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THE STRESS SYNDROME AND MEAT QUALITY

POST-MORTEM CHANGES AND ATPase ACTIVITY IN BOVINE SKELETAL MUSCLE

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Aspects of post-mortem changes (37°C under nitrogen) were studied in two skeletal muscles of beef cattle. M. longissimus dorsi contained more creatine phosphate, ATP and glycogen, less lactate and had a higher pH soon (15 min.) after death than m. psoas major. The main difference between the two muscles appeared to be related to the extent of the changes which occurred at or immediately after death rather than to the subsequent rates at which they continued as the muscles went into rigor. The inherent ATPase activity of natural actomyosin and preparations of myofibrils from both muscles were similar. Study of the actomyosin ATPase suggested that this enzyme may have two sites, one active at high, the other at low concentrations of ATP-Mg^{-2}.

ABSTRACT

FRENCH

CHANGEMENTS APRES DECES ET ACTIVITE' DE ATPase DANS LE MUSCLE SQUELETTIQUE DU BOVIN

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Les aspects des changements après décès (37°C sous nitrogène) ont été étudies dans deux muscles du squelette de bovins. Le m. longissimus dorsi contenait plus de phosphate de créatine, ATP et glycogène, moins de lactate et avait un pH plus élevé rapidement (15 minutes) après la mort que le m. psoas major. Les principales différences entre les deux muscles semblaient etre liées à l'étendue des changements qui se sont produits à la mort ou immédiatement après la mort, plutôt qu'à la vitesse consécutive à laquelle ils ont continué tandis que les muscles devenaient rigides. L'activité de l'ATPase inhérente de l'actomyosine naturelle et les préparations de myofibriles des deux muscles etaient similaires. Une étude de l'ATPase actomyosine a suggéré que cet enzyme peut avoir deux zônes, l'une active à des concentrations élevées, l'autre à des concentrations basses de ATP-Mg-2.

ПОСЛЕУБОЙНЫЕ ИЗМЕНЕНИЯ И АТФ-азная АКТИВНОСТЬ В СКЕЛЕТНЫХ МЫШЦАХ КРУПНОГО РОГАТОГО СКОТА

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Исследовались виды послеубойных изменений (при 37°C под азотом) в двух скелетных мышцах крупного рогатого скота. **M. longissimus dorsi** содержала большее количество фосфорно-креатина, АТФ и гликогена, меньшее лактата, и имела высшую pH скоро (через 15 мин) после убоя по сравнению с **m. psoas major** Оказалось, что главная разница между двумя мышцами относится к изменениям при убое или сразу после, а не к последующим скоростям по которым они продолжались при наступлении **rigor** в мышцах. Присущая АТФ-азная активность естественного актомиозина и препаратов миофибрилов из обеих мышец походили друг на друга. Исследование АТФаз актомиозина указывает на возможность, что этот фермент имеет два местоположения, одно активное при высоких, другое при низких концентрациях АТФ-M^{-2}.

ABSTRACT

GERMAN

LEICHENÖFFNUNGSVERÄNDERUNGEN UND ATPase TÄTIGKEIT IN RINDER SKELETAL MUSKEL

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Aspekte der Leichenöffnungsveränderungen (37° C unter nitrogen) wurden in zwei skeletal Muskeln von Rindern studiert. M. longissimus dorsi enthielten mehr creatine phosphate, ATP und glycogen, weniger lactate und hatten einen höheren pH bald (15 Min.) nach dem Tod als m. psoas major. Die hauptsächlichen Unterschiede zwischen den zwei Muskeln schienen mehr dem Ausmass der Veränderung, welche sofort nach dem Tode stattfand zuzuschreiben zu sein als dem darauffolgenden Verhältnis bei Übergang der Muskeln in Totenstarre. Die inhärierende ATPase Tätigkeit von natürlicher actomyosin und Herstellung von myofibrils von beiden Muskeln waren ähnlich. Studien der actomyosin AtPase wiesen darauf hin, dass dieses Enzym zwei Seiten haben kann, eine aktive bei höher, die andere bei niedriger Konzentration von ATP-Mg-2.

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INTRODUCTION.

The biochemical changes which occur post-mortem in pig muscle have been widely investigated in relation to the development of pale soft exudative (PSE) muscle and the porcine stress syndrome. Similar studies on bovine muscle have not been carried out on so extensive a scale, nevertheless, fundamental information on the course of post-mortem glycolysis in this species, with particular emphasis on its temperature dependance, has been published by several authors (Cassens and Newbold, I966, I967; Disney, Follett and Ratcliff, I967; Newbold and Scopes, I967). Studies of post-mortem changes in bovine muscle are especially relevant to the understanding and control of the phenomenon of cold-shortening which causes toughening of beef (Locker and Hagyard, I963; Marsh and Leet, I966; Bendall, I973).

MATERIALS AND METHODS.

Eighteen beef cattle (approximately 2 years old) were stunned using a captive bolt which penetrated the forebrain and exsanguinated. A section of m. longissimus dorsi (lumbar region) and m. psoas major were immediately removed from the carcase. Samples of muscle were taken (I5 min post-mortem) for biochemical analysis and pH measurements. The remainder of the tissue was placed at 37°c under a stream of moist nitrogen and further samples taken at I hr intervals up to 6 hr.

Biochemical Analysis. Samples of tissue were extracted in ice-cold perchloric acid(5%w/v). Creatine phosphate (C~P), ATP and glucose-6-phosphate (G-6-PO_4) were assayed (triethanolamine buffer, pH 7.6) using G-6-Po_4 dehydrogenase, hexokinase, creatine phosphate kinase, NADP, glucose and ADP. Lactate was assayed using lactic dehydrogenase and NAD. The reactions were measured spectrophotometrically using the change in absorption at 340 mμ due to the reduction of NADP or NAD. The pH of muscle was measured on suspensions (20% w/v) in iodoacetate (5mM, nutralised to pH 7.0) using a Radiometer pH meter 22.

Preparation of actomyosin. Actomyosin was prepared by a modification of the method of Heffron and Duggan (I97I). Samples of muscle were taken (30 min post-mortem) and homogenised in 4 volumes of cold Weber Edsall solution (0.6M KCl, 0.04M $NaHCO_3$, 0.0I M Na_2CO_3) containing dithiotreitol (ImM) and extracted for 24 hrs. The extract was diluted with an equal volume of Weber Edsall solution, centrifuged (30 min at 20,000xg) and the actomyosin precipitated by the addition to the supernatant of 9 volumes of cold, distilled, deionised water. The precipate was collected by centrifugation (I5 min at 2000xg). The precipitate was redissolved by the addition of KCl to a concentration of 0.6M. The solution was clarified by centrifugation (30 min at 20,000xg). The actomyosin was reprecipated by dilution with water (9 volumes) collected by centrifugation, washed and suspended in deionised water. The pH of the suspension was adjusted to 7.2 with Tris HCl buffer (25mM) and the protein concentration adjusted to 3 to 6 mg/ml.

ATP hydrolyase activity. Activity was measured on a final volume of 1ml actomyosin suspension (pH 7.2 in 25mM Tris-HCl buffer). The reaction was stopped (5 min) by the addition of TCA solution (I0% w/v). The inorganic phosphate (Pi) liberated was measured by the method of Tanssky and Shorr (I953). The protein content of the actomyosin suspension was determined using the biuret method (Layne I957). The ATP hydrolase activity was expressed as umole Pi liberated per mg of protein per min.

Statistical Analysis. The statistical significance of differences between the two muscles was assessed using the Student T-test.

RESULTS

The changes which occurred post-mortem in the ATP content of m. longissimus dorsi and m psoas major are shown in Fig.I. There was significantly ($p<0.05$) more ATP in the longissimus dorsi (5.6±0.2 μmole/g) than in the psoas (4.4±0.5 μmole/g) immediately after death. The rate of ATP loss from both muscles was slow but appeared to accelerate somewhat in m psoas major after about 3 hr at 37°c under nitrogen. M. longissimus dorsi also contained significantly ($p<0.00I$) more C~P (7.2±6 μmole/g) than the psoas (2.3±0.5 μmole/g immediately post-mortem (Fig.2). The C P level fell sharply in the longissimus dorsi during the first 2 hr. but declined very slowly during the subsequent 4 hr. Post-mortem pH/time curves for both muscles are shown in Fig.3. The pH at I5 min after death was significantly ($P<0.00I$) lower in the psoas (6.52±0.05) than in the longissimus dorsi (6.84±0.05). The rate of pH fall appeared to be somewhat higher in the psoas than in the l. dorsi. The pH of the longissimus dorsi was only just below 6.5 at 6 hr., that of the psoas had reached approximately 5.6 at this time. M. psoas also had initially a significantly ($P<0.00I$) higher lactate content (49.0±5.6 μmole/g) than the longissimus dorsi (28.7±2.8 μmole/g). The lactate content of the psoas remained higher than that of the longissimus dorsi as the muscles went into rigor. The differences between the two curves in Fig.4 were significant at $P<0.00I$ initially and also at subsequent time intervals, with the exception of the 3 hr. difference which was not significant. There appeared to be an acceleration in the rate of lactate formation in both muscles at 2 to 3 hr. post-mortem. The glycogen content (I3 animals) of m. longissimus dorsi (I0.4±I 0.5mg/g) was significantly ($P<0.0I$) higher than that of m psoas major (7.9±0.5 mg/g) at I5 min after death and remained higher at hourly intervals up to 6 hr. At 24 hr. post-mortem the psoas contained a residual I.57±0.6 mg glycogen/g, the longissimus dorsi I.8±0.3 mg/g. The difference between the two muscles was not significant at this time. The G-6-PO_4 content of the muscles at each time interval were assayed but no significent differences were found. The concentration of this substance found ranged from 2.4 to 4.6 μmole/g.

The ATP hydrolase activity of preparations of natural actomyosin from both muscles were examined at a range of $MgATP^{2-}$ concentrations. The curves in Fig. 5 represent average values for eight preparations from each muscle. The enzyme from m.psoas major had a significantly ($P<0.05$ and $P<0.0I$) greater activity at four substrate concentrations between I.5 and 4.5 mM $MgATP^{2-}$ than that from m. longissimus dorsi.

The (S)/activity curves for the individual preparations from the psoas muscle consistenly showed a small fall in activity at (S)=3.0 mM so that two peaks were present, one at (S)=I.5 mM where activity was 0.290±0.036 μmole Pi/mg protein/min, the other at (S)=4.5 mM with an activity of 0.294±0.0I5 μmole Pi/mg protein/min. Maximum activity of the enzyme preparations from m. longissimus dorsi was 0.24I±0.0I4 μmole Pi/mg protein/min at (S)=3.0mM. There was no indication of two peaks on the (S) activity curve for this muscle.

The relationship between ATP hydrolase activity and pH for eight preparations from both muscles is shown in Fig.6. There was a steady fall in ATP splitting with fall in pH. The enzyme from m. longissimus dorsi showed a maximum activity at approximately pH 7.8 with a fall in activity at higher pH. The activity of the preparations from m.psoas major continued to rise to pH 8.25.

DISCUSSION.

The results presented in this paper show that significant differences existed between the ATP? C~P, lactate and glycogen contents and pH of bovine m.psoas major and m.longissimus dorsi immediately and up to 6 hr. after death. The main difference between the two muscles, however, lay in the initial values for these parameters. The subsequent differences appeared to be largely a reflection of the initial differences. Nevertheless the results also suggested that the rate of post-mortem ATP loss, lactate formation, and pH fall were somewhat higher in the psoas than in the longissimus dorsi.

Since the main difference between the two muscles was due to the fall in ATP levels and to glycolysis resulting from the death reaction, the inherent ATP hydrolase activity of actomyosin (the contractile enzyme) of both muscles was studied. The results showed that preparations from m.psoas were significantly more active in the splitting of $MgATP^{2-}$ at optimal substrate concentration than were those from m. longissimus dorsi. The optimal substrate concentrations observed were in the range encountered in muscle post-mort

When the initial burst of activity was completed ATP loss continued at a reduced rate.

pH

Lactate (μmole/gm)

This slow loss of ATP might be explained on the basis of a continuing activity of a less active ATP hydrolase during the onset of rigor.

The association of a higher ATP hydrolase activity with the psoas muscle, which responded to slaughter with a greater burst of glycolysis, is in agreement with previous observations (Hefferon and McLoughlin, 1971) on ATP hydrolase activity and glycolysis in muscle. These workers noted that the white fibre areas of pig m. semitendinosus responded either to death or electrical stimulation in vivo with a greater depletion of the high energy phosphates, greater formation of lactate and fall in pH than did the red fibre areas. The white areas also had an inherently more active ATP hydrolase than the red.

An examination of the effects of $MgATP^{2-}$ concentration on ATP hydrolase activity suggested that psoas actomyosin had two sites, one active at low, the other at higher concentrations of substrate. The observation might partly explain the acceleration in the rate of ATP loss from m.psoas as rigor progressed, particularly since the C~P levels, the immediate source of ATP resynthesis, were virtually depleted at death. The relationship between pH and ATP hydrolase activity at I.5mM substrate and 37° C did not show that low pH had any activating effect on the engyme. Whether or not low pH may have such an effect under the various combinations of pH , substrate concentrations and temperature which exist in muscle post-mortem remains to be clarified.

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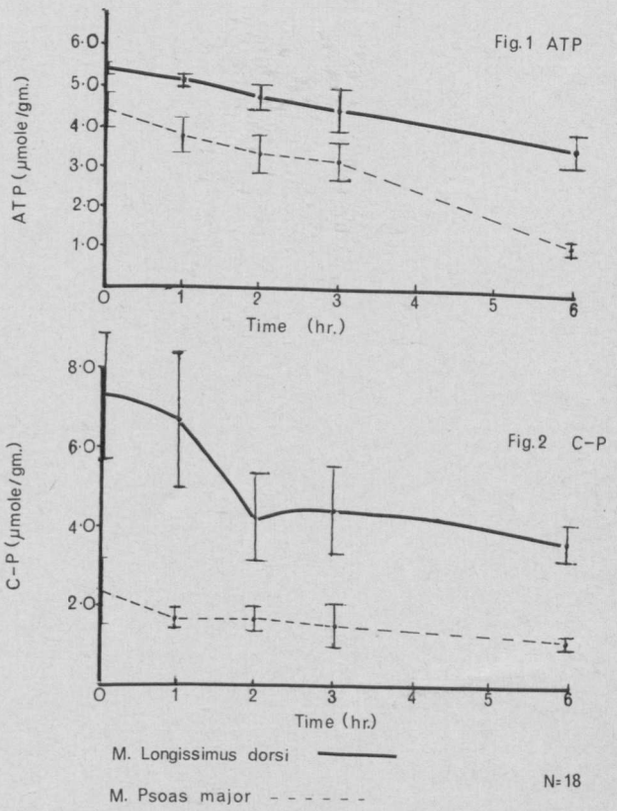
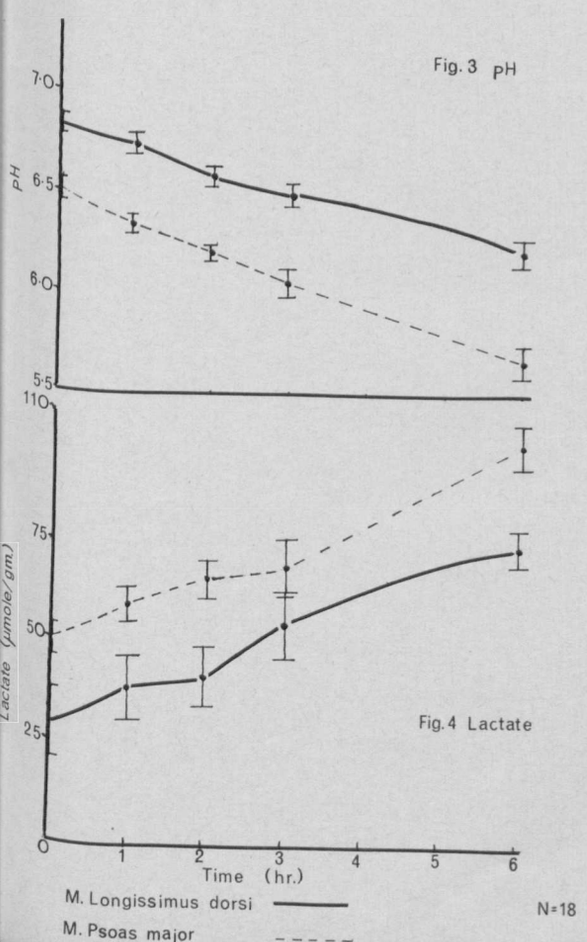


Post-mortem Changes In Beef Muscle



Properties Of Beef Actomyosin