EFFECT OF BRINE INJECTION ON ENERGY CATABOLISM : P-31 NMR STUDIES ON *BICEPS BRACHII* RABBIT MUSCLE

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MARY

atabolism of high energy phosphate compounds was examined by P-31 NMR spectroscopy, in rabbit muscle. The time-dependent *post* w changes were analyzed for different technological treatments. The rabbits were anaesthetized, and the *Biceps Brachii* muscle was ed, perfused by artery. The simulated death of the muscle was performed by stopping the perfusion. The brine was immediately ed into the arterial system. NMR experiments are performed at 162 MHz on a home-built P-31, H-1 tuned probe. The temperature in NMR probe was maintained either at 20° or 4°C. Thus, it is possible to keep muscle metabolism stable, and to monitor the initial ntrations of phosphorylated metabolites. The rate of pH fall and Pcr were determined. The results showed a dependence on the osition of the brine.

TRODUCTION

ng of *pre-rigor* meat induces a higher Water Holding Capacity. Hamm (1977) pointed out that salt accelerated the breakdown of ATP. im of this work is to study, by P-31 NMR spectroscopy, the catabolism of high energy phosphate compounds in muscle injected with e. The time-dependent *post mortem* changes, for different technological treatments, are analyzed.

TERIAL AND METHOD

Animals

e rabbits were anaesthetized using intravenous administration of sodium pentobarbital (50 mg/kg). The *Biceps Brachii* muscle (73% of twitch glygolytic fibres) was exposed, axillary arteries were ligated and cut before the Krebs perfusion initiated by artery. The rate of fusion was 0.15 ml/min/g muscle. The muscle was removed to be placed in the specifically designed probe. The simulated death of the scle was performed by stopping the perfusion. The brine (5 M or 3 M NaCl) was immediately injected 15% w/w into the arterial system.

NMR Experiment

MR spectra were acquired on a Bruker AM 400 wide-bore ectrometer operating at 162 MHz using a home-built double tuned e, designed to perform experiments with perfused muscle. The o radio-frequency coils are made of copper film on a removable rt. The ^{31}P and ^{1}H saddle coils have diameters of 28 mm and 22 m respectively . The matching and tuning capacitors are at the top of e main part of the probe. The muscle was fixed onto a removable pport placed at the head of the probe. Temperature was controlled (20 or 4°C) with a water jacket placed at the top of the NMR probe.

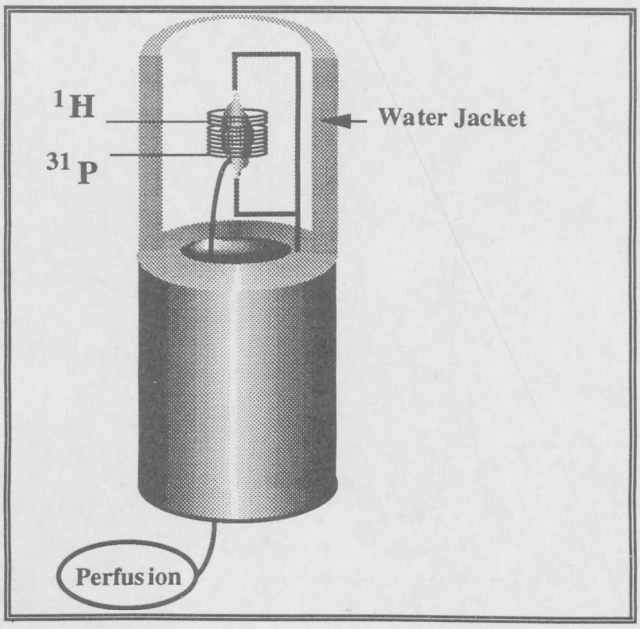


Fig 1 : NMR probe for perfused muscle →

Maximum field homogeneity was obtained by optimization of the water proton spectrum of the muscle to obtain the half width of about 3
Hz. Each spectrum is an average of 256 scans accumulated over a total time of 7 minutes, with 8 K points. 10° pulse of 20 µs and a
bandwidth of 6200 Hz were used. Data manipulation were carried out on a SUN4 work-station. An exponential line-broadening of 20 Hz
was applied before Fourier transformation. Chemical shifts were expressed with respect to phosphocreatine.

RESULTS AND DISCUSSION

Stability of muscle

With this experimental system, it was possible to keep muscle metabolism stable, and thus to monitor the initial concentrations of
phosphorylated metabolites. Some traits of catabolism were investigated without producing twitching or damage to the tissue during
insertion into NMR tubes. The whole muscle was examined in the probe.The figure 2 shows the P-31 NMR spectrum from *Biceps Brachii*
muscle perfused for 40 minutes. The five peaks correspond to phosphorylated metabolites : inorganic phosphate (Pi) phosphocreatine (PCr)
and γ, α, β phosphate group of ATP. The 7.2 pH value measured from the chemical shift of Pi agrees with the pH measured *in vivo*
(Bendall 1973).

Post mortem changes and intracellular acidosis

The *post mortem* pH variation was well fitted with a linear regression program giving a pH drop rate of 1.9 x 10^{-3} pH unit /min at 20°C and
1.7 x 10^{-3} at 4°C. When temperature was lowered, a decrease in pH rate was observed in agreement with the work of Bendall (1973b). For
salted muscles, the pH experimental data were always best fitted with a non linear regression program. The figure 3 shows the pH change
during the first 500 min *post mortem* at 4°C. Temperature affects the pK values of the muscle buffers (carnosine) and the pH is expected to

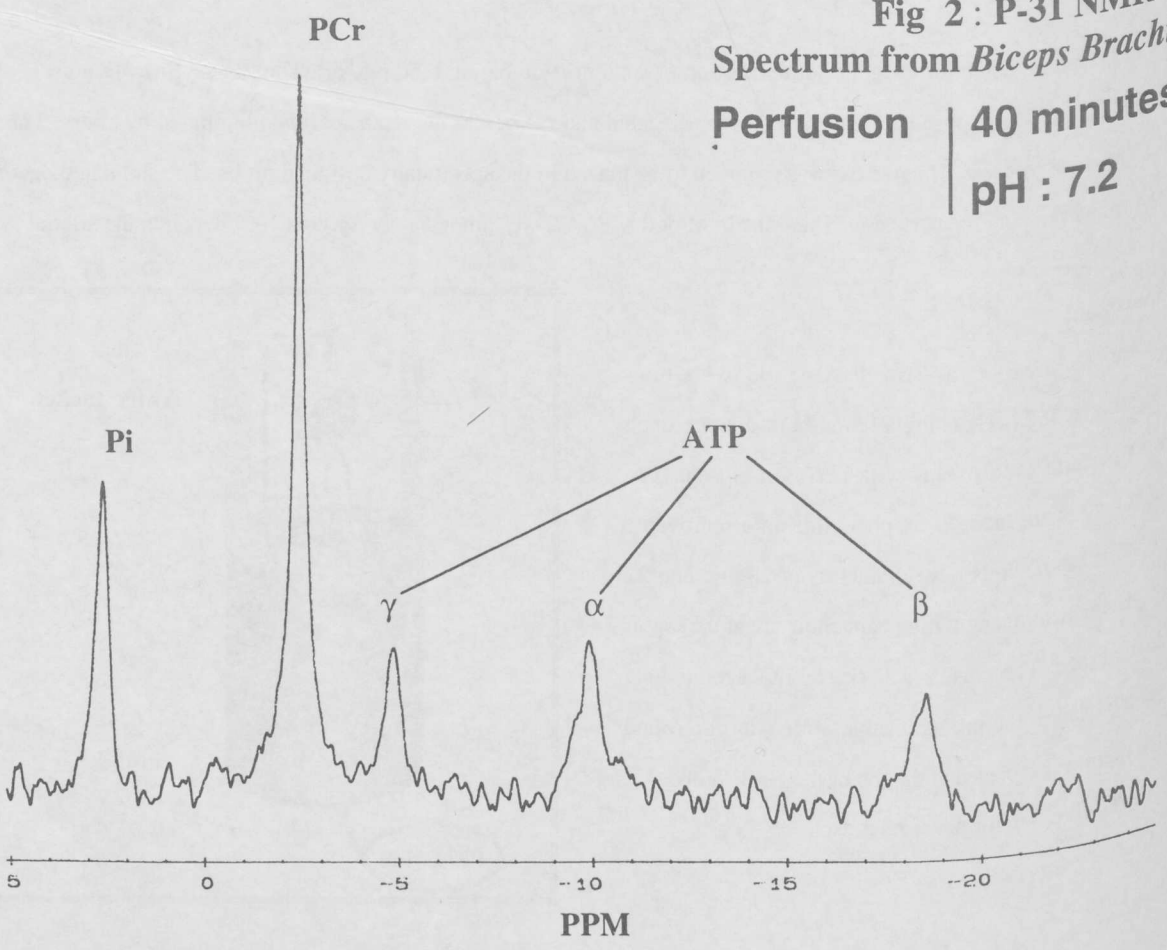
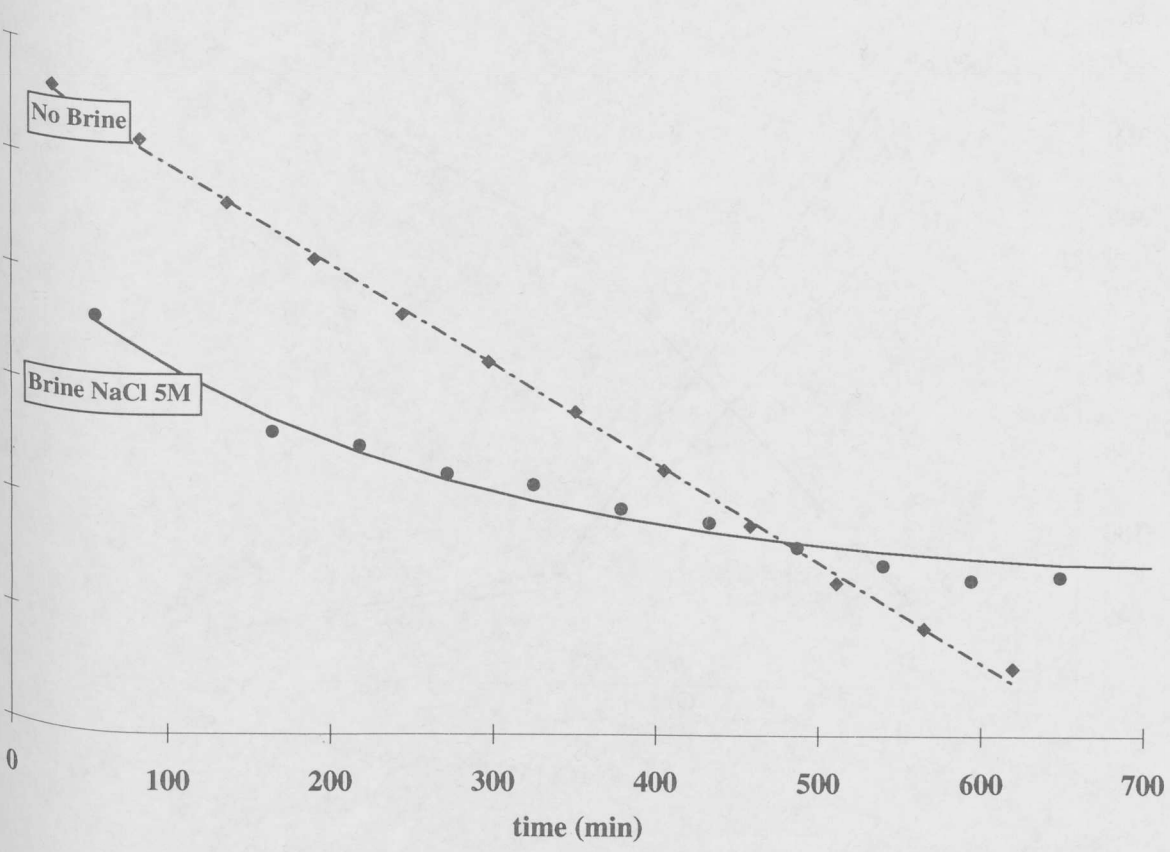


Fig 2 : P-31 NMR Spectrum from *Biceps Brachii* Muscle
Perfusion | 40 minutes
pH : 7.2

Fig.3 : Brine effect on intramuscular post mortem pH variation (4°C)

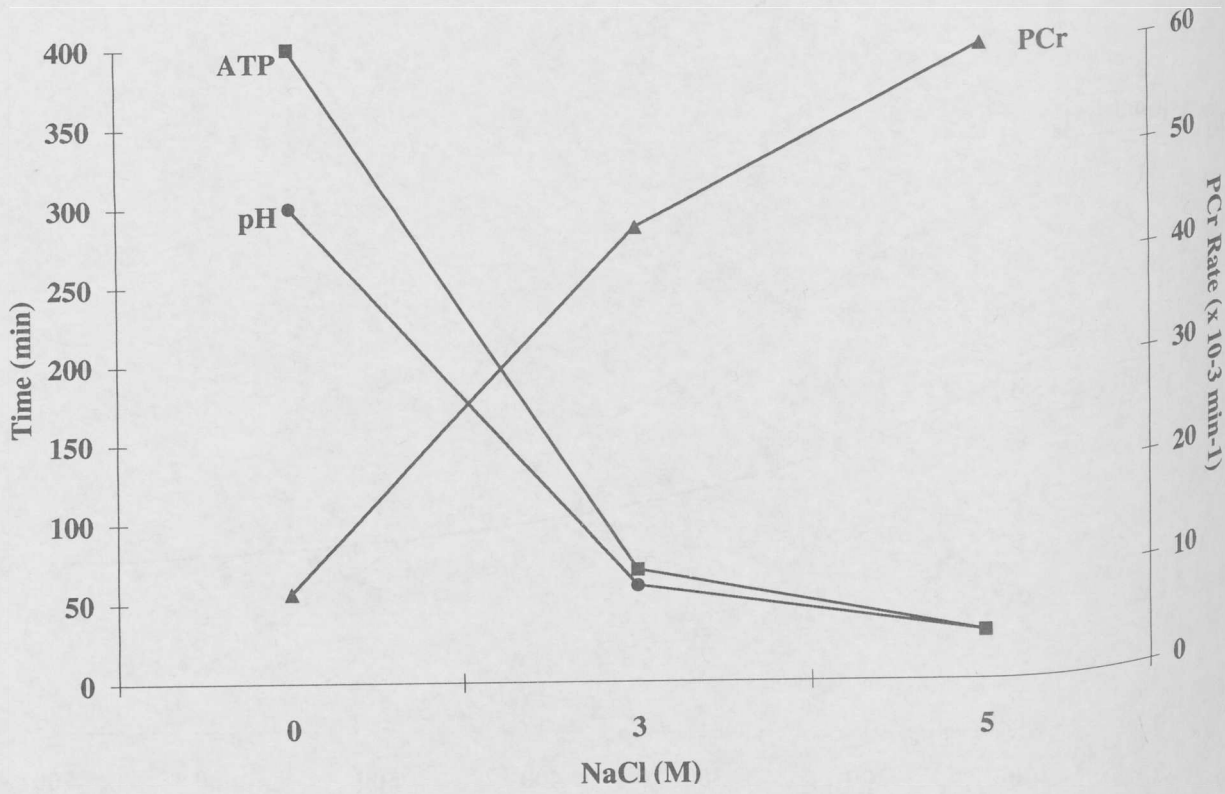


y about 0.2 units on lowering the temperature from 20 to 0°C. To permit comparison of the data, the time for pH to reach 6.4 (20°C) or
4°C) is determined and reported in table 1. This time decreases drastically with the injection of brine and is dependent on the NaCl
ntration (Figure 4). Dalrymple & Hamm (1974) noted a slight increase in the rate of lactate build-up during the first hours *post mortem*
o change in the rate of glycogen breakdown in ground salted pre-rigor beef muscle. In *post mortem* muscle, glycogen is broken down
h mainly responsible for the formation of lactic acid which accounts for the pH decline. In our experimental conditions, our NaCl
ntration (3 & 5M) in brine was 10 times higher than in their experiments (0.3M) and the increase in NaCl increased the pH drop rate.
ming that ATP level remains constant in the presence of phosphocreatine, the rate of PCr drop was also informative of glycolysis rate.
g these experiments, the ATP, and PCr levels were followed (Fig.4). The PCr data were fitted with a mono exponential decay giving a
which increased with NaCl concentration in brine (Table 1 and fig. 4). The ATP variation is described by its time of half life, which
ased with brine injection. Our results on ATP breakdown are in agreement with Hamm (1977) who noted an increase in the rate of
kdown of ATP into IMP with the addition of 2% NaCl to ground pre-rigor beef muscle.

Table 1 : Effect of temperature and NaCl Concentration on *post mortem* kinetics parameters

Temperature	20°C			4°C		
[NaCl]	Time (pH=6.4) (min)	ATP $t_{1/2}$ (min)	$R_{Pcr} \times 10^{-3}$ (min^{-1})	Time (pH=6.6) (min)	ATP $t_{1/2}$ (min)	$R_{Pc} \times 10^{-3}$ (min^{-1})
0 M	300	400	8.4	600	500	3.8
5 M	30	< 30	> 60	300	150	8.9

Fig.4 : Effect of NaCl concentration on post mortem kinetics parameters (20°C)



CONCLUSION

The energy metabolism in *ex vivo* muscle can be monitored with the designed NMR probe. The level of high-energy phosphorylated metabolites in *ex vivo* muscle can be determined and the *post mortem* changes were determined for different technological processes. The NaCl concentration in brine increased the catabolism : increase in pH fall rate, in ATP and PCr breakdown. The temperature induced changes in the kinetics of *rigor mortis* onset. Further studies are in progress to confirm and extend these experiments.

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