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A study of the post-mortem pH changes in the muscles of Danish Landrace pigs, in relation to the occurrence of watery pork.

by J.R.Bendall[x], O. Hallund and J. Wismer-Pedersen

Danish Meat Research Institute, Denmark

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During the last ten years many workers in different countries have attempted to discover the cause of the pale and watery meat-structure which is more or less frequently found in pig carcases. The phenomenon occurs in most countries which produce large numbers of pigs and has been variously described as 'muskel-degeneration', white muscle disease, 'myopathie exudative depigmentaire du porc', two-toned pork and watery pork. Although many articles describing the physical and chemical differences between the normal and watery pork have been published, there is still a great deal of confusion on the subject, which mainly arises because the various authors do not all mean the same thing by the names they have given the condition. We must therefore make clear that what we understand by watery pork is a pale and watery meat structure, which is usually found only in those carcases which have a low pH (≤ 6.20) in the musculature $\approx$ 45 mins after death, particularly in the long. dorsi and biceps femoris muscles. Ludvigsen (1954, 1955, 1957) was the first to show that low pH values after death were an almost invariable symptom of the condition and this has subsequently been confirmed by many observations in Danish slaughterhouses (Wismer-Pedersen 1959). We must, however, emphasise that it is impossible to predict from the behaviour or the appearance of the pigs before death which will give watery meat and which will not, since all of them appear healthy and well-fed. Watery pork can occur in most breeds of pigs, but is much more prevalent in some breeds than in others.

The basis of the phenomenon is still very imperfectly understood, and has been variously attributed to hormonal imbalance, particularly lack of thyroxin and ACTH (Ludvigsen 1954, 1955, 1957); to a lack of deoxy-corticosterone, which controls the K/Na balance in the blood and cells (Lawrie 1960); to excitement immediately before slaughter (Wismer-Pedersen 1959) and to stress combined with enzyme and hormonal defects (Henry et al. 1958). Most of these explanations are rather unsatisfactory, particularly those which attribute the condition to a generalised stress syndrome, first because stress has exactly opposite effects in other animals such as the bullock (Howard & Lawrie 1957) and the rabbit (Bendall 1960), where it usually produces a high ultimate pH and very firm and dry meat, and secondly because the behaviour of those pigs which will later yield watery meat in no way suggests that they were in a condition of stress just before death. Moreover, there is no evidence that the muscles of the watery carcases were in any way abnormal or 'degenerated' before they went through

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[x] Present address; Low Temperature Research Station, Cambridge

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Results

(1) pH/time curves

The results of the experiments at a constant temperature of 37°C showed that the pigs could be divided into two groups, A and B, with reference to all the observed post-mortem physical and chemical changes. Group A was characterised by low, and group B by high rates of change. In the present paper, we will report only the pH changes. Table 1 summarises the initial (pH_{10}) values, the maximum rates of fall and the ultimate pH values. Fig. 1 shows how the pH changes with time at 37°C, for the two groups, A and B, of table 1. Mean values are given and the vertical lines represent the standard errors of the means.

We note that the initial pH values (pH_{10}) are nearly identical for the two groups (6.79 for A and 6.62 for B), the difference between them becoming quite insignificant when the curves are extrapolated back to zero time, because of the higher rate of fall of group B. From fig. 1, we see that in group A the pH falls slowly (0.25 units/hour) until it reaches 6.5, after which the rate increases to a maximum of 0.65 units/hour at pH 6.0 and then rapidly declines again. After 270 mins the ultimate pH has been reached. In group B, on the other hand, the rate is high from the start and reaches a maximum at pH 6.0 of 1.05 units/hour after only 70 mins. The ultimate pH is reached in this group after 150 mins. Put in another way, it takes group A 170 mins and group B only 88 mins to reach pH 5.7, the lowest pH at which the rate is still high and constant in both groups. It is also clear that the rate of fall is quite independent of the ultimate pH, which is the same in both groups (5.39). The marked differences in rate between the two groups must, therefore, be due to some other factor or combination of factors.

(2) Temperature coefficient (Q_{10}) and energy of activation of glycolysis

When the rates of pH fall in samples of long. dorsi muscle from the same pig, are compared at temperatures of 36 and 41°C, it is found that the average Q_{10} in this temperature range is 2.70 with a standard deviation of ± 0.13 (6 estimations). There is no significant difference between the Q_{10} values for the fast and slow portions of the pH/time curves, nor does the Q_{10} depend on the absolute rates of change, whether of type A or B. The average apparent energy of activation of the processes of glycolysis, calculated from the Hood-Arrhenius equation, is 19 K. cals per g. mole lactic acid, from 36 to 41°C.

(3) Comparison of pH values calculated from time curves with pH_1 values of the carcase.

When we compare the pH values reached after ½ hour in the samples of excised muscle at 37°C with the pH_1 values obtained from the carcases by probe electrode measurement, we find from fig. 1 that the mean value for group A is 6.68 compared with the mean pH_1 value of 6.45, and for group B it is 6.30 compared with the mean pH_1 of 6.08. Considering the very different way in which the laboratory samples were handled, the small discrepancies

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the long-drawn out processing necessary in the manufacture of bacon. Quite on the contrary, Briskey and Wismer-Pedersen (1961) were able to show that if a sample of muscle were removed from the animal immediately after death and cooled rapidly to room temperature it was invariably of normal appearance and stucture, whether the carcase from which it was taken subsequently showed a watery structure or not. The corollary also applies, that meat heated for ½ to 1 hour at 37°C at low pH ($\ll$ 6.00) will invariably become watery (Briskey and Wismer-Pedersen 1961). Similarly, meat cut quickly from the freshly killed carcase will be normal if allowed to go into rigor at 20°C, but watery if allowed to go into rigor at 37°C providing the ultimate pH is below $\approx$ 5.90 (Bendall and Wismer-Pedersen 1962).

Thus we may conclude that it is the combination of a low pH with a high temperature in the carcase which is the principal cause of watery structure, and it is precisely these conditions which lead to denaturation of a part of the muscle proteins, and consequent loss of water-binding ability. It has, in fact, been shown (Bendall and Wismer-Pedersen 1962) that it is mainly the 'soluble' sarcoplasmic proteins which are denatured in this way and that this denatured protein becomes deposited upon, and bound to, the contractile actomyosin filaments of the muscle fibril, where it appears as irregular cross-bands within the muscle membrane (Bendall, King and Wismer-Pedersen 1962). This reduces the extractability and water-binding capacity of the actomyosin filaments, so that they lose water to the surrounding sarcoplasmic fluid, which is already more dilute than normal, due to the loss of about 30% of its protein to the filaments.

The problem therefore resolves itself into discovering the cause of the low pH values shortly after death. These could be due either to a high production of lactic acid during the death struggle, or to an intrinsically high rate of anaerobic glycolysis, or to a combination of both. The object of the present study was to determine the rates of the post-mortem physical and chemical changes in the long. dorsi muscles of Danish Landrace pigs at a constant temperature of 37°C, with particular reference to the pH changes, so as to be able to distinguish between these possibilities.

Methods

The pigs used in these experiments were all healthy well-fed animals of Danish Landrace, of live weight 85 to 95 Kg. Within 5 minutes after slaughter by stunning and sticking a sample of $\sim$ 500 g was cut from the long. dorsi muscle at the last rib and brought quickly to the laboratory. The carcase from which the sample had been taken was allowed to go through the normal slaughtering procedure, and at ½ hour after slaughter the pH values of its long. dorsi muscles were measured in the region of the last rib by probe electrodes. Next day the ultimate pH was measured and the meat quality judged with reference to wateriness and colour. From the samples brought to the laboratory a piece of $\sim$ 30 g was cut immediately and placed in an atmosphere of moist N_2 at 37°C for the measurement of pH values at 20 mins' interval by the maceration procedure of Bendall and Davey (1957). In cases where the Q_{10} of the rate of pH-fall was to be measured samples were held at 36 and 41°C, and sampled as above. In some cases the buffering capacity of the meat was measured, after homogenisation in 0.1 M KCl/0.004 M sodium iodoacetate, by titration with 0.1 N NaOH at intervals from pH 5.4 to 7.2.

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So far as our faster group B is concerned, we have shown that its rate of pH fall is sufficiently high to account for the low pH_1 values observed in those carcasses which yield watery meat, because the mean temperature of the carcase during the first hour after death is $> 37^{\circ}C$. Even exceptionally low pH_1 values of 5.70 or below could be accounted for, if it were assumed that such carcasses had a slightly lower initial pH (pH_{10}) than normal, or that their mean temperature was slightly higher than normal. In fact, some pigs, whether belonging to groups A or B, have been found during this work, which show considerably lower pH_{10} values than those given in table 1. If these pigs were to belong to the fast group B, they would correspond to the very fast group 4 pigs of Briskey and Wismer-Pedersen's classification (1961). The slower groups, 1 to 3, defined by these authors, could then be reckoned to belong either to our slow group A or to group B, according to the rates.

It is of some interest that groups, corresponding to our A and B, can often be discerned when distribution curves of large numbers of pH_1 values are drawn. Thus it is a common feature of such curves to show two maxima, one of which lies between pH 6.2 and 6.5, corresponding to the slow group A, and the other at 5.5 to 5.8 corresponding to the fast group B. The actual position of the maxima on the pH scale is to some extent dependent on the season of the year and to the particular conditions in the slaughter-house.

The underlying reason for the two markedly different rates of pH fall cannot be decided from the results of the present experiments alone, although these enable us to eliminate certain possibilities. For instance, the difference in rate is not due to differences in the buffering-capacity of the muscles, as Ludvigsen (1955) proposed, because no significant difference can, in fact, be shown between the two groups. Similarly temperature differences can be eliminated, because the present experiments were carried out at a constant temperature of $37^{\circ}C$, and also differences in initial pH and in initial creatine phosphate (CP) content, because these did not differ sufficiently to account for the different rates. We must, therefore, conclude that the differences are due to real differences in the activity of the enzymes responsible for the various changes. This in itself however, is an exceedingly complicated question in the case of a tissue such as muscle, because the activity of its enzymes is governed to such a large extent by electrical and chemical changes at the boundary membrane, the sarcolemma. To illustrate the importance of the membrane, we may cite the example of thaw-rigor, where damage has been caused to it by quick-freezing in the pre-rigor condition. Depending on the rate of thawing, the subsequent rate of pH fall and of the other chemical changes can increase to 10, 20 or even 50 x the maximum rate observed in normal rigor at $37^{\circ}C$, although control over the rates of the reactions is quickly re-established once thawing is complete (Bendall 1960). On these grounds alone it is tempting to suggest that the higher rates of pH fall, characteristic of group B pigs, are due to slight changes during life in the delicate salt balance at the membrane interface, for which only a very small shift in Na ions inwards or K ions outwards would be necessary (Sandow, quoted by Bendall 1960). A similar idea has been suggested by Ludvigsen (1955), although it was founded upon the incorrect assumption that the salt changes, observed in watery meat after rigor was complete, represented the actual state of affairs in the living animal.

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To assess the relevance of these ideas, we must first decide on the nature of the primary reaction which determines the rate of breakdown of glycogen to lactic acid under anaerobic conditions. There is little doubt that this is the splitting of ATP to ADP and inorganic phosphate, the latter being used to phosphorylate glycogen in the first step of glycolysis and the former being resynthesised to ATP at two of the later steps in the cycle (Bendall 1960). The enzyme responsible for the splitting reaction has by no means been decided, but it seems likely that it is mainly a sarcoplasmic ATP-ase at temperatures below $\approx 30^{\circ}C$, where the energy of activation of the processes is known to be low (< 6 K.cals per mole), whereas at higher temperatures the main fibrillar ATP-ase of the muscle seems to become more and more implicated, because the energy of activation rises quickly to a value of ≈ 20 K.cals, characteristic of it. It is primarily this enzyme also, and not the sarcoplasmic ATP-ase, which is extremely easily activated by salt changes at the membrane, and because of its very high intrinsic ATP-ase activity only a slight change would be necessary to account for the differences in rate between groups A and B. Experimental proof of this idea could be obtained by studying the energy of activation over a wide range of temperature, and research is in progress along these lines.

Speculating even further, one might suppose on the basis of the above arguments, that the most likely hormone to be involved would be deoxycorticosterone which controls the Na/K balance in the body. If so one might expect that adrenalectomised animals would show extreme examples of high rates of pH fall, and even of watery meat, if the pH could fall low enough. Experiments along these lines are in progress.

Summary

A study of the post-mortem pH changes at constant temperature in the long. dorsi muscles from Danish Landrace pigs showed that the pigs could be divided into two groups, A and B, which differed with regard to maximum rates of pH fall. Maximum rate in group A was 0.65 pHu/h and maximum rate in group B was 1.04 pHu/h. Q_{10} for the pH fall was found to be 2.70 ± 0.13 in the interval 36 - 41 °C. The meat from the carcases belonging to group A was of good colour and quality, whereas meat from group B carcases was of watery and pale appearance. No difference could be found in buffering capacities of meat from good or watery pigs. It is suggested that the difference in rate of pH fall is due to a slight activation of the myofibrillar ATPase.

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OH/AT

Table 1

Group A

Pig No.	pH_{10}	pHu/h	pH ultm. sample 37°C
1	6.75	0.53	5.43
2	6.80	0.73	5.47
6	6.80	0.78	5.38
D	6.84	0.60	5.36
F	6.79	0.57	5.37
5/10	7.02	0.66	5.34
17/10	6.80	0.60	5.50
26/10	6.60	0.66	5.23
30/10	6.72	0.76	5.42
	6.79	0.654	5.39

Group B

S4	6.58	.06	5.34
S5	6.51	0.85	5.35
SB	6.80	1.17	5.43
SH	6.73	1.02	5.40
SI	6.70	1.08	5.34
23/10	6.40	1.00	5.45
	6.62	1.03	5.39

Figure 1.