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Studies on the post mortem glycolytic changes in the M. semimembranosus of lamb and beef.

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INTRODUCTION

The overall programme being undertaken in this laboratory in connection with the prepackaging of fresh meats includes research into the post mortem changes which contribute to ultimate overall meat quality. One aspect of this research involves the study of the biochemical changes which occur within the initial 48 hours post mortem, with emphasis on the glycolytic changes.

The present report deals with the post mortem biochemistry of 12 English lambs, and highlights some interesting features of lamb muscle during its conversion to meat. Changes in the glycolytic intermediates, as well as adenosine triphosphate and its degradation products, are compared with similar data obtained on beef muscle post mortem. Variations between shackled and unshackled legs are also discussed.

METHODS

Animals

Twelve Dorset Down × Devon Close Wool lambs (live weights ~ 80 lbs.) were used in this experiment. The age of the animals was 8 months.

Slaughter and Cooling

The lambs were stunned by captive bolt and killed by severing the throat. The dressed carcasses were then placed in a cold air tunnel for one hour and then placed in a holding room at 3 ° for the subsequent 47 hours.

Sampling

For six of the animals samples were excised from the M. semimembranosus of the left (unshackled) leg, whilst the remainder were sampled from the M. semimembranosus of the right (shackled) leg. Samples (10 g.) were excised at recorded times between ½ and 48 hours post mortem, frozen in

liquid nitrogen, placed in a polythene bag and stored below $-40°$ until required for biochemical analyses.

pH

pH values were recorded on a macerate of 1 g. of *unfrozen* tissue in 7 ml. of 0.005 M. sodium iodoacetate solution.

Extraction of Tissue

1 g. of the frozen sample was macerated in cold perchloric acid solution, filtered and then neutralised with 5 M.K_2CO_3 to pH 6.5 (final volume 16 ml.). This solution was used for all enzymic analyses.

Chemical Methods (glycogen)

Glycogen was extracted from 1 g. of frozen tissue as described by Hawk (1). The precipitate obtained was dissolved in 50 ml. of water and 0.5 ml. of this solution used in the Anthrone reaction for glucose determination (2).

Enzymic Methods

Concentrations of ATP*, ADP, AMP, CP, NAD and all the glycolytic intermediates including lactate, GP and glycerol were determined by methods described in Bergmeyer's »Methods in Enzymatic Analysis» (3).

* The following abbreviations have been used throughout this report:

ATP, ADP and AMP = Adenosine tri-, di- and mono-phosphates
G-1-P, G-6-P = glucose 1 and 6 phosphate
CP = creatine phosphate
NAD = nicotinamide adenine dinucleotide
Gly-3-P = glyceraldehyde 3 phosphate
2-, 3-PGA = 2-, 3-phosphoglyceric acid
F1: 6DP = fructose 1: 6 diphosphate
DHAP = dihydroxyacetone phosphate
a GP = glycerol-1-phosphate
PEP = phosphoenol pyruvate.

RESULTS

All results for chemical analyses are expressed as uM/g. of wet tissue. Hours quoted are from the time of slaughter. The words 'initial' and 'ultimate' refer to ½ and 48 hour samples respectively. Values quoted represent the averages for six animals.

pH and Lactate

pH values and lactate concentrations are presented in Fig. I.

A large difference of 12 uM/g. was observed in the initial lactate con-

centrations between the left and right legs. Corresponding pH values were also different, 6.70 (S. D. ±0.14) in the left legs compared with 6.93 (S. D. ±.06) in the right legs.

Ultimate pH values and lactate concentrations were 5.84 (S. D. ± .09) and 72.4 uM/g. (S. D. ±11.7) respectively for the left legs, and 5.93 (S. D. ±.06) and 65.8 (S. D. ±10.0) for the right legs.

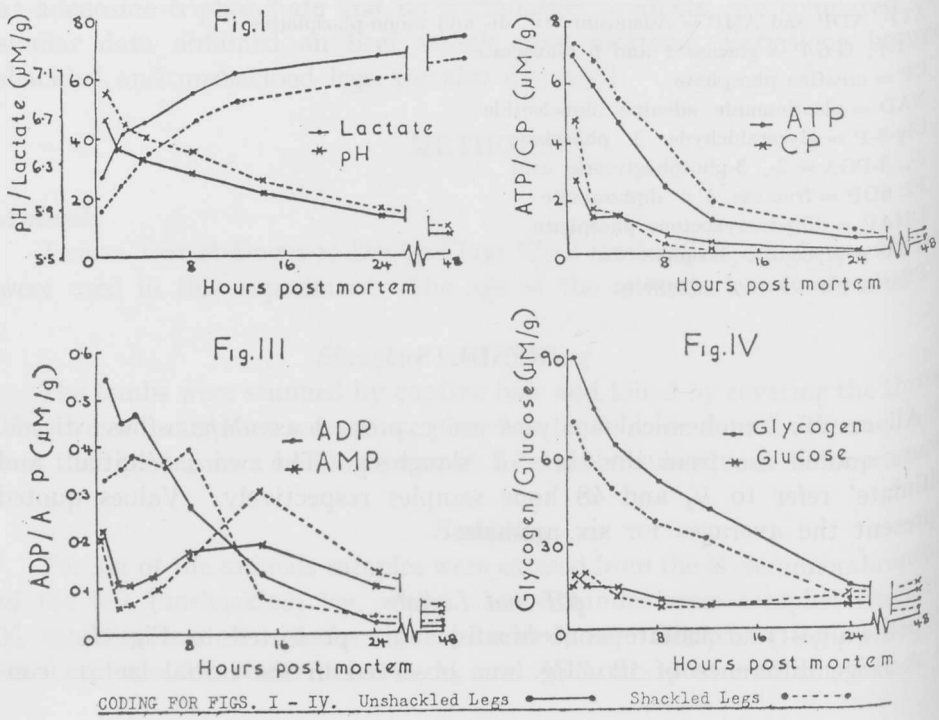
ATP and CP

Differences in ATP initially were only marginal (Fig. II): in the unshackled legs 6.67 uM/g. (S. D. ±0.62) and in the shackled legs 7.00 uM/g. (S. D. ±0.58). Both groups of legs then showed a rapid decline in triphosphate and within 24 hours they contained approximately the same levels. Subsequent rates of dephosphorylation were very similar giving ultimate values of 0.20 uM/g. in both groups.

Initial levels of CP were higher in the shackled legs than in the unshackled legs, the respective values observed being 4.6 uM/g. and 2.9 uM/g. (Fig. II). By 14 hours less than 0.5 uM/g. of CP was present in both groups of legs.

AMP and ADP

Concentrations of both of these nucleotides were small compared with that of ATP (Fig. III).



Variations in the concentration of AMP were very small, with a general rise between 4—14 hours, followed by a slow decline up to 48 hours. ADP declined throughout the whole experimental period in the unschackled legs, whereas in the shackled legs the concentration rose slightly from the initial level to 0.4 uM/g. and then gradually decreased.

Ultimate values of both nucleotides were below 0.1 uM/g.

NAD (oxidised + reduced forms)

Initial concentrations were very similar in samples from both the left and right legs (0.64 uM/g. and 0.67 uM/g. respectively). For 8 hours NAD was maintained above 0.60 uM/g. in both groups and then steadily declined to 0.20 uM/g. ultimately.

Glucose and Glycogen

Concentrations of glucose, initially 19.0 uM/g. for the unshackled legs and 17.4 uM/g. for the shackled legs (Fig. IV), fell within 24 hours to 10.1 uM/g. and 9.2 uM/g. respectively. Subsequent changes were slight.

Initial glycogen levels were different in the two groups, values for the unshackled and shackled legs amounting to 68.0 uM/g. (S. D. $\pm$22.1) and 89.0 uM/g. (S. D. $\pm$15.5) respectively. Both groups then showed a marked decline to ultimate concentrations of 2.0—4.0 uM/g. Thus the total glycogen hydrolysed was 66 uM/g. for the unshackled legs compared with 85 uM/g. for the shackled legs.

G-6-P, G-1-P and F-6-P

A large difference in the half-hour values for G-6-P in the two groups was observed. Thus for the unshackled legs the concentration was 2.9 uM/g. (S. D. $\pm$0.6) and for the shackled legs 1.6 uM/g. (S. D. $\pm$0.4). During the next 7½ hours the concentration/time pattern for this ester was different in each group, the unshackled legs showing a decline followed by a sharp rise, whereas in the shackled legs there was little change from the initial level. Both groups reached peak concentrations by 14 hours (5.2—5.7 uM/g.). Ultimate concentrations were 5.2 uM/g. and 4.9 uM/g. in the unshackled and shackled legs respectively.

Initial levels of G-1-P amounted to only 0.1 uM/g., increasing to a maximum value of 0.4 uM/g. in the unshackled legs, and to 0.5 uM/g. in the shackled legs. Ultimate values were between 0.2 and 0.3 uM/g.

Fructose-6-Phosphate levels were initially 0.6 uM/g. and 0.4 uM/g. for the unshackled and shackled legs respectively. A slight decline then occurred in both groups, after which the levels rose to a maximum of 0.9 uM/g. in the unshackled legs, and 0.6 uM/g. in the shackled legs. Ultimate concentrations recorded were 0.6 uM/g. in both groups.

F1: 6DP, DHAP and Gly-3-P

Data presented for F1: 6DP, DHAP and Gly-3-P apply equallly to both groups of legs.

Concentrations of F1: 6DP were very low throughout, declining from the maximum at 6 hours, 0.5 ± 0.1 uM/g, to 0.2 uM/g. ultimately.

DHAP started off at a relatively high value of 1.1 uM/g. and rose to 1.5 uM/g. within the first 2 hours. This concentration was not maintained however and a steady decline was observed from thereon to give ultimate concentrations between 0.5 and 0.6 uM/g.

Glyceraldehyde-3-phosphate concentrations were fairly steady throughout, varying by only $\pm$ 0.2 uM/g. from the 0.6 uM/g. found initially.

a GP and Glycerol

A difference of 1.0 uM/g. was observed in the initial concentrations of *a* GP in the unshackled and shackled legs, but by 8 hours both groups contained 1.5 uM/g. This level then diminished to give ultimate values of $\sim$ 0.4 uM/g.

Glycerol was only analysed in three legs from each group. The initial concentrations, 1.3 uM/g., increased to 3.5 uM/g. in the unshackled legs and to 2.3 uM/g. in the shackled legs by 5 hours, and to ultimate levels of 5.8 and 2.5 uM/g. respectively.

2-PGA, 3-PGA, PEP and Pyruvate

Pyruvate concentrations varied within the range 0.3 ± 0.1 uM/g. whilst the other three intermediates were always found at < 0.1 uM/g.

DISCUSSION

It would appear that the overall rates of pH fall and of lactate production in the M. semimembranosus of lamb were very similar to those observed in beef muscles (4) (5). The initial pH value was higher in the shackled legs and this was reflected in a lower initial lactate concentration of 16 uM/g. in these legs compared to 28 uM/g. in the unshackled legs. As differences of this order were maintained throughout the 48 hours, then clearly the rates of lactate production in this period were unaffected by the initial difference in lactate levels. Similar observations have been made in beef semimembranosus and rabbit L. dorsi (5).

The buffering capacity of both left and right legs was 44 uM lactate/pH/g. This agrees closely with that recorded for beef M. semimembranosus (6) but is lower than the value quoted for M. sternomandibularis of beef (7).

Initial concentrations and patterns of degradation of ATP were also not dissimilar to those reported for beef muscle (7) (8). Initial differences in

concentration between left and right legs were marginal although the subsequent rate of fall was more rapid in the unshackled legs. Assuming that the onset of rigor occurred when $^3/_4$ of the initial concentration of ATP had disappeared (9), then the shackled legs reached this stage 3 ½ hours later than the unshackled legs. Under conditions of rapid chilling this delay could markedly influence the degree of cold shortening (10) (11).

By 14 hours, triphosphate levels were almost identical in both groups. The ultimate values observed (0.15 uM/g.) were lower than those recorded in beef M. semimembranosus (1 uM/g.) were lower than those recorded in beef M. semimembranosus (1 uM/g.) at the same time post mortem.

The total nucleotide concentration (ATP + ADP + AMP) observed for the unshackled legs was 7.46 uM/g., and for the shackled legs 7.54 uM/g. These values are very close to those previously quoted for beef muscle (6) (7). Concentrations of ADP were small throughout and never rose above their intial values (Fig. III) although ATP was being rapidly lost within the first 12 hours. Similarly levels of AMP never rose above a concentration of 0.3 uM/g. and it must be assumed that any adenylic acid produced from ADP was swiftly deaminated to IMP (12) (6).

As in beef muscle the role played by CP in rephosphorylating ADP one hour after slaughter can only be of minor importance when compared with the phosphorylation which accompanies lactate production. In beef, a high level of ATP may be maintained in the muscle for several hours post mortem, during which the contribution through the Lohman reaction is of minor importance.

Only small changes in glucose concentrations were observed during the whole of the experimental period and it is therefore concluded that this sugar plays no major role in the overall glycolytic changes post mortem.

Initial concentrations of glycogen were widely different in the two groups although subsequent rates of hydrolysis were not dissimilar. An interesting feature was that glycogenolysis proceede at a rate which was not stoichiometric with lactate production. Thus in the unshackled legs the ratio of total glycogen degraded to total lactate produced (both expressed in glucose units) was 1.5 : 1 and in the shackled legs 1.75 : 1. Similar rates have been recorded for beef M. semimembranosus in this laboratory (5).

Increases in G-6-P and minor changes in the remaining intermediates of glycolysis throughout the 48 hour period were not sufficient to account for the discrepancies between the glycogen hydrolysed and lactate produced. Thus the question as to what metabolites are being formed from the 'excess' glycogen degraded is not obvious. One possible explanation may be that a large proportion of the fructose diphosphate passes down the DHAP — *a* GP — glycerol pathway to be built up as triglycerides.

Observed patterns of accumulation and degradation of *a* GP and glycerol

tended to support this suggestion; thus while *a* GP was decreasing (between 24—48 hours), glycerol increased significantly, especially in the unshackled legs.

Significant differences in NAD between left and right legs were not apparent throughout the 48 hours. Initial levels of the cofactor were slightly higher than those reported for beef (7) although the present values ere expressed as the total of oxidised and reduced forms. The overall disappearance of NAD amounted to 0.4 uM/g. representing two thirds of the initial concentration.

Standard deviations calculated for ATP, lactate, pH, G-6-P and NAD, although not all presented in the data and graphs were found to be much larger for the unshackled legs.

It must be remembered that these legs were unrestrained and thus allowed to kick violently in the early stages post mortem. This undoubtedly resulted in a poorer muscle consistency which ultimately lead to larger variations in the biochemical analyses.

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   (d) G-1-P; p. 131
   (e) F1: 6DP, DHAP and Gly-3-P; p. 246
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   (g) Glycerol; p. 220
   (h) Pyruvate, 2- and 3-PGA and PEP; p. 224.
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