# 31P NMR STUDY OF POST MORTEM CHANGES IN PIG MUSCLE

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## INTRODUCTION

Post mortem changes affecting energetic metabolism in pig muscles have been extensively investigated. It is well-known that pH evolution faithfully reflects both extent and intensity of post mortem metabolism. A fast rate of pH fall leads to pale soft exudative (PSE) muscle through extensive protein denaturation (Bendall & Wismer-Pedersen, 1962). Limited pH change gives rise to dark firm dry (DFD) meat. Metabolic changes were studied using biochemical techniques (Briskey & Wismer-Pedersen, 1961; Kastenschmidt et al., 1968; Charpentier, 1968) and more recently 31P NMR (Vogel et al., 1985; Renou et al., 1986). The latter technique is of great interest, because it allows to make successive measurements on a single sample, thus avoiding the part of imprecision inherent to serial sampling. It is possible to get from a single NMR spectrum the pH and the concentrations of the main phosphorylated metabolites involved in energetic metabolism, namely inorganic phosphate (Pi), adenosine-triphosphate (ATP), creatine-phosphate (CP) and phosphomonoesters (PME). Swedish researchers extensively studied post mortem metabolism in muscle from cattle, pigs and sheep undergoing various technological traetments, using 31P NMR (Vogel et al., 1985; Lundberg et al., 1987) The present study was designed to investigate in more details the changes occuring after slaughter in muscle of pigs giving PSE or DFD meat, due to genetic disposition or pre-slaughter treatment.

## MATERIAL AND METHODS

### Animals

Fifteen pigs, i.e. 7 Large White pigs and 8 Pietrain pigs, were used. Five pigs of each breed, referred to as control pigs, were slaughtered taking care to minimize preslaughter stress . The other pigs (3 Large Whites and 2 Pietrains) were injected i.m. with 0.1 mg adrenaline per kg liveweight before slaughter, in order to increase meat ultimate pH. All the animals were killed by electronarcosis and exsanguination.

### Sampling and determinations

As soon as possible after slaughter, a sample of Longissimus dorsi muscle was taken from each animal at the level of the first lumbar vertebra. This muscle sample was divided into 3 parts: i/ one part was dipped in paraffin oil then put into a 10 mm diameter NMR tube for NMR measurements; ii/ another part was crushed in liquid nitrogen ; iii/ the third part was homogenized in 0.005 M iodoacetate for pH determination by combined glass electrode.
The part kept in liquid nitrogen was extracted by homogenization in 0.6 M perchloric acid, centrifugation and neutralization of the supernatant using 3 M $K_2CO_3$. The extract was then used for ATP determination by bioluminescence.
Around 24 h after slaughter, ultimate pH was measured directly in Longissimus dorsi muscle using a combined glass electrode.

### NMR measurements

31P NMR spectra were recorded at 162 MHz on a Bruker AM400 spectrometer. Field frequency and proton decoupling were not required. The field homogeneity was obtained by

optimization of the water proton spectrum of the muscle. Each spectrum was an average of 224 scans accumulated in a total time of 10 min with a recycle time of 2 s. 45° pulse angles, a sweep width of ± 3000 Hz and an exponential line broadening of 20 Hz were used. NMR measurements began between 17 and 32 min after slaughter and lasted 2 h.

Calculations

ATP, PC, SP and Pi were estimated from NMR spectra and biochemical ATP determination; pH was deducted from NMR spectra using chemical shift of inorganic phosphate.

## RESULTS AND DISCUSSION

Three of the adrenalin-treated pigs (1 Large White and 2 Pietrains) gave meat with ultimate pH above 6. The pigs with normal ultimate pH, i.e. 6 Large Whites and 6 Pietrains, had very variable rates of post mortem muscle metabolism. At 30 min post mortem, according to the NMR data, pH varied from 6.04 to 6.66 and ATP varied from 1.1 to 6.8 µmol/g. From the distribution of pH and ATP at this time (figure 1), animals were divided into 2 groups: pigs with a pH above 6.2 and ATP level above 4 µmol/g were considered as having normal muscle, the other ones were considered as having muscle with an abnormally fast rate of post mortem metabolism (PSE-prone muscle).

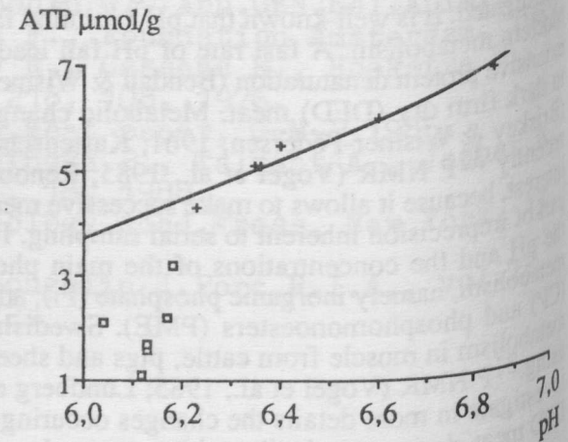


*Fig. 1. Relationship between pH and ATP level at 30 min post mortem*

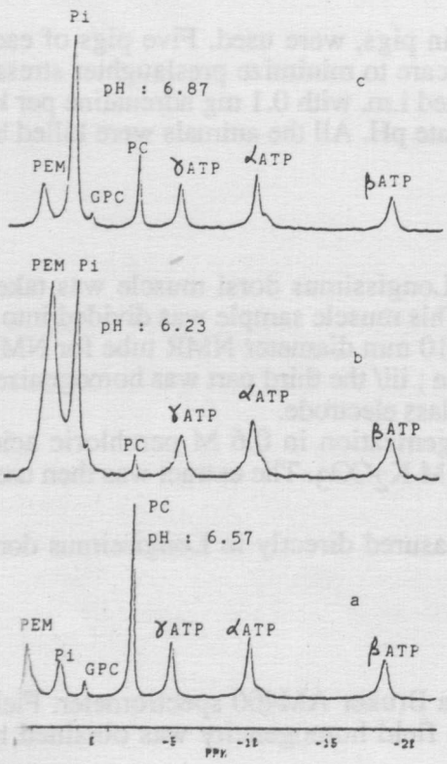


Figure 2a shows a typical NMR spectrum obtained around 30 min post mortem from a normal muscle. It was possible to distinguish 7 peaks, among them 6 peaks corresponding to the main phosphorylated metabolites involved in the energetic metabolism, i.e. from left to right: the PME (mainly sugar-phosphates), the Pi, the PC and the γ, α and β phosphate groups of ATP. The signal between the Pi and PC resonances was assigned to glycerophosphorylcholine. Figure 2b shows a NMR spectrum obtained from a PSE-prone muscle: ATP and PC were very low, whereas PME level was high and showed little change during the period of measurement. Figure 2c shows a spectrum from a DFD muscle. Pi level was very high, whereas PME content was low.

*Fig. 2. Examples of spectra from: (a) normal muscle; (b) PSE-prone muscle; (c)DFD muscle.*

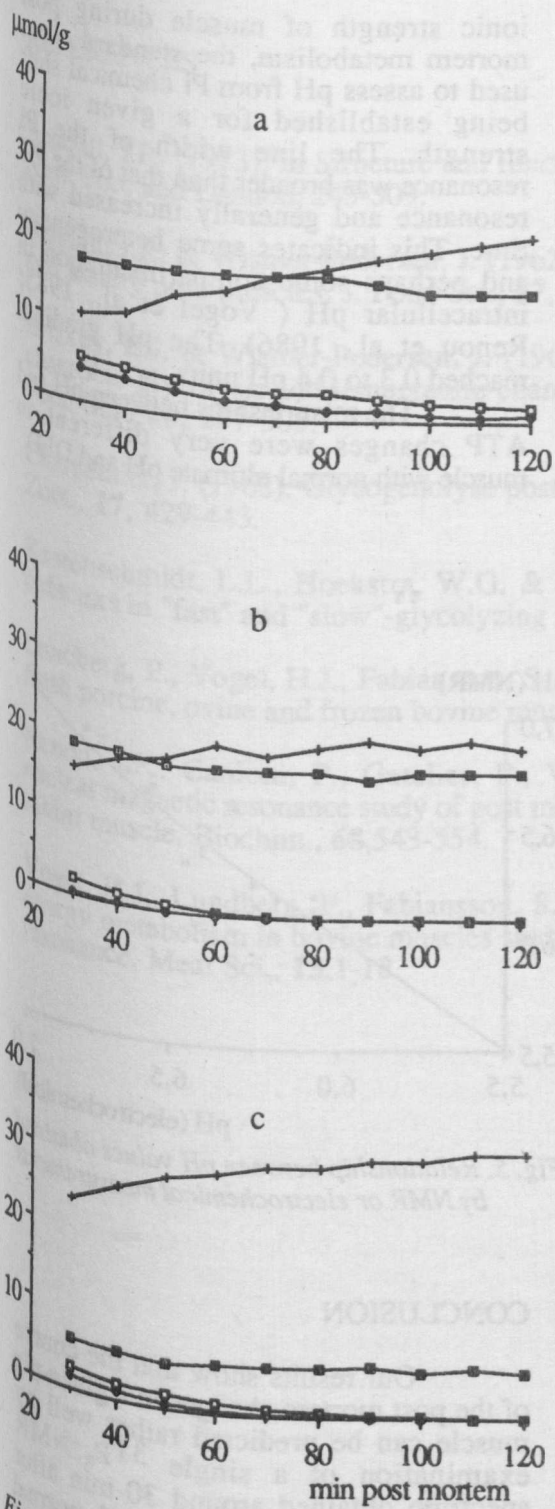


*Fig. 3. Post mortem changes in phosphorylated metabolites*
*(a) normal*
*(b) PSE-prone*
*(c) DFD*

The post mortem changes in the levels of the phosphorylated metabolites according to muscle condition (normal, PSE-prone or DFD) are described in figure 3. In normal muscle, ATP and PC decreased slowly, while Pi increased steadily (figure 3a). In PSE-prone muscle, ATP and PC were quickly exhausted; Pi level increased as long as ATP and PC declined (figure 3b). In DFD muscle, changes in ATP, PC and Pi were similar to those observed in PSE-prone muscle, although Pi was initially markedly higher (figure 3c). PME levels were similar in normal and PSE-prone muscles, and in both cases much higher than in DFD muscle; they underwent very small change with time whatever the muscle condition. In normal muscle, pH decreased from 6.5 at 30 min post mortem to approximately 6,1 at 120 min, i.e. a rate of around 0.27 pH unit per hour (figure 4). The PSE-prone muscle had a low pH at 30 min post mortem i.e. 6.1, it decreased to approximately 5.9 at 120 min post mortem. At the same time, in DFD muscle, pH was high i.e. 6.6 at 30 min post mortem and showed little change with time.

The total level of phosphorylated metabolites detected by NMR stayed almost constant during post mortem metabolism at values of 38 to 39 µmol/g. This value was the same as that found by Vogel et al. in beef Longissimus dorsi muscle, which was 38 µmol/g, but was lower than the value of 54 µmol/g given by Bendall (1973) for the same muscle. Vogel et al. (1985) explained the lower value obtained by $^{31}P$ NMR by the fact that a part of Pi contained in muscle at the end of post mortem metabolism is likely to be sequestred in cell organelles and so remain undetected by NMR.

Figure 5 illustrates the comparison between the pH values obtained by either NMR or electrochemical measurements. There was a good agreement above 6.2, but below 6.2 the NMR values were systematically higher than the electrochemical values. This phenomenon was previously described by Vogel et al. (1985). It can be at least partly attributed to: i/ local heterogeneity in pH values within the muscle tissue, Pi resonance allowing to estimate only intracellular pH, ii/ to the known change in

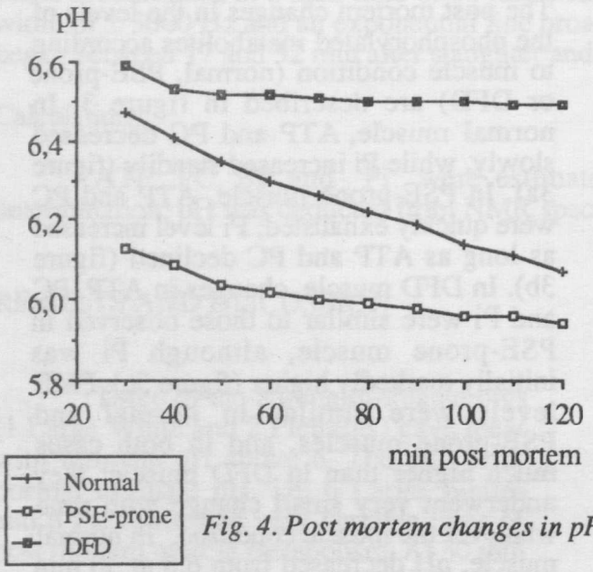


*Fig. 4. Post mortem changes in pH*

muscle (figure 6). In the former, ATP disappeared at pH values between 5.9 and 6.2 whatever the rate of pH fall. In the latter, ATP almost completely disappeared (level < 0.5 µmol/g) at a pH value around 6.5, corresponding to the ultimate pH. This is due to the fact that glycogenolysis is ended and so there is no more ATP resynthesis, when ultimate pH is reached.

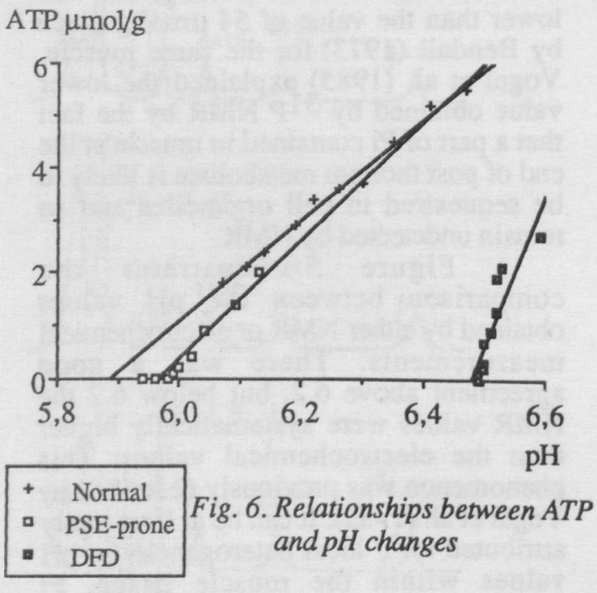


*Fig. 6. Relationships between ATP and pH changes*

ionic strength of muscle during post mortem metabolism, the standard curve used to assess pH from Pi chemical shift being established for a given ionic strength. The line width of the Pi resonance was broader than that of the PC resonance and generally increased with time. This indicates some heterogeneity and perhaps some compartmentation in intracellular pH ( Vogel et al., 1985; Renou et al., 1986). The pH gradient reached 0.3 to 0.4 pH units.

The relationships between pH and ATP changes were very different in muscle with normal ultimate pH and DFD

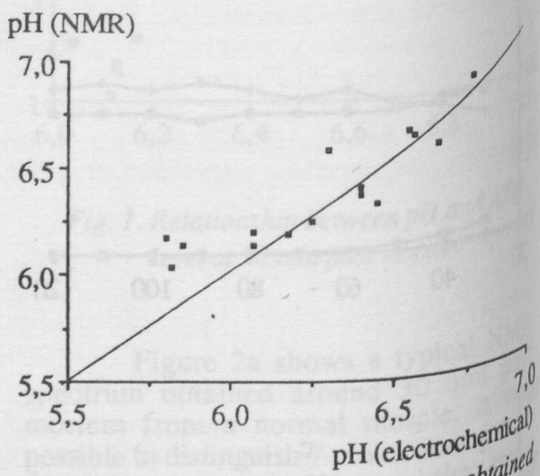


*Fig. 5. Relationship between pH values obtained by NMR or electrochemical measurements*

## CONCLUSION

Our results show that the course of the post mortem changes in a given pig muscle can be predicted rather well by examination of a single $^{31}P$ NMR spectrum obtained around 30 min after death. At this time, muscle with normal rate of metabolism simultaneously showed medium to high pH, high ATP level and rather low Pi level; muscle with fast rate of metabolism (PSE-prone muscle) had low pH, low to medium ATP level and generally high SP level; muscle with high ultimate pH (DFD-prone muscle) had high pH, low SP level and high Pi level .

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