PROCEEDINGS

(SUPPLEMENT)

14 EUROPEAN MEETING TH OF MEAT RESEARCH WORKERS

BRNO CZECHOSLOVAKIA AUGUST 26th – 31st 1968

RESEARCH INSTITUTE OF MEAT INDUSTRY BRNO 12, PALACKÉHO 1–3 CZECHOSLOVAKIA

14TH EUROPEAN MEETING OF MEAT RESEARCH WORKERS
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SECTION

ANAEROBIC GLYCOLYSIS IN MUSCLE TAKEN IMMEDIATELY POST-MORTEM AND FROM LIVE PIGS UNDER ANAESTHESIA

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Low pH values (below 6.0) are frequently reached in certain muscles of the pig within 1 hr. after death (Ludvigsen, 1954; Wismer-Pedersen, 1959; Briskey and Wismer-Pedersen, 1961; Wismer-Pedersen and Briskey, 1961; McLoughlin, 1963a; Eliott, 1965). At this time post-mortem, the temperature of the deep-lying musculature of the carcase is usually still above 35°C. The combination of high temperature and low pH brings about changes in the properties of the muscle proteins (Bendall and Wismer-Pedersen, 1962; McLoughlin, 1963b; Sayre and Briskey, 1963; Goldspink and McLoughlin, 1964) which make muscle, normal at the time of death, become pale, soft and exudative as it goes into rigor mortis (Sayre and Briskey 1963; Sayre et al., 1964; Borchert and Briskey, 1964 and 1965; Sayre et al., 1966).

The breed of pig and ante-mortem factors which influence the physiological condition of the animal at the time of death, affect the

course of post-mortem glycolysis in muscle (see a review by Briskey, 1964). Theoretically, the slaughter procedure itself will considerably influence post-mortem glycolysis because struggling at death lowers the initial levels of ATP and phosphocreatine in muscle and thus accelerates the onset of rigor. McLoughlin (unpublished) observed that the average pH was 6.3 at 3 min. post-mortem in the longissimus dorsi muscle of pigs which were stunned using a captive bolt pistol and exsanguinated; when transmission of neural impulses into the muscle was prevented using curare, the initial pH was raised to 6.8 and the subsequent rate of pH fall considerably slowed. McLoughlin (unpublished) also observed that the initial pH was lowered and the rate of pH fall accelerated when the gastrocnemius muscle was stimulated via the sciatic nerve in vivo. Bendall and Hallund (1965) and Bendall (1966) reported that direct stimulation of the longissimus dorsi muscle had a continued accelerating effect on the rate of post-mortem pH fall. Differences in the pH value of pig muscle at 45 min. after death have been associated with commercial methods of stunning and slaughter (Bendall, 1965; McLoughlin, 1965; McLoughlin and Davidson, 1966).

These observations suggest that neural stimuli reaching the muscle at the time of death may have a very marked effect on post-mortem glycolytic changes. In this work, glycolysis was studied in muscle taken from the live animal under anaesthesia and in muscle taken immediately after slaughter to assess the influence of the death reaction on post-mortem changes in muscle. The response of muscle to direct stimulation was also examined.

EXPERIMENTAL

Animals

Pure-bred pedigree Landrace and Large White pigs were used. The animals were between 5 and 6 months old and were in the liveweight range 160 to 180 lb. They came from herds kept under the same conditions on one farm. They were individually transported (17 miles) to the laboratory the day before each experiment, were fed on arrival and kept in the heated laboratory overnight. Maximum care was taken to see that the animals were not subjected to stress.

Anaesthesia

Animals were held in a folding table and a bag placed over the head. The bag had two apertures, one to let in nitrous oxide and oxygen, the other to let out expired gases. Nitrous oxide was delivered at a rate of 7l./min., oxygen at 3l./min. The gases were passed through halothane (2-bromo-2-chloro-1, 1, 1-trifluoroethane) so that the emerging gas mixture contained about 1% to 2% of this substance. When tha animal had become unconscious, an incision was made in the mid-line of the throat and the larynx and the upper part of the trachea exposed by dissection. The trachea was sectioned transversely but was not severed. A tracheal tube was inserted and the cuff inflated. After intubation, the animal was placed on an operating table and when it had respired normally for 20 min., specimens of muscle were taken.

Slaughter

Pigs were stunned by shooting through the forebrain using a captive bolt

pistol and exsanguinated.

Direct stimulation of the longissimus dorsi muscle

Sections of the longissimus dorsi muscle were removed under anaesthesia and divided. One portion was taken as a control and the other stimulated to contract for 30 sec. Stimulation was carried out by inserting electrodes in the ends of the muscle section and applying a current of 5 volts intensity, a frequency of 100 shocks / sec. and a pulse width of 10 millisec.

Materials

Sections of the longissimus dorsi were taken from the lower thoracic region. After removal, specimens of muscle were taken immediately for pH measurement and chemical analysis. The muscle was then placed at 37°C in a stream of moist nitrogen and samples taken for pH measurement and chemical analysis at hourly intervals.

Chemical analysis

Glycogen was determined using the anthrone reagent (Seifter et al., 1950) lactic acid using lactic acid dehydrogenase (Hohorst, 1963); and ATP using phosphoglycerate kinase and phosphoglyceraldehyde dehydrogenase.

Measurement of pH was made on a suspension (20% W/V) of muscle tissue in iodoacetate solution (0.005 M).

The fat content of muscle was determined by soxhlet extraction of the tissue; and results of chemical analysis were expressed on a fat-free basis.

RESULTS

1. POST-MORTEM CHANGES

Post-mortem changes (at 37°C., under nitrogen) in (1) pH, (2) glycogen, (3) lactic acid and (4) ATP were studied in muscle taken from the live animal under total anaesthesia and from animals immediately after slaughter. The following results were obtained:

(1) pH Changes

The initial (5 min.) pH in muscle from anaesthetised pigs (12) was 7.0 ± 0.04. The pH fell fairly steadily (0.2 units / hr.) over the following 7 hr., although some acceleration in the rate of fall appeared to occur when pH 6.1 had been reached. In contrast to this, the initial pH of muscle taken immediately after death was 6.3. Due to the very considerable difference in initial pH, it is difficult to validly compare the subsequent rates of pH fall in the two types of muscle. However, a pH of 6.0 was reached at 1 hr. post-mortem in muscle from the slaughtered animals while muscle taken from the live animals, which were not subjected to a death reaction, took about $4\frac{1}{2}$ hr. to reach this pH. From the point of view of meat quality, the pattern of pH change found in muscle not subjected to the death reaction would produce meat of satisfactory colour and water-holding capacity. The death reaction, however, altered the pattern of pH change to one conducive to the formation of pale, soft, exudative muscle.

(2) Glycogen

The initial level of glycogen in muscle taken from the live animal

was 13.9 ± 1.0 mg. / g. tissue; after 8 hr. glycolysis, the glycogen concentration had fallen to 1.1 mg. / g. tissue. The relationship between the fall in glycogen concentration and time was not quite linear but indicated a some what slower rate of pH fall during the first 2 to 3 hr. The rate of disappearance then accelerated slightly and slowed again after about 5 hr., at which time the glycogen concentration had fallen to low levels (ca. 4 mg. / g. tissue) and the low pH (5.8) reached in the muscle was perhaps exerting an inhibitory influence on glycolysis.

The initial level of glycogen in muscle taken immediately after stunning and exsanguination was 6.8 mg. / g. tissue and the concentration fell steadily to reach a value of 1.3 mg. / g. tissue at 5 hr. post-mortem.

(3) Lactic Acid

The initial level of lactic acid in muscle taken from the live animal was 17.0 ± 3.0 μ mole / g. tissue and 80 μ mole in muscle taken from animals immediately post-mortem. Lactic acid was produced steadily (16.5 μ mole / hr.) for 6 hr. in the muscle excised from the live animal. Production of lactic acid then slowed and the concentration of this substance in the muscle after 8 hr. was 125 μ mole / g. tissue.

There was a linear relationship between lactic acid formation and glycogen disappearance, and between lactic acid formation and the fall in pH over the range 7.0 to 5.5, in muscle from the anaesthetised animals.

(4) ATP

The initial level of ATP in muscle taken from the live animal was 6.3 ± 0.2 μ mole / g. tissue. The ATP level fell slowly (0.5 μ mole / g. / hr.)

during the first 2 hr. This time it corresponded to the interval after which muscle pH reached 6.5. The rate of ATP decay then accelerated to 1.0 μ mole / g. / hr. to reach a concentration of 1.5 μ mole after a further 4 hr. The rate of ATP decay then slowed considerably and 8 hr. after excision of the muscle had reached 0.7 μ mole / g. tissue.

The initial level of ATP in muscle taken post-mortem was 4.8 ± 0.8 μ mole / g. tissue. There was no delay phase. The ATP level fell to 2 μ mole / g. in 2 hr. and subsequently, fell rather more slowly to reach 0.8 μ mole / g. tissue at 5 hr. post-mortem.

The half - life of ATP in muscle taken from the live animal was 4 hr., and 90 min. in muscle taken after death.

2. EFFECT OF MUSCLE CONTRACTION ON POST-MORTEM GLYCOLYSIS

Four Landrace pigs were anaesthetised and were given myanesin (50 mg.) intravenously 15 min. before excision of muscle specimens. Myanesin is a muscle relaxant which acts by depressing the motor activity of the anterior horn cells of the spinal cord. The excised section of the longissimus dorsi muscle was divided transversely into two sections and one stimulated electrically so that it went into a sustained contraction for 30 sec. The initial pH of the stimulated section was somewhat (0.1 pH unit) lower than that of the unstimulated control section. Stimulation considerably increased the rate of glycolysis. The pH reached 6.0 after 3 hr. in the stimulated sections, but took 6 hr. to attain this value in the unstimulated sections. At 6 hr. after excision, only 1 mg. glycogen / g. tissue remained in the stimulated sections, while 8.5 mg / g. remained in the control sections. The initial levels of

lactic acid were similar (16.1 μ mole / g. of tissue) for both sections of muscle but lactic acid was formed at a rate of 18 μ mole / 1 g. / hr. in the stimulated muscle, and 10 μ mole / 1 g. / hr. in the unstimulated muscle; and the average half-life of ATP in the stimulated muscle was $2\frac{1}{2}$ hr. compared to 6 hr. in the unstimulated muscle.

DISCUSSION

According to the theory of rigor (Bendall, 1960) muscle contraction at the time of death accelerates the rate of post-mortem glycolysis and the onset of rigor mortis by lowering the initial levels of ATP and phosphocreatine in muscle. Pig muscle is subject to very considerable variations in patterns of post-mortem glycolysis and would appear to be very susceptible to factors which influence the course of post-mortem changes in muscle. The results reported here show that the initial pH and the initial levels of glycogen, lactic acid and ATP in resting muscle taken from the live animal are very different from those obtained if the muscle is removed after slaughter. Similarly, post-mortem glycolysis in the resting muscle taken from the live animal is different to that in muscle removed after death. The entire pattern of post-mortem glycolysis observed in the latter type of muscle resembles that section of the pattern attained after glycolysis had proceeded for several hours in muscle not subjected to the death reaction.

Observations by McLoughlin (unpublished) indicated that the major component of the death reaction appeared to be contraction of the musculature at death. When curare was given just before slaughter, thus preventing muscle contraction, the initial pH was substantially elevated and the rate of pH

fall was slowed. The differences found here between muscle from slaughter animals and muscle from live animals supports this contention. So also does the observed effect of stimulation of excised muscle. Direct stimulation of pig muscle did not merely have the transient effect on glycolysis generally associated with direct stimulation but caused a continued acceleration of glycolysis. This observation is in accord with those of Hallund and Bendall (1965) and Bendall (1966) and suggests that pig muscle responds to stimuli in a peculiar and, as yet, obscure way which has the overall effect of continually increasing the rate of post-mortem glycolysis.

In conclusion, two general points can be made. The first is that if one wishes to obtain absolute biochemical values for post-mortem glycolytic changes in pig muscle, and the influence of ante-mortem treatments on such changes, then the effect of the death reaction should be eliminated either by removal of muscle from the live animal under anaesthesia or the use before slaughter of substances, such as curare, which prevent neural stimuli entering the muscle. In the latter case, it would, of course, be necessary to give oxygen to the animal and to maintain respiration artificially.

The second point is that, because of its effect on post-mortem glycolysis, slaughter may very markedly influence the colour and water-holding of fresh pork meat. This may be especially important where breeds of pig particularly prone to develop pale, soft, exudative muscle are involved.

Fourteenth European Meeting of Meat Research Workers,
Brno, Czechoslovakia, August 25-31, 1968.

ANAEROBIC GLYCCLYSIS IN MUSCLE TAKEN IMMEDIATELY POST-MORTEM AND FROM LIVE PIGS UNDER ANAESTHESIA.

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SUMMARY.

Post-mortem changes in pig longissimus dorsi muscle were studied at 37°C under nitrogen. Muscle was taken (i) from live animals under anaesthesia and (ii) from animals immediately after slaughter. High initial pH values (7.0) and slow rates of pH values were found in muscle taken from the live animal; the initial levels of lactic were low, the initial levels of glycogen high and the half-life of ATP about 4 hr.

Slaughter considerably altered the pattern of post-mortem changes. The initial pH was low (6.3). Glycogen levels were reduced to about 50% of those found in muscle from anaesthesised animals and the

lactic levels were considerably elevated. The half-life of ATP was about 1½ hr.

Excised sections of longissimus dorsi were made to contract by means of electrical stimulation and were compared with unstimulated control sections. Stimulation lowered the initial pH somewhat and had a very marked accelerating effect on the rate of post-mortem changes. This effect was continuous over the entire course of post-mortem glycolysis.

It appears (a) that the death reaction can have a very dramatic effect on post-mortem changes in muscle and (b) that pig muscle responds to direct stimulation in a curious way, i.e., by a continued acceleration of glycolysis.

References

Bendall, J.R., 1960 in "Structure and Function of Muscle", Vol. 111, Ed. G.H. Bourne, Acad. Press.

Bendall, J.R., 1966, J. Sci. Fd. Agric., 17, 333.

Bendall, J.R., Cuthbertson, A. and Gatherum, D.P., 1965, Paper read to the Eleventh European Meeting of Meat Research Workers, Belgrade.

Bendall, J.R. and Wismer-Pedersen, J., 1962, J. Fd. Sci., 27, 144.

Borchert, L.L. and Briskey, E.J., 1964, J. Food Sci., 29, 203.

Borchert, L.L. and Briskey, E.J., 1965, J. Food Sci., 30, 138.

Briskey, E.J., 1964, "Advances in Food Science", Vol. 13, Acad. Press Inc., N.Y., p. 89.

Briskey, E.J. and Wismer-Pedersen, J., 1961, J. Food Sci., 26, 297.

Eliott, R.J., 1965, Nature (Lond.), 206, 315.

Goldspink, G. and McLoughlin, J.V., 1964, Irish J. Agr. Res., 3, 9.

Ludvigsen, J., 1954, Beretn. fra forsøglab. (Kbhn.), No. 272.

McLoughlin, J.V., 1963a, Irish J. Agr. Res., 2, 27.

McLoughlin, J.V., 1963b, Irish J. Agr. Res., 2, 115.

McLoughlin, J.V., 1965, Irish J. Agr. Res., 4, 151.

McLoughlin, J.V. and Davidson, V.G.J., 1966, Irish J. Agr. Res., 5, 55.

Sayre, R.N. and Briskey, E.J., 1963, J. Food Sci., 28, 675.

Sayre, R.N., Para, J. and Briskey, E.J., 1966, J. Food Sci., 31, 189.

Wismer-Pedersen, J., 1959, Food Res., 24, 711.

Wismer-Pedersen, J. and Briskey, E.J., Nature (Lond.), 189, 318.