to normal rats deprived of food for 6 h, and the stomach contents were collected after
15 min. About 10–12% of the radioactivity of the dose administered was found in the
stomach contents, which, when passed through the Sepharose 6B column, gave 65–75% of
the radioactivity in the lipid peak fractions (4–10) collected just after the void volume,
as shown in Fig. 1. The position of this peak is identical with that observed in the prepara-
tion and separation of liposomally entrapped insulin from free insulin, and it corresponds
to the liposomes. We have concluded, therefore, that most of the insulin in still entrapped
within liposomes in the stomach contents so examined. The remaining 25–35% of the
radioactivity found in the fractions between 18 and 30 (Fig. 1) we assume to be hydro-
lysed products of the 125I-labelled insulin, as the free 125I-labelled insulin is normally
eluted between fractions 13 and 20. Moreover, the radioactivity in fractions 18 to 30
(Fig. 1) is not precipitated with 10% (w/v) trichloroacetic acid. Most of this radio-
activity probably originates from the 125I-labelled insulin associated with the outside
surface of the liposomes, which was not truly entrapped during the preparation of
insulin-containing liposomes or from degraded liposomes. In both cases such insulin
would be readily hydrolysed by the proteolytic enzymes of the stomach.

To investigate the action of intestinal enzymes on liposomally entrapped 125I-labelled
insulin and to compare it with the free hormone, incubations with rat intestinal washings
were carried out. Although extensive degradation (as assessed by loss of trichloroacetic
acid precipitability) of free insulin occurs (60% in 5 min), as would be expected, very
little degradation of liposomally entrapped insulin occurred under similar conditions
(15% in 25 min). Further, the rapid degradation of the free insulin in the presence of rat
intestinal washings was inhibited in the presence of liposomes containing no insulin. A
similar effect was observed when purified trypsin was incubated with liposomally
associated or entrapped insulin; here again, the degradation of the hormone was much
less than that obtained by incubation without liposomes.

Thus these preliminary studies show that some at least of the liposomes given by the
oral route to rats remain intact in the stomach, and thus protect the entrapped protein
from proteolytic digestion by gastric enzymes. Proteolytic activity of the intestine
appears to be inhibited by liposomes.

We gratefully acknowledge the British Diabetic Association for financial support.


Post-Mortem High-Energy Phosphate and Glycolytic Changes in Two
Skeletal Muscles of the Ox

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The concentration of ATP in skeletal muscle falls after death as a result of the continuing
activity of intracellular ATP hydrolases and the virtual absence of oxidative resynthesis
of this high-energy phosphate under the anaerobic conditions that prevail in muscle post mortem. The progressive loss of ATP causes the formation of actin–myosin cross-
linkages in the absence of the concentrations of Ca2+ that are required for this inter-
action to occur under physiological conditions. The formation of actomyosin leads to
the loss of muscle extensibility known as rigor mortis. The rate of onset of rigor mortis
depends on the initial concentrations of ATP, creatine phosphate and glycogen in

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muscle at the time of death and on the temperature of the muscle. Considerable variations in the rate of rigor-mortis onset have been reported between species, individual animals within a species, different muscles and myofibre types (cf. Bendall, 1973). In the present work, post-mortem changes in two muscles of the ox were compared.

Psoas major and longissimus dorsi muscles were removed from 18 cattle at approx. 15 min after slaughter by stunning and exsanguination at the laboratory. Samples were immediately taken for analysis and the remainder of the tissue placed at 37°C under moist N₂. Further samples were taken at hourly intervals up to 6 h. After this time, the muscles were placed at 2°C and final samples taken at 24 h. Specimens of tissue were frozen in liquid N₂ and the frozen material was subsequently extracted as previously described (McLoughlin & Mothersill, 1976). ATP and creatine phosphate were determined by a procedure based on that described by Lamprecht & Stein (1963) and involving the use of glucose 6-phosphate dehydrogenase, hexokinase and creatine kinase in a sequence of NADP-linked reactions. Lactate was assayed with lactate dehydrogenase and NAD (Hohorst, 1963), and glycogen was determined by means of the anthrone reagent (Seifter et al., 1950). Muscle pH was measured on suspensions of tissue (20%, w/v) in iodoacetate (5 mM, neutralized to pH 7.0) with a Radiometer 26 pH-meter with scale expander. Spectrophotometric measurements were made with a Pye-Unicam SP. 8000 spectrophotometer.

The initial concentration of creatine phosphate was significantly \((P < 0.001)\) higher in the longissimus dorsi muscle \([7.5 \pm 1.0 \text{ (s.e.m.) } \mu \text{mol/g}]\) than in the psoas major muscle \((2.1 \pm 0.1 \mu \text{mol/g})\) (Fig. 1). The initial concentrations of ATP (longissimus dorsi, 5.6 \pm 0.2; psoas major, 4.4 \pm 0.5 \mu \text{mol/g}) were also significantly \((P < 0.05)\) different (Fig. 2). The [ATP]-time curves did not show a delay phase where resynthesis just balanced hydrolysis. The half-life of ATP was 3.5 h in psoas major and 7 h in longissimus dorsi. The residual concentrations of ATP were similar in both muscles after 24 h. The initial concentration of lactate was significantly \((P < 0.001)\) higher in psoas major \((49.8 \pm 4.8 \mu \text{mol/g})\) than in longissimus dorsi \((29.4 \pm 2.4 \mu \text{mol/g})\). Lactate was formed at similar rates in both muscles over the subsequent 6 h \(\text{in vitro}\) (psoas major, 12.6; longissimus dorsi, 15.0 \mu \text{mol/h per g}). The pH of both muscles fell linearly with respect to time over 6 h. The initial pH of longissimus dorsi \((6.85 \pm 0.03)\) was significantly higher \((P < 0.001)\) than that of psoas major \((6.48 \pm 0.03)\). The subsequent rates of pH change and the pH values at 24 h were not significantly different. The calculated pH of 7.2 at zero [lactate] was obtained by extrapolation of the linear pH-[lactate] relationship. The relationship between pH and [creatine phosphate] was exponential, but the direct relationship between log [creatine phosphate] and pH gave a calculated concentration of

![Fig. 1. Changes in creatine phosphate concentration in psoas major muscle (○) and in longissimus dorsi muscle (●) at 37°C and under N₂. Mean values for 18 animals are shown.](image)
Fig. 2. Changes in ATP concentration in psoas major muscle (o) and in longissimus dorsi muscle (●) at 37°C under N₂.

Mean values for 18 animals are shown.

22.8 µmol of creatine phosphate/g at zero [lactate], i.e., in resting muscle. The buffering capacity expressed as ΔpH/Δ[lactate] per g of tissue was 67.5 and 61.2 for longissimus dorsi and psoas major respectively. The rate of glycogenolysis was similar in both muscles (0.8 mg/h per g) and extrapolation of the pH–[glycogen] curves gave a calculated concentration of 10.5 mg of glycogen/g of tissue at pH 7.2 for both muscles.

The total high-energy phosphate (~P) turnover during 6h in vitro was calculated from the equation:

\[ \Delta \sim P = \Delta[\text{creatine phosphate}] + 2(\Delta[\text{ATP}]) - 1.5(\Delta[\text{lactate}]) \]

The turnover rates were not significantly different (longissimus dorsi, 14.2; psoas major, 14.9 µmol of ~P/h per g) between the initial (15 min) and 6h samples. The theoretical high-energy-phosphate turnover for the period between slaughter and removal of muscle at 15 min post mortem was also calculated. To do this, the [creatine phosphate] at zero [lactate] (22.8 µmol/g) was used and the initial [ATP] was assumed to be 5.6 µmol/g in both muscles. The results of the calculation indicated that the turnover rate of high-energy phosphate at and just after death was substantially higher in psoas major (286 µmol/h per g) than in longissimus dorsi (172 µmol/h per g).

The results show that psoas major responded to slaughter with a much greater burst of high-energy-phosphate splitting and anaerobic glycolysis than did longissimus dorsi. The major differences between the patterns of post-mortem change between the two muscles appear to be due to the extent of motor stimulation of the tissues at death. Heffron & Dreyer (1975) found similar differences between these two muscles in the Boer goat. The initial values for ATP, creatine phosphate and pH, and the subsequent rates of changes in these parameters reported by Heffron & Dreyer (1975), were similar to those described in the present paper. The rates of high-energy-phosphate and glycolytic change post mortem in these two ruminant species were considerably slower than those found in the pig. Pig muscles, particularly the psoas major, longissimus dorsi and semitendinosus frequently show extremely rapid rates of rigor-mortis onset after slaughter without prior tranquillization (Tarrant et al., 1972).


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Muscle Calcium Accumulation during Magnesium Deficiency in the Rat

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Experimental Mg²⁺ deficiency in the rat is usually associated with disturbances in Ca²⁺ metabolism manifested by hypercalcaemia, nephrocalcinosis and increased tissue Ca²⁺ (Aikawa, 1971). Loss of cellular K⁺ is also a consistent finding during experimental Mg²⁺ deficiency (Whang & Welt, 1963; Ryan et al., 1969, 1973). Hypercalcaemia itself has been shown to be associated with renal K⁺ wasting and muscle K⁺ deficiency (Ferris et al., 1961). The membranes of many cells exhibit a K⁺-selective permeability mechanism with a gating process controlled by the concentration of Ca²⁺ in the cytosol (Lew & Ferreira, 1976). Accordingly, it might be suggested that the cellular K⁺ loss accompanying Mg²⁺ deficiency may not be due to lack of Mg²⁺ itself, but rather to associated abnormalities, such as hypercalcaemia and increased tissue Ca²⁺. We decided to investigate Ca²⁺ metabolism in soleus muscle (which consists predominantly of red fibres), superficial white gastrocnemius muscle (which consists predominantly of white fibres) and cardiac muscle of both control and Mg²⁺-deficient rats.

Male Wistar rats (120g) were housed individually and given a Mg²⁺-deficient diet. Controls were pair-fed on a Mg²⁺-supplemented diet. After 36–41 days on the diet, animals were injected intravenously with ⁴⁵Ca (5μCi/100g body wt.), placed in individual metabolism cages and killed 24h later. Samples of plasma, urine and muscles were taken for analysis.

Mg²⁺-deficient animals had hypercalcaemia (P < 0.005) and showed decreased urinary output of Ca²⁺ (P < 0.005) and ⁴⁵Ca (P < 0.005). Mg²⁺ deficiency resulted in increased Ca²⁺ concentration in all three muscle types studied, but by far the greatest proportional increase over control values was detected in cardiac muscle (P < 0.001). ⁴⁵Ca accumulation (expressed as d.p.m./g dry wt.) was unaltered by Mg²⁺ deficiency in either soleus or gastrocnemius muscle, but was slightly increased in cardiac muscle (P < 0.05). The specific radioactivity of ⁴⁵Ca was decreased in both gastrocnemius and cardiac muscle during Mg²⁺ deficiency. The relative specific radioactivity of ⁴⁵Ca was significantly decreased during Mg²⁺ deficiency in cardiac muscle (P < 0.001).

The results indicate that Mg²⁺ deficiency in the rat causes much greater calcification in cardiac muscle than in either red or white skeletal-muscle fibres. Furthermore the Ca deposited in the cardiac muscle during Mg²⁺ deficiency does not exchange with injected ⁴⁵Ca over the 24h period studied here. The most likely sites of the Ca deposits are the mitochondria, as suggested by published morphological studies (Heggveit et al., 1964).

We thank the Medical Research Council of Ireland for the award of a grant.

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