtained [$pH = -0.00532$ (lactate+pyruvate)$+7.06$; $r = 0.96$]. Values obtained from experiments where the return of local blood flow preceded sampling of muscle specimens (i.e. release of tourniquet occlusion) are excluded in this comparison, since recovery of lactate content and muscle pH probably occurs at different rates.

Discussion

Muscle pH

The concept intracellular pH usually denotes the average pH of the cell. As this is an abstraction not corresponding to an existing or calculable parameter (Siesjö & Pontén, 1966) the obtained value will be highly dependent on the analytical method used. In this study muscle pH was obtained by direct measurements of muscle homogenates with a glass electrode. By this method the values will be determined by the H^+ activity and the buffer capacity of all involved compartments, i.e. cytosol, mitochondria, blood, interstitial fluid etc. Of these the cytosol makes the major contribution in a muscle sample and measured pH represents therefore primarily this compartment. In an investigation by Hermansen & Osnes (1972) on human subjects it was found that muscle pH measured by a similar technique was decreased to 6.46 after dynamic exercise sustained to exhaustion, from a mean value of 6.98 at rest. We found a decrease of muscle pH from 7.09 at rest to 6.56 after isometric exercise sustained to fatigue. Considering the differences in experimental design and analytical methods the similarity of the decrease in pH is remarkable.

Creatine kinase equilibrium in relation to muscle pH

One of the main aims of the present study was to investigate the changes in log (apparent K_{CK}) in relation to muscle pH during isometric contraction.

Before muscle biopsies were taken for the analyses of pH and metabolites, 1 min equilibration with

occluded circulation was allowed (except when samples at rest were taken). This time was considered long enough to equilibrate pH between the different compartments (i.e. to obtain about the same pH gradients as at rest). The catalytic activity of creatine kinase is known to be very high in muscle tissue (Kuby *et al.*, 1954) and the reaction is probably at (or very near) to equilibrium in all muscle biopsies taken after exercise (R. C. Harris, E. Hultman & K. Sahlin, unpublished work).

In a previous report it was found that circulatory occlusion increased apparent K_{CK} from a value of 4.9 at rest to 8.7 after 15 min circulatory occlusion (Harris *et al.*, 1975). This change was not believed to be an effect of altered intracellular pH but rather of a change in that portion of ADP and ATP which is available to creatine kinase. Our results in this study are in accordance with this hypothesis. Circulatory occlusion for 15 min caused an increase of apparent K_{CK} from 4.62 at rest to 9.59, whereas no major change in muscle pH occurred. It must, however, be noted that the results in this respect are somewhat uncertain because of the few observations made. Values obtained at rest and after 15 min occlusion were both therefore included in the linear-regression analysis between muscle pH and log(apparent K_{CK}). The results indicate a linear relationship between muscle pH and log (apparent K_{CK}). The equation obtained from linear-regression analysis of the data was, however, different from the theoretical value derived from eqn. (1).

$$\text{Theoretical: } pH = -\log(\text{apparent } K_{CK}) + \log K$$

$$\text{Obtained: } pH = -0.42 \log(\text{apparent } K_{CK}) + 7.38$$

This means that the measured decrease in muscle pH was lower than that expected from the changes in log(apparent K_{CK}). Some possible explanations for this discrepancy will be discussed below.

(*a*) *Mg^{2+}–nucleotide complexes.* As in most reactions where ATP and ADP participate, the Mg^{2+} complex is the reactive species. Creatine kinase is believed to catalyse the following reaction (Nihei *et al.*, 1961; Watts, 1973):

$$x \cdot H^+ + (MgADP)^- + (\text{creatine phosphate})^2 \rightleftarrows (MgATP)^{2-} + \text{creatine} \quad (3)$$

where x ($\leqslant 1$) denotes the extent to which H^+ is involved in the reaction.

In Fig. 4 the main reactions involving ATP, ADP, Mg^{2+}, K^+ and H^+ which occur under intracellular conditions are shown. $HATP^{3-}$ and $HADP^{2-}$ both have dissociation constants in the intracellular pH region $pK_{aATP} = 6.9$; $pK_{aADP} = 6.7$ (Kuby & Noltman, 1962). When ATP and ADP are fully ionized (i.e. pH > 8) one H^+ ion will be taken up (x = 1) for each molecule of creatine phosphate broken down (Kuby & Noltman, 1962). However, under intracellular conditions where the pH is approx. 6.5–7, the stoicheiometry of the creatine kinase reaction will depend on the free concentration of Mg^{2+}, intracellular pH, pK_{aATP}, pK_{aADP}, and the dissociation constants of the Mg and K complexes of the nucleotides. When Kuby & Noltman (1962) considered the different complexes involved and reviewed the data of Noda *et al.* (1954), they calculated x to be in the range 0.5–0.95 (free $Mg^{2+} = 0.026$–16.0 mM; pH 7.4–9.0). In the present study it was found that $1/x = 0.42$, i.e. $x = 2.38$, under intracellular conditions [free $Mg^{2+} \simeq 0.5$–1.0 mM (Veloso *et al.*, 1973); pH 6.5–7.1].

(*b*) *Unavailable pools of metabolites involved in the creatine kinase reaction.* When equilibrium constants are calculated from the total tissue content of reactants it is assumed that these are proportional to the concentration at the enzymic site. If some intracellular compartmentalization of the four metabolites (creatine, creatine phosphate, ATP and ADP) exists, calculated values of apparent K_{CK} will be in error and so will the relationship of log(apparent K_{CK}) towards pH. The extent of compartmentalization and therefore also the error in the calculated apparent K_{CK} are not known.

It is believed that a major fraction of the muscle ADP content is protein-bound. From theoretical considerations of equilibrium constants and of the

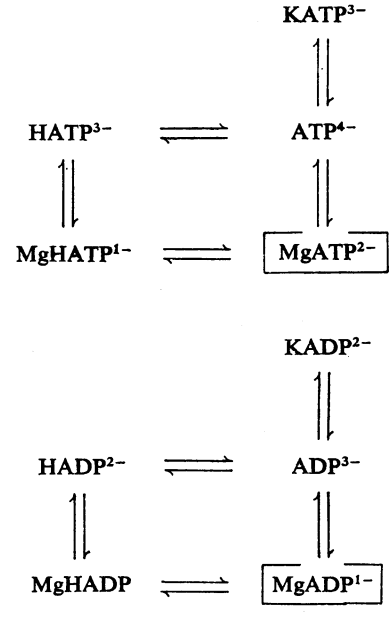

Fig. 4. *Different forms of ATP and ADP occurring under intracellular conditions when complexed with Mg^{2+} and K^+*

The species in boxes are involved in the creatine kinase reaction and constitute the major amounts.

role of ADP in cellular regulation it has been calculated that about 90% of the ADP content must be protein-bound and unavailable to creatine kinase (Carlsson & Siger, 1959; McGilvery, 1975). Experimental attempts to estimate the bound portion give a value of about 50% (Seraydarian *et al.*, 1962; Valin & Charpentier, 1969). Though the true amount is uncertain, protein binding of ADP will increase the importance of the observed change in the ATP/ADP ratio relative to creatine/creatine phosphate in log (apparent K_{CK}). This will tend to increase the value for observed x.

(*c*) *Changes in free* Mg^{2+}. The apparent equilibrium constant for the creatine kinase reaction depends on the free intracellular Mg^{2+} concentration (Rose, 1968). Changes in free Mg^{2+} due to altered contents of complex-forming metabolites would affect apparent K_{CK}. However, there was no change in the apparent equilibrium constant of the adenylate kinase reaction, and as this constant also depends on free Mg^{2+} major changes in the latter are unlikely.

General comment. We have put forward the hypothesis that muscle creatine phosphate content after exercise obtained from the analysis of needle-biopsy samples is a reflexion of the equilibrium state of the creatine kinase reaction and that the major factor determining the change in equilibrium state from that at rest is intracellular pH (R. C. Harris, E. Hultman & K. Sahlin, unpublished work). The following observations from this study support this hypothesis. (1) A linear relationship between values of log(apparent K_{CK}) and muscle pH before and after isometric exercise was obtained. (2) No recovery after isometric exercise (work for 25s or to fatigue) of either creatine phosphate content or muscle pH occurred when the blood circulation was occluded. (3) When the circulation after isometric exercise was restored both creatine phosphate content and muscle pH recovered concomitantly.

Lactate content and muscle pH

When the local blood supply is occluded the muscle could be regarded as a closed system that does not exchange matter with the surroundings. Under the relatively short time-periods studied transport via diffusion can be considered negligible compared with the metabolic changes obtained. When the muscle contracts isometrically, lactate and pyruvate are accumulated in an amount proportional to the sustained force and duration (Bergström *et al.*, 1971*b*; Ahlborg *et al.*, 1972). These changes will result in an equimolar release of H^+, which will decrease the intracellular pH. The extent of this decrease will be modified by (*a*) a dynamic contribution that corresponds to the amount of acidic and basic equivalents formed through the metabolic production and consumption of acids and bases other than lactate–pyruvate, and (*b*) the static buffer capacity of muscle, which equals the buffer capacity of resting muscle if no enzymic activity occurred.

The major contribution to the dynamic portion will come from the rearrangement of phosphoryl groups, i.e. decrease of creatine phosphate and increase principally of P_i, glucose 6-phosphate, fructose 6-phosphate and glycerol 1-phosphate. Because the dissociation constant of the phosphoryl group in creatine phosphate ($pK_{a2} = 4.5$) is higher than in the other compounds mentioned ($pK_{a2} = 6$–7) the net result will be a net uptake of H^+. The amount of H^+ released due to accumulation of malate and citrate in muscle tissue during isometric contraction is small compared with lactate, 1% Bergström *et al.*, 1971*b*) and <1% (R. C. Harris, unpublished work) respectively. The same will probably hold for the other tricarboxylic cycle acids. The amount of H^+ released due to formation of HCO_3^- can only at the most equal the local muscle store of O_2 trapped at the onset of contraction. This has been estimated to be 2mmol of O_2/kg dry wt. of muscle (Harris *et al.*, 1975). The amount of H^+ so released will correspond to about 2% of that from lactate accumulation at fatigue.

It has previously been shown *in vitro* that decrease in muscle pH is linearly related to the accumulation of lactate. In rabbit muscle stored at different temperatures after death Bate-Smith & Bendall (1949) obtained a ratio Δlactate/$-\Delta$pH of 65mmol of lactate/pH unit per kg of muscle (pH7.3–5.7). In ox skeletal muscle stored in an atmosphere of N_2 Newbold & Scopes (1967) found a value of 60mmol of lactate/pH unit per kg of muscle (pH6.8–5.7). It has been shown in the present paper that accumulation of lactate is linearly related to the fall in muscle pH during isometric contraction *in vivo*. Assuming a mean water content of 3.3ml/4.3g of muscle (Bergström *et al.*, 1971*a*) a value of 43.7mmol of lactate+pyruvate/pH unit per kg of muscle is obtained. The higher buffer capacity obtained in the studies *in vitro* is probably due to slow buffering processes which occur with the long experimental times used (about 5h to reach pH6.5), among which loss of CO_2 from tissue is the most conspicuous. When titrating muscle breis in which all labile phosphorus had been converted into P_i and from which all CO_2 had probably escaped, Bate-Smith (1938) obtained buffer capacities with a range of 48–63mmol/pH unit per kg of muscle (pH6–7) within different animal species. With the simplified closed system used in the present study for investigations of muscle metabolism, it is tempting to regard the lactate accumulation as an internal titrating system operating *in situ*.

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