PREDICTION OF THE RATE OF *POST MORTEM* pH FALL IN PIG MUSCLE BY ^{31}P NMR MEASUREMENTS ON A MUSCLE BIOPSY.

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INTRODUCTION

The rate of *post mortem* pH fall in pig muscle is of prime importance with respect to pork quality (Briskey and Wismer-Pedersen,1961; Briskey, 1964). Very fast pH fall often occurs and leads to production of pale soft exudative (PSE) meat. So techniques for prediction of metabolism intensity in muscle after slaughter have been matter of intensive research for many years. The techniques which have been proposed can be classified in three groups: 1/ determination of blood enzyme activities, such as creatine kinase or lactate dehydrogenase (Hessel de Heer, 1969; Addis and Kallweit, 1969); 2/ determination of metabolites (Schmidt *et al.*,1971), enzyme activities or other biochemical traits on muscle biopsies (for reviews, see Hennebach *et al.*,1983 and Lahucky, 1987); 3/ halothane test (Eikelenboom and Minkema,1974; Christian, 1974) and blood typing (for a review, see Andresen,1985). Blood creatine kinase measurement, blood typing and halothane test have been extensively used to select pigs against stress susceptibility and PSE-proneness.

The metabolism of phosphorylated energy-rich compounds in a muscle fragment separated from an animal body and kept anoxic can be supposed to mimic that will take place in the same muscle after slaughter. ^{31}P nuclear magnetic resonance (NMR) allows to easily follow the metabolism of phosphorus compounds and the pH changes in biological samples, particularly muscle (Gadian, 1980).The present experiment was a preliminary study about the possibility to predict intensity of *post mortem* metabolism in pig muscle and ultimate meat quality from measurements of metabolism in a muscle biopsy.

MATERIAL AND METHODS

Animals, biopsy sampling and treatment

Ten pigs (5 Large Whites, 1 Large White x Landrace and 4 Pietrains) of about 90 kg liveweight were anaesthetized using ketamine (Imalgene,Rhone Merieux) and sodium pentobarbital (Pentobarbital sodique, Sanofi). A biopsy of around 10 g was taken from the *Biceps femoris* (BF) muscle. This muscle was chosen because of its superficial location and easiness of sampling. The biopsy sample was divided into 3 parts: one part was immediately crushed in liquid nitrogen using iron tongs for measurement of ATP; another one was homogenized in 1M perchloric acid for determination of R value (R 0') according to Honikel and Fischer (1977); the third part was dipped into paraffin oil, then it was introduced in a 10 mm diameter tube for NMR measurements. The latter was submitted to NMR measurements till 1 h after biopsy taking then used for determination of R value (R 60').

The ^{31}P NMR spectra were recorded at 162 MHz on a Bruker AM400 spectrometer The probe temperature was maintained at 30°C. The total ^{31}P spectrum was recorded with a sweep width of 8400 Hz and a 45° pulse of 7.5µs. The recycle time was 2 s. Each spectrum was a result of 224 transients. A maximum field homogeneity was obtained by adjusting the shims to obtain a half width of the phosphocreatine signal of ≈ 20 Hz. An exponential line broadening of 20 Hz was applied before Fourier transformation.

For ATP determination, muscle tissue was freeze-dried, then homogenized in 8 M guanidine ; ATP was determined by bioluminescence (kit 567 736, Boehringer).

Slaughter and meat quality measurements

Three weeks after the biopsy, the pigs were killed by electrostunning and exsanguination. Samples were taken from the *Longissimus dorsi* (LD) 45 min after death for immediate determination of pH and R value (respectively pH_1 and R_1). pH was measured after homogenization of 2 g of muscle in 18 ml of 0.005 M iodoacetate .

The day after slaughter, carcasses were cut and some meat quality traits were determined on LD and BF muscles. pH (pH_2) was determined directly on muscle tissue. Reflectance was assessed using a Manuflex 2 reflectometer. Water holding capacity (WHC) was measured according to the paper imbibition technique (Charpentier *et al.*, 1971).

Calculations

Absolute quantification of phosphorylated compounds from NMR spectra in a sample of intact tissue is generally made from the ATP level biochemically determined on a similar sample. This was not possible here, because NMR measurements began 4 to 13 min after biopsy sampling (time necessary to put the sample in the spectrometer and adjust the magnetic field). During this time, it seemed impossible to us to quantify the more or less pronounced evolution of ATP .To express the rate of change of the phosphorylated compounds,we used the ratio of either ATP or phosphocreatine (PC) - both decreasing with time - to the sum of inorganic phosphate (Pi) and sugar-phosphates (SP) - both increasing with time. These ratios give an index of the rate of catabolism of energy-rich compounds.As measurements were not begun at a constant time after taking the biopsy, the values of the ratios at 20 and 30 min were obtained by interpolation. These values were used in the calculations of correlation coefficients between NMR parameters and meat quality traits.

RESULTS AND DISCUSSION

Muscle ATP levels showed little variations between animals at the time of biopsy : mean: 4.8 µmol/g; s.d.: 0.5; minimum: 4.4; maximum: 5.2 .There was no significant difference between Large Whites(mean: 4.9) and Pietrains (mean: 4.7).

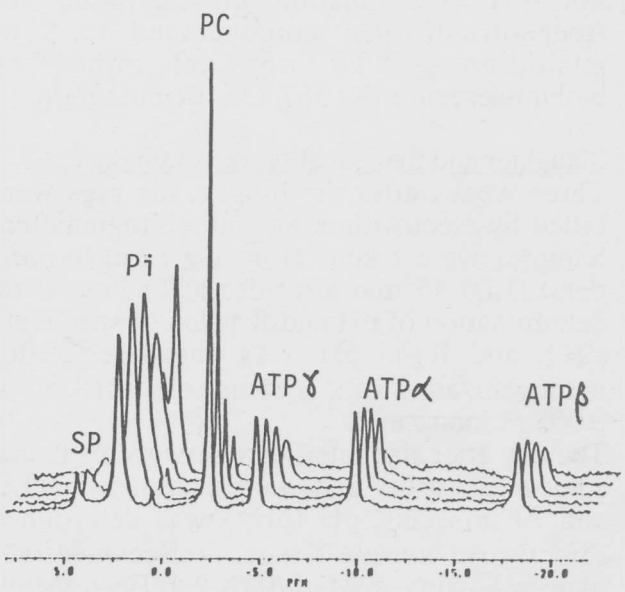

Fig.1. Series of spectra obtained from a biopsy between 15 and 55 min after taking the sample. *Each spectrum corresponds to 10 min of observation.*

Fig. 1 shows a series of spectra obtained from a biopsy between 15 and 55 min after taking.The rate of changes in pH and phosphorus compounds was very variable from a biopsy to another as illustrated in fig.2 and fig.3. In every case PC and ATP decreased with time, whereas Pi and to a lesser extent SP increased, as it is well-known in anoxic muscle (see Bendall, 1973). In animal a of fig.2, there was still a high level of PC at the beginning of the NMR measurements and ATP level was kept high till the end; conversely, in animal b, PC was almost exhausted 9 min after taking the biopsy and subsequently ATP disappeared very quickly.

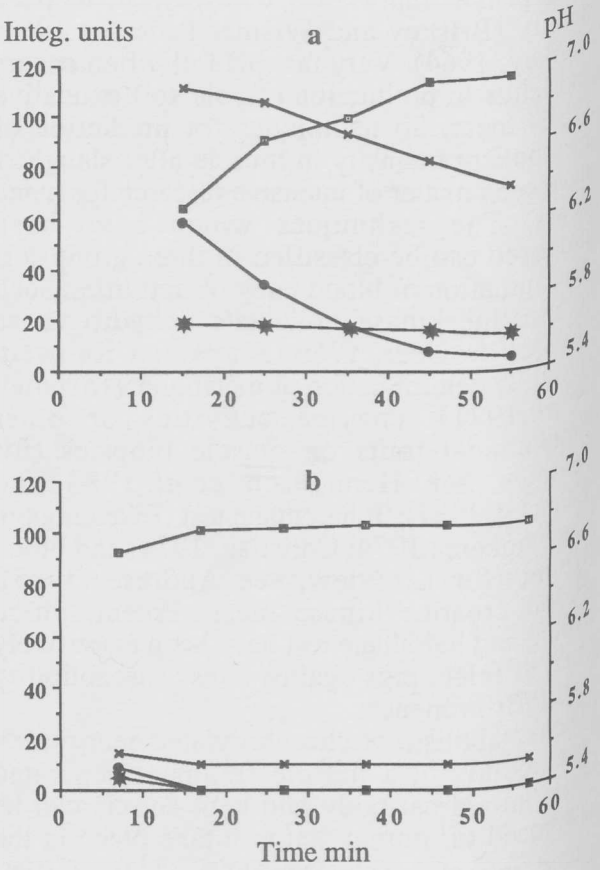

Fig.2. Evolution of pH, ATP, PC and Pi in biopsies from a Large White pig (a) and from a Pietrain pig (b).
pH —×— ATP —✱— PC —●— Pi —▬—
Each point was derived from a 10 min spectrum. Times in abscissa correspond to the half-times of spectra; time 0: taking the biopsy. As phosphorus compounds were not quantified, they were expressed in integration units of the spectrometer.

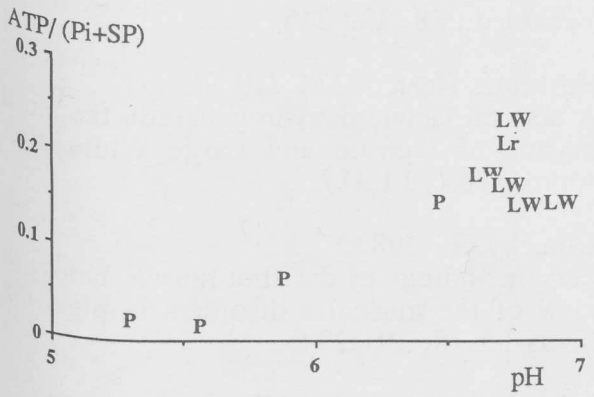

Fig.3. pH and ATP/ (Pi+SP) values at 20 min after taking the biopsy.
LW : Large White; Lr : crossbred; P : Pietrain

Correlations between NMR measurements or R values in the biopsy on the one hand, and rate of *post mortem* pH fall or meat quality traits on the other hand, are reported in table 1. It appears that the parameters calculated from NMR measurements are well correlated with the indicators of *post mortem* pH fall rate, i.e. pH_1 and R_1.Some significant correlations are observed between pH as determined on biopsy and meat quality traits, particularly WHC and reflectance of BF muscle. By contrast, R values were poorly related with *post mortem* pH fall or meat quality. Other researchers found much higher correlations between R values and meat quality traits (Lahucky,1987), but they incubated the biopsy at 37-39°C before R determination; this difference in techniques could explain the discrepancy between their results and ours.
Among the Pietrain pigs, 3 pigs showed a very high metabolism rate in the biopsy , whereas the fourth had a metabolism rate similar to that observed in Large White pigs (see fig.3). It can be hypothetized that the former were halothane-positive and that the latter was halothane-negative. ^{31}P NMR measurements on a muscle biopsy could perhaps be a tool for a diagnosis test of halothane sensitivity. Renou *et al.* (1989) previously showed that ^{1}H NMR study of muscle biopsies can be used to identify halothane-sensitivity.

CONCLUSION

In the present study, ^{31}P NMR compared well as meat quality predictor with the biochemical methods applied on biopsies by other researchers (Schmidt *et al.*,1971; Pfeiffer *et al.*, 1981; Lahucky, 1987). This technique appears promising to identify PSE-proneness and perhaps halothane- sensitivity, although its practical interest is presently limited by its high cost. Also, the biopsy technique used here is expensive, time-consuming and harmful for the animals; the possibility of using the shot biopsy method (Schöberlein,1976; Talmant *et al.*,1989) is currently under investigation.

	pH1 LD	R 1 LD	pH 2 LD	pH 2 BF
pH 20'	**0.85**	**-0.84**	-0.23	0.16
pH 30'	**0.84**	**-0.83**	-0.24	0.16
PC 20'	**0.88**	**-0.85**	-0.29	0.23
PC 30'	0.26	-0.32	0.52	0.10
ATP 20'	**0.79**	**-0.79**	-0.31	0.20
ATP 30'	**0.78**	**-0.80**	-0.05	0.48
R 0'	-0.20	0.27	**-0.60**	-0.14
R 60'	-0.43	0.38	**0.74**	-0.23

	Reflec. LD	Reflec. BF	WHC LD	WHC BF
pH 20'	-0.25	**-0.64**	**0.62**	**0.63**
pH 30'	-0.18	**-0.66**	**0.65**	**0.68**
PC 20'	-0.23	**-0.72**	0.48	0.59
PC 30'	-0.21	-0.40	0.58	0.11
ATP 20'	-0.45	**-0.72**	0.30	0.42
ATP 30'	-0.42	**-0.83**	0.39	0.29
R 0'	0.23	0.33	-0.52	-0.03
R 60'	-0.20	0.34	-0.53	-0.48

Table 1. Correlations between NMR measurements and some meat quality traits.
Reflec.: reflectance. Standard characters: non significant; bold: $P< 0.05$.

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