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THE EFFECT OF RAPID POST-MORTEM pH FALL ON THE EXTRACTION OF THE SARCOPLASMIC AND MYOFIBRILLAR PROTEINS OF PIG MUSCLE

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ABSTRACT

The proteins of porcine longissimus dorsi muscle were studied using protein extraction techniques. The solubility of the sarcoplasmic and myofibrillar proteins from exudative post-rigor muscle was reduced at low and high ionic strengths respectively. Changes in the extractability of the proteins of the post-rigor muscle occurred when low pH values were attained in the muscle soon after death. The extent of these changes was related to rate of pH fall post-mortem.

INTRODUCTION

Porcine longissimus dorsi muscle which is exudative and pale in colour has been described by various workers, e.g. Ludvigsen (1,2), Henry et al. (3,4), Wismer-Pedersen (5), Wismer-Pedersen and Briskey (6), Briskey and Wismer-Pedersen (7,8), Lawrie et al. (9), Lawrie (10), Bendall and Wismer-Pedersen (11), McLoughlin and Goldspink (12). In general, muscle of this type is associated with a rapid fall in pH post-mortem so that low pH values (below 6.0) are attained in the muscle at a time when the temperature of the muscle is still high (above 30°C). The pH about 45 minutes after death (arbitrarily designated "pH_1") has been used by numbers of workers (5,6,7,8,11,12) as an indication of the rate of pH fall post-mortem. When the pH_1 is below 5.6 the changes in the water-retaining properties and in the colour of the post-rigor muscle are particularly marked. Low ultimate pH values (pH when post-mortem anaerobic glycolysis has ended) have also been found in muscle of this type (3,4,9,10).

Changes take place in certain properties of the muscle proteins when pH falls rapidly after death. Wismer-Pedersen (5) reported that pH_1 values below 6.0 were associated with a reduced solubility of the proteins of the post-rigor muscle in 0.6 M KCl solution. Bendall and Wismer-Pedersen (11) studied the properties of preparations of washed myofibrils from exudative muscle and showed that their capacity to bind water and protons was reduced and that their extractability at high ionic strength was less than that of myofibrils from normal meat. The titration curves and the isoelectric points of the myofibrillar proteins from both watery and normal muscle were

similar to each other but were quite different from those of myofibrils from heat-coagulated meat in which denaturation of the myofibrils had taken place. Since watery muscle was found to have an apparently elevated content of myofibrillar protein and since the extra protein was shown to be derived from the sarcoplasm, it was concluded that the altered properties of the myofibrillar protein of exudative muscle were not due to denaturation of the proteins in the classical sense but rather to the adsorption of denatured sarcoplasmic protein onto sites on the myofibrils which normally bound water and protons.

Scopes and Lawrie (13) examined the sarcoplasmic proteins of beef and pig muscle by electrophoresis in starch gel and observed differences in electropherograms from pre- and post-rigor muscle. These differences were particularly marked where accelerated glycolysis had occurred post-mortem in pig muscle. McLoughlin and Goldspink (14) pointed out that, since conditions of pH and temperature which cause precipitation of the sarcoplasmic proteins can be attained in pig *longissimus dorsi* muscle after death, the change in the apparent colour of the muscle associated with rapid pH fall post-mortem may be due to a masking of muscle pigment by precipitated protein.

Following the finding of exudative muscle in pig carcases (McLoughlin and Goldspink, 12), the extractability of the sarcoplasmic and myofibrillar proteins of muscle of this type was studied at low and high ionic strengths. The composition of the post-rigor muscle was related to the pH_1, value of the muscle and the results of the analyses of specimens of muscle taken at 45 minutes and 24 hours after death were compared.

EXPERIMENTAL

Materials. Specimens of muscle used were taken from the lumbar region of the *longissimus dorsi* muscle. At an early stage of the work materials were obtained at about 24 hours post-mortem from carcases which were obviously exudative and muscle which exhibited low ultimate pH (below 5.3) was subjected to analysis. Later, pure-bred Large White pigs (about 150-180 lb. live weight) were killed and samples of muscle taken from the right and left-hand sides of the carcases at 45 minutes and at 24 hours post-mortem respectively. The pH_1 (pH 45 minutes after death) was recorded. The carcases were kept at 2°C during the 24-hour period. The animals were killed using a captive bolt humane killer and were immediately exsanguinated. In a few instances about 40 mg. of tubocurarine chloride (Burroughs Wellcome) were given intramuscularly 3 to 5 minutes ante-mortem to immobilise the animals and thus ensure a high pH in the muscle after death. It was possible to collect material which exhibited low pH_1 values in the range 5.0 to 5.9 (in the course of normal rates of post-mortem glycolysis the pH_1 is well above 6.0).

Methods

Protein extraction. The analysis of specimens of muscle was commenced immediately after removal from the carcase and all subsequent operations were carried out at 2°C. The material was thoroughly minced (to a size which finally passed through a 2 mm. mincer plate) and duplicate samples (25 mg. each time) were taken for protein extraction. The sarcoplasmic proteins were washed out of the tissue by extracting the material three times

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(30 minutes each time) with ten volumes of potassium phosphate buffer (0.03 M ; pH 7.4). The myofibrillar proteins were extracted from the sarcoplasm-free residue by stirring three times (1 hour each time) with 10 volumes of a solution (pH 7.1 - 7.2 ; I = 0.68) containing KI (0.6 M), KH_2PO_4 (0.009 M) and K_2HPO_4 (0.041 M). Bendall and Wismer-Pedersen (11) used a solution (pH 6.5 ; I = 0.55) containing KCl (0.5 M) and KH_2PO_4/K_2HPO_4 (0.04 M) to demonstrate differences between myofibrils from normal and from watery muscle.

Soluble, non-protein nitrogen. An equal volume of ice-cold trichloroacetic acid (20% w/v) was added to an aliquot of a solution of sarcoplasm in phosphate buffer. The precipitated protein was removed by centrifugation and the nitrogen content of the protein-free supernatant solution assayed.

pH measurement. pH was measured using a Radiometer 23 pH meter with glass and calomel electrodes (types G 200 C and K 400 respectively). The pH at 45 minutes post-mortem was measured on a suspension (10 per cent) of muscular tissue in iodoacetate solution (0.01 M). The pH at 24 hours post-mortem was measured on a similar suspension in distilled water.

Total nitrogen determination. Samples (3.3 g.) of tissue were digested with sulphuric acid and mercuric oxide catalyst. Ammonia was distilled into a boric acid-indicator mixture.

Fat determination. Samples (15 g.) were allowed to stand *in vacuo* at 2°C. over silica gel for 16 hours. The dried materials were extracted with petroleum ether (40 - 60°C) by soxhlet extraction for 6 hours.

Expression of results. The composition of muscle was expressed either as g. of nitrogen per 100 g. of wet tissue (on a fat-free basis) in the sarcoplasmic protein, myofibrillar protein, soluble non-protein nitrogen and residual stroma or as the percentage of the total nitrogen (on a fat-free basis) comprising each of these components. The nitrogen content of the stroma residue (true stroma and any unextracted protein) was not determined directly but was calculated as the difference between the total nitrogen and the sum of the sarcoplasmic and myofibrillar nitrogens and the soluble non-protein nitrogen.

RESULTS

At the beginning of the present study protein extractions were carried out on five specimens of longissimus dorsi muscle which exhibited low ultimate pH values (below 5.3) and which were pale-coloured and markedly exudative. The results of these analyses are shown in Tables 1 and 2. The values obtained for a muscle of normal ultimate pH (ca. 5.5) are also included. In Table I the amount of nitrogen in each of the muscle components examined (sarcoplasmic protein, myofibrillar protein, soluble non-protein nitrogen and stroma) are given as g. of nitrogen per 100 g. of muscular tissue, while in Table 2 the distribution of nitrogen is expressed as a percentage of the total nitrogen content of the materials. By comparison with a muscle of relatively normal ultimate pH (specimen No. 6, in Tables I and 2) it was evident that the materials of low ultimate pH showed a reduction in the amount of protein soluble in dilute salt solutions, a reduced extractability of the myofibrillar proteins at high ionic strength

and a consequently elevated value for residual stroma nitrogen. The lowered content of soluble sarcoplasmic protein and the fact that the total nitrogen contents of the tissues were in the normal range suggested that denaturation of the sarcoplasmic proteins had occurred with loss of their solubility at low ionic strength. When the myofibrillar and stroma nitrogen values in Table I are added together (Table 3) the nitrogen contents of the fibrillar residues remaining after washing away the sarcoplasm are obtained. The nitrogen content of the washed fibrils from muscle of low ultimate pH was in all instances higher than that of the fibrillar material from muscle of normal ultimate pH. Bendall and Wismer-Pedersen (11) reported that washed fibrils from exudative longissimus dorsi muscle had an increased content of protein and showed that the extra fibrillar protein was denatured sarcoplasmic protein attached to sites on the myofibrils which normally bound water and protons. These authors quoted a value of 0.122 g. of fibrillar protein per g. of tissue for normal meat and 0.150 g. of protein per g. for muscle allowed to go into rigor at 40°C. Taking the nitrogen content of the fibrillar proteins as 16.7% (Bailey, 15), the above values become 2.10 g. and 2.5 g. of nitrogen per 100 g. of muscular tissue respectively. These values are similar to those shown in Table 3 and suggested that the low content of soluble sarcoplasmic protein found at low ultimate pH was due to denaturation of the proteins followed by loss of solubility at low ionic strength. The composition of one muscle of low ultimate pH (No. 5) is rather similar to that of normal muscle (No. 6). It seems unlikely that the ultimate pH attained in a muscle will determine the apparent distribution of nitrogen in the post-rigor tissue, since ultimate pH is attained at a time when the temperature of the muscle is falling. On the other hand,

when low pH is attained in muscle soon after death, i.e. at a time when the temperature of the muscle is still high (above 30°C), marked changes occur in the properties of the muscle proteins (Bendall and Wismer-Pedersen, 11). The relationship between the pH_1 value and the composition of the post-rigor muscle was therefore next studied.

Materials of low pH_1 were obtained without undue difficulty as it had been observed that rapid rates of post-mortem pH fall very often occurred when pigs were killed using a captive bolt humane killer followed by exsanguination. The animals usually struggled very violently after exsanguination. According to Bendall (16) the onset of rigor is more rapid when an animal struggles than when it is immobilised at death, because struggling results in lowered levels of ATP, phosphocreatine and glycogen, high content of lactic acid and low pH in the muscle, which conditions bring about a rapid onset of rigor. The accelerated rates of pH fall observed here were presumably due to the activity of the skeletal musculature at and immediately after death. This suggestion is supported by the observation that intramuscular administration of 40 mg. of tubocurarine chloride 3 - 5 minutes ante-mortem resulted in a slow, steady fall in pH after death (McLoughlin, 17). Some animals were given tubocurarine chloride before death during the present study to ensure obtaining some materials with as high as possible a pH_1.

The results of the extraction of the proteins of longissimus dorsi muscle 24 hours post-mortem are shown in Tables 4 and 5, where they are listed in order of ascending pH_1. It is clear that at low pH_1 values less protein is soluble in dilute solution (phosphate buffer, pH 7.4; 0.03 M)

and less myofibrillar protein is soluble in more concentrated solution (KI, 0.6 M; KH_2PO_4/K_2HPO_4, 0.05 M; pH 7.1 - 7.2). The amounts of extractable sarcoplasmic and myofibrillar protein are [illegible]y related to the pH_1, and progressively decrease at lower pH_1 values. The reduced extractability of the muscle proteins is reflected in the high nitrogen values for the residual stroma material. Where high pH_1 values (6.7, 6.8) were recorded the soluble sarcoplasmic protein comprised 28.3 and 30.5 per cent of the total nitrogen content of the tissues; on the other hand, for example, only 20.8 per cent of the total nitrogen content of the muscle could be extracted in dilute phosphate buffer where the pH_1 was 5.1. Similarly, over 50 per cent of the total nitrogen of the post-rigor muscle appeared as myofibrillar nitrogen when the pH_1 value was high, while pH_1 values below 6.0 were associated with considerable reductions in this figure.

In Table 7 the results of extractions of specimens of muscle taken at 45 minutes post-mortem and analysed immediately, are given. The pH_1 of each of these materials was below 6.0. The results of analysis of the corresponding muscles taken from the other side of the carcase 24 hours after death are also shown. It seems that some loss of protein extractability may already have occurred at 45 minutes post-mortem in these materials and that further loss of extractability subsequently occurred.

DISCUSSION

Post-rigor porcine longissimus dorsi muscle which is exudative and pale in colour has been associated with rapid pH fall post-mortem, so that pH values at or below 6.0 are reached in the muscle before the temperature of the muscle has fallen below 30 - 35°C (5, 6, 7, 8, 18).

Under these conditions changes occur in the muscle proteins, e.g. their solubility in 0.6 M KCl is reduced (5). Bendall and Wismer-Pedersen (11) showed that the number of sites available for water and proton-binding is reduced in preparations of myofibrils from watery muscle, although the isoelectric point and titration curves of such myofibrils are similar to those of myofibrils obtained from normal muscle. They also showed that when the sarcoplasm was washed out of exudative muscle using dilute phosphate buffer (0.04 M; pH 6.5 ; I = 0.05) some of the sarcoplasmic protein was retained by the myofibrillar residue. In the present study, the sarcoplasmic proteins were washed out of post-rigor muscle which had exhibited low pH soon after death (pH_1) using potassium phosphate buffer (0.03 M; pH 7.4 ; I = 0.06). It was found that with decreasing pH_1 values the amount of sarcoplasmic protein extracted was reduced. The elevated nitrogen content of the residue (myofibrils plus stroma) remaining after removal of the proteins soluble at low ionic strength indicated that insoluble (presumably denatured) sarcoplasmic protein was attached to the residual material. The solubility at higher ionic strength of the contractile proteins was also reduced when low pH_1 values were attained in the muscle. The fact that reduced extractability of the myofibrillar proteins is accompanied by a loss in the solubility at low ionic strength of some of the sarcoplasmic protein, and that the denatured sarcoplasmic material remains attached to the washed myofibrillar residue, appears to support the observations of Bendall and Wismer-Pedersen (11) that the loss of solubility at high ionic strength characteristic of the contractile

proteins of watery muscle is due to the adsorption onto the myofibrils of denatured sarcoplasmic protein.

In conclusion, it appears that loss in solubility at low and high ionic strengths of the sarcoplasmic and myofibrillar proteins is characteristic of post-rigor exudative muscle. The changes which occur in the solubility of the muscle proteins are brought about when low pH values are attained in the muscle soon after death. The extent of these changes appear to be determined by the pH_1 value.

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TABLE I

Ultimate pH (pH_u) and composition of porcine _l. dorsi_ muscle

No.	pH_u	Distribution of Nitrogen*				Total N*
		Sarcoplasmic N	Myofibrillar N	Non-Protein N	Stroma N	
1	5.05	0.66	0.63	0.43	2.11	3.8
2	5.15	0.72	1.78	0.45	0.86	3.8
3	5.15	0.80	1.68	0.46	0.73	3.7
4	5.20	0.59	1.38	0.42	1.34	3.7
5	5.20	0.82	1.93	0.49	0.37	3.6
6	5.55	0.85	1.93	0.46	0.18	3.4

* g. per 100 g. of wet tissue

TABLE 2

Ultimate pH (pH_u) and composition of porcine <u>l. dorsi</u> muscle.

No.	pH_u	Distribution of Nitrogen*			
		Sarcoplasmic N	Myofibrillar N	Non-Protein N	Stroma N
1	5.05	17.4	16.6	11.3	55.5
2	5.15	18.9	46.8	11.8	22.6
3	5.15	21.8	45.8	12.5	19.9
4	5.20	15.9	37.3	11.4	36.2
5	5.20	22.1	53.5	13.6	10.24
6	5.55	24.9	56.4	13.5	5.3

* Expressed as percentage of the total nitrogen.

TABLE 3

Nitrogen content of the washed fibrillar residue from exudative and normal muscle.

No.	1	2	3	4	5	6
pH_u	5.05	5.15	5.15	5.20	5.20	5.55
*N content of washed fibrillar residue	0.274	0.264	0.241	0.272	0.230	0.211

* g. of N per 100 g. of tissue expressed on a fat-free basis.

TABLE 4

pH_1 and composition of *l. dorsi* muscle 24 hours post-mortem

No.	pH_1	Distribution of Nitrogen*				Total N*
		Sarco-plasmic N	Myo-fibrillar N	Non-protein N	Stroma N	
1	5.0	0.78	1.09	0.46	1.34	3.7
2	5.1	0.74	1.06	0.45	1.32	3.6
3	5.15	0.78	1.08	0.45	1.22	3.5
4	5.30	0.80	1.36	0.45	1.15	3.8
5	5.4	0.85	1.59	0.45	0.76	3.6
6	5.5	0.70	1.51	0.43	0.80	3.4
7	5.6	0.86	1.41	0.45	0.84	3.6
8	5.8	0.92	1.64	0.44	0.71	3.7
9	5.85	0.89	1.76	0.47	0.32	3.4
10	6.2	0.92	1.84	0.44	0.21	3.4
11	6.7	1.02	1.90	0.46	0.22	3.6
12	6.8	1.10	1,95	0.45	0.13	3.6

* g. N per 100 g. of wet tissue expressed on a fat-free basis.

TABLE 5

pH_1 and composition of l. dorsi muscle 24 hours post-mortem

No.	pH_1	Distribution of Nitrogen*			
		Sarco-plasmic N	Myo-fibrillar N	Non-protein N	Stroma N
1	5.0	21.1	29.5	12.4	36.2
2	5.1	20.8	29.6	12.6	36.9
3	5.15	21.9	30.7	12.6	34.7
4	5.31	21.2	35.0	11.9	31.8
5	5.4	20.0	40.1	12.4	25.5
6	5.5	20.4	43.8	12.5	23.4
7	5.6	24.2	39.1	12.5	23.7
8	5.8	24.9	44.3	11.8	18.9
9	5.85	25.9	51.2	13.7	9.3
10	6.2	27.1	54.1	12.8	6.0
11	6.7	28.3	52.8	12.8	6.1
12	6.8	30.5	54.2	12.5	3.6

"expressed as percentage of the total nitrogen

TABLE 6

pH_1 and composition of l. dorsi ¾ hr. and 24 hr. post-mortem

No.	pH_1	Distribution of Nitrogen*				
		Time post-mortem	Sarco-plasmic N	Myo-fibrillar N	Non-protein N	Stroma N
2	5.1	¾ hr.	0.90	1.67	0.48	0.62
		24 hr.	0.74	1.06	0.45	1.32
3	5.15	¾ hr.	0.96	1.62	0.46	0.37
		24 hr.	0.78	1.08	0.45	1.22
6	5.5	¾ hr.	0.85	1.62	0.46	0.37
		24 hr.	0.70	1.51	0.43	0.80
7	5.6	¾ hr.	1.00	1.70	0.45	0.50
		24 hr.	0.86	1.41	0.45	0.84
8	5.8	¾ hr.	1.07	1.76	0.45	0.37
		24 hr.	0.92	1.64	0.44	0.71
9	5.85	¾ hr.	0.97	1.61	0.49	0.33
		24 hr.	0.89	1.76	0.47	0.32

g. per 100 g. tissue, expressed on a fat-free basis

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Neuvième congrès des investigateurs faisant des recherches sur la Viande.

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L'effet de la diminution rapide de pH, après la mort, sur l'extraction des protéines du sarcoplasma et des fibrilles musculaire du muscle du porc.

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SOMMAIRE

Les protéines du muscle long dorsal du porc ont été etudiées en utilisant les techniques d'extraction des protéines. La solubilité des protéines du sarcoplasma et des fibrilles musculaires provenant du muscle exsudant, après la rigidité cadavérique, fut réduite à basse et haute puissance ionique respectivement. Des changements quant à la possibilité d'extraction des protéines du muscle après la rigidite cadaverique se produisirent quand un faible degré de pH fut atteint dans le muscle peu après la mort.

L'importance de ces modifications était en rapport avec le degré de diminution de pH après la mort.

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DIE WIRKUNG EINES SCHNELLEN POSTMORTALEN pH-ABFALLIS AUF DIE EXTRAKTION DER SARKOPLASMISCHEN UND MYOFIBRILLÄREN EIWEISSSTOFFE VON SCHWEINEMUSKEL

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ZUSAMMENFASSUNG

Die Eiweissstoffe des M. longissimus dorsi des Schweines wurden bei der Verwendung von Eiweissextraktionsverfahren studiert. Die Lösungsfähigkeit der sarkoplasmischen und myofibrillären Eiweissstoffe aus ausschwitzendem Muskel nach der Totenstarre war bei niedriger beziehungsweise hoher Ionenstärke vermindert. Veränderungen entstanden in der Extraktionsfähigkeit der Eiweissstoffe des Muskels nach der Totenstarre, wenn niedrige pH-Werte im Muskel bald nach dem Tode erreicht wurden. Der Ausmass dieser Veränderungen wurde auf die postmortale pH-Abfallsgeschwindigkeit bezogen.