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Influence of Temperature Variation on the Metabolism of Pig Muscle *in situ* and After Excision

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ABSTRACT

The experiment involved six halothane-positive (HP) and six halothane-negative (HN) pigs of about 100 kg *liveweight. Under general anaesthesia the* tibialis cranialis *was exposed. The temperature of the muscle was maintained at either* 35 *or* 40°C (in situ). *The muscle was stimulated via the peroneal nerve at* 0·1 Hz *for* 10 min *then* 1 Hz *for* 10 *further min. Twitch contractions were recorded throughout the stimulation, after which the muscle was excised and split into two parts which were kept in paraffin oil at either* 35 *or* 40°C *(post-excision temperature) for* 2 h. *Samples were taken just before and after the* 20 min *stimulation period, and at* 1 *and* 2 h *after muscle excision for determination of pH and concentrations of PC, ATP, IMP, glycogen, G-6-P and lactate. As soon as one muscle was removed, the operation was repeated on the other leg. Both temperatures* (35 *and* 40°C) *were applied to each animal in a balanced design. However data were obtained from only* 11 *pigs at* 40°C *as one HP pig died accidentally at the beginning of the* 40°C *experiment.*

Halothane sensitivity influenced the pH *value and the levels of PC, G-6-P and lactate of the muscle* in situ (P < 0·01 *in all cases). Temperature affected contraction time* (P < 0·01). *Both halothane sensitivity and post-excision temperature affected the* pH *values and the levels of PC, ATP, IMP, G-6-P and lactate* (P < 0·01 *in all cases) in the excised muscle. By contrast, the* in situ *temperature treatments did not affect any of the muscle*

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traits measured after excision. It was concluded that the effects of the various treatments on the rate of metabolism in the excised muscle were wholly explainable in terms of temperature from the time of excision, and that the in situ *temperature treatments may not be responsible for the differences after excision.*

INTRODUCTION

Several authors have shown that an increase in the body or muscle temperature of pigs at the time of slaughter has unfavourable effects on *post mortem* muscle metabolism and meat quality (Sayre *et al.*, 1963; Monin, 1973; Gariepy *et al.*, 1989; Warriss, 1991; Klont, 1994). However, the biochemical mechanisms underlying these effects have not been investigated in detail. It remains a question whether the *post mortem* acceleration of muscle metabolism is due purely to the activating effect of temperature on biochemical reactions, or if it is elicited by the continuation of the action of *ante mortem* physiological factors. Indeed, changes in the body temperature are generally induced by placing the animal in stressful situations, and so they are associated with other physiological disturbances susceptible to affect muscle metabolism, such as circulatory and respiratory changes (Forrest *et al.*, 1968; Charpentier *et al.*, 1971: Judge *et al.*, 1973) as well as hormonal changes (Aberle, 1974; Lundström, 1976). The effects of catecholamines administered before slaughter on the rate of *post mortem* metabolism are controversial (Bendall & Lawrie, 1962; Hedrick *et al.*, 1964). Monin *et al.* (1992) concluded from experiments with perfused isolated rabbit muscle that catecholamines released before slaughter are unlikely to have any significant influence. On the other hand, temperature variations in the physiological range above the normal resting level affect markedly the rate of the energy metabolism in anoxic muscle independently of any hormonal or nervous influence (Klont *et al.*, 1994).

There is some difficulty in assessing specifically the metabolic effects of temperature variations in the muscle tissue of live animals. Klont *et al.* (1994) used isolated muscle strips to overcome this difficulty. However, with the latter technique, energy metabolism is markedly disturbed in muscle from halothane-positive pigs. An other way is to use the experimental preparation developed by Campion *et al.* (1972). In this preparation, the *tibialis cranialis* muscle is exposed while keeping its natural nerve and blood supply. The temperature can be modified locally without influencing the temperature of the rest of the body; the muscle can be stimulated to contract and biopsy samples can be obtained to establish the metabolic changes. The aim of the present experiment was to assess the effects of temperature variations on the metabolism of the muscle *in situ* and of the muscle made anoxic by excision. The *tibialis cranialis* was prepared as described above. It was stimulated to work isometrically at a rate inducing a moderate fatigue. The effects of a temperature rise of 5°C above the normal resting value (about 35°C according to Quinlan *et al.*, 1986, confirmed by preliminary measurements) on contraction and metabolic traits were investigated. Then the muscle was excised and split in two parts which were placed in anoxic conditions at 35°C and 40°C, and biochemical changes were studied for 2 h.

MATERIAL AND METHODS

Animals and muscle preparation

Six Belgian Landrace pigs and six Large White × Dutch Landrace crossbred pigs were used. All Belgian Landrace were halothane-positive (nn) and all crossbred were halothane-negative (NN) according to a DNA-test (Fujii *et al.*, 1991) practised on DNA purified from blood according to Te Pas and Erkens (1994).

The animals were fasted for about 16 h before they were transported to the laboratory for the experiment. They were injected intramuscularly with azaperone (Stresnil, Janssen Pharmaceutica, 4 mg/kg liveweight) about 30 min before being anaesthetized by an intravenous injection of sodium pentobarbital (Sanofi, 10 mg/kg liveweight). The animals were intubated and ventilated with N_2O/O_2 (2/1). An intravenous pentobarbital infusion was applied *via* an ear vein to maintain anaesthesia. The hind legs were tightly fixed in order to prevent any movement during the experiment and the *tibialis cranialis* was exposed and dissected free from surrounding muscles. The distal tendon was cut and attached to a force transducer (Sedeme type AC, 20 daN) with a non compliant chain. Two platinum electrodes were put on the peroneal nerve. Two type T thermocouples were inserted inside the muscle (one from the outer side and the other from the inner side) and the temperature was displayed on an electric thermometer (Comark Electronics) throughout the experiment. Finally, the muscle was covered with parafilm in order to avoid desiccation.

Physiological measurements

The temperature of the muscle was brought to either 35 or 40°C by an infrared lamp, and kept at the same temperature (±0·5°C). Twitch contractions were elicited by stimulation of the peroneal nerve through the platinum electrodes (stimulator model 302, WP Instruments Inc.; stimulation: duration 1 ms; frequency 0·1 Hz; voltage 50 V). The electric signal was amplified (amplifier W1532R, Beta) and recorded using a MAC II microcomputer equipped with a MacAdios card. Recording began 20 ms before the stimulation pulse and was performed for 500 ms at a frequency of 1000 Hz. The length-twitch tension relationship was established from 12 twitch contractions and the subsequent measurements were made at the optimal length. The nerve was stimulated at 0.1 Hz for 10 min, then at 1 Hz for 10 further min. Twitch contractions were recorded every 30 s. Two muscle samples of 50–100 mg were taken from the surface of the muscle just before and just after the stimulation period for determination of pH and metabolites. After the second sampling, the muscle was carefully excised, weighed and split into two parts. Each part was put into paraffin oil at either 35 or 40°C ('post-excision temperature'). The time between the end of stimulation and putting the muscle into oil was less than 5 min. Samples of 50–100 mg for determination of pH, and samples of 200–300 mg for determination of metabolites were taken at 60 and 120 min after excision. As soon as one muscle was excised, the operation was repeated on the other leg. Both temperatures (35 and 40°C) were applied to each animal in a balanced design.

Analytical techniques

Contraction parameters were computed using the MacAdios programme. Peak tension, contraction time and half relaxation time were determined. Peak tension was expressed in relation to the cross-sectional area of the *tibialis* approximated according to Quinlan *et al.* (1986), i.e. cross area = 0·8 $(\text{weight})^{3/2}$

At sampling, one muscle sample was put into an Eppendorf plastic tube and dipped into liquid nitrogen. The other one was put into 0·5 ml of 0·005 M Na iodoacetate in a glass microextractor (Radnoti, Bioblock) and immediately crushed with a glass pestle. The pH was measured with a glass microelectrode (Ingold 104023522 or 104063123). On eight samples, the homogenate was added five times with 0·5 ml of iodoacetate and the pH was determined after each of the five additions, to assess the pH change induced by the variations of the muscle/liquid ratio.

The frozen samples were freeze-dried and stored at –20°C under vacuum. They were dissected free of fat, blood and connective tissue and crumbled. Ten to 30 mg of tissue was put in each of two Eppendorf tubes with 1 ml perchloric acid and the mixture was homogenized using a pulverizing machine (Retsch MM2). From one tube, 0·5 ml of homogenate was taken for determination of glycogen (Dalrymple & Hamm, 1973). The rest was centrifuged at 2500 *g* for 10 min and used to determine G-6-P and lactate (Bergmeyer, 1974). The other tube was centrifuged (2500 *g* for 10 min) and the supernatant was neutralized (pH 6·5–7) with 2·5 M K_2Co_3 for determination of phosphorus compounds.

Phosphocreatine (PC), adenosine triphosphate (ATP) and inosine monophosphate (IMP) were separated and quantified by HPLC (Gilson Medical Electronics). Samples were diluted 10 times with eluent and injected automatically. Elution was carried out under isocratic conditions on a reversed phase C18 column (Dynamax 4·6 × 150 mm, 5 μm ODS, Rainin Instruments Co.) guarded with a C18 precolumn (4·6 × 50 mm), with a flow rate of 1·25 ml/min. The eluent was 100 mM NH4 dihydrogenphosphate (pH 6·5) with 0·05% tetrabutylammonium hydroxyde (Aldrich Chemie) and 2% acetonitrile. Metabolites were detected at 212 and 254 nm. Data were recorded and computed using the Gilson software.

Statistics

Data were obtained from 12 muscles for the 35°C *in situ* temperature and from 11 muscles for the 40°C *in situ* temperature (six halothane-negative and five halothane-positive animals, as a halothane-positive pig died by accident at the beginning of the 40°C experiment). They were analyzed by variance analysis, data before and after muscle excision being analyzed separately. Factors in the analyses were 'halothane status', '*in situ* temperature' and 'time' for the data before excision, and the same plus 'post-excision temperature' for the data after excision. When significant interactions were found, differences between temperatures and times were assessed by t-test. All calculations were carried out using the Statview SE + Graphics programme.

RESULTS

The temperature of the *tibialis cranialis*, in anaesthetized pigs before muscle dissection, was 34·2 ± 0·2°C in halothane-negative pigs and 34·5 ± 0·3°C in halothane-positive pigs.

TABLE 1
Effects of Genetic Type, *in situ* Temperature and Time on Contraction Traits in Pig *Tibialis cranialis*

		*PT (kN/m²)**	*CT (s)*	*1/2 RT (s)*
Genetic type†	HN	31·3	53	56
	HP	28·8	53	59
Temperature (°C)	35	29·1	57^{A}	58
	40	31·2	50^{B}	57
Time (min)	0	$34·6^{A}$	55	55^{A}
	10	$33·6^{A}$	51	53^{A}
	20	$22·0^{B}$	55	65^{B}

*PT: peak tension; CT: contraction time; 1/2 RT: half-relaxation time.
†HN: halothane-negative; HP: halothane-positive.
^{A,B}: means with different superscripts in the same source within a column are significantly different at the $P < 0·01$ level.

The changes in contraction parameters during muscle work are illustrated in Table 1 and Fig. 1. The genetic type did not influence significantly any trait. Muscle contracted faster at 40°C than at 35°C. The peak tension remained approximately constant during stimulation at 0·1 Hz whatever genetic type and temperature, then it decreased during stimulation at 1 Hz. The temperature affected only the contraction time. The half-relaxation time increased during 1 Hz stimulation. There were interactions between genetic type and time for the peak tension ($P < 0·01$) and the contraction time ($P < 0·05$) (Table 2). The peak tension decreased more in halothane-positive pigs than in halothane-negative pigs (–50% vs –19%, $P < 0·01$) during 1 Hz stimulation. The contraction time tended to decrease in halothane-negative pigs while it tended to increase in halothane-positive pigs ($P < 0·10$) with time.

The influence of dilution of the muscle homogenate in iodoacetate on the measured pH values is illustrated in Fig. 2.

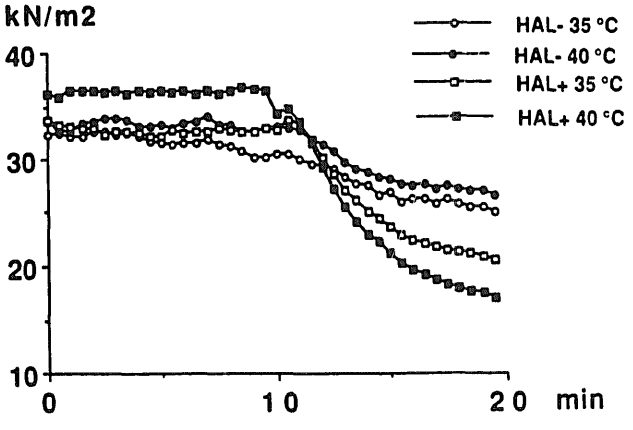

Fig. 1. Changes in peak tension during the stimulation period. 0–10 min: stimulation at 0·1 Hz; 10–20 min: stimulation at 1 Hz. HAL–: halothane-negative; HAL+: halothane-positive.

TABLE 2
Genetic Type × Time Interactions on Peak Tension (PT) and Contraction Time (CT) in Pig *Tibialis cranialis*

		PT (kN/m^2)		*CT (s)*	
*Genetic type**		*HN*	*HP*	*HN*	*HP*
Time (min)	0	$34{\cdot}3^{a}$	34.9^{A}	57	53
	10	$32{\cdot}8^{a}$	$34{\cdot}5^{A}$	51	52
	20	$26.6^{b,C}$	$17{\cdot}1^{B,D}$	50^{c}	60^{d}

*HN: halothane-negative; HP: halothane-positive.
Means with different superscripts in the same line or the same column are significantly different. Effect of time: ^{a}, ^{b}, $P < 0{\cdot}05$; ^{A}, ^{B}, $P < 0{\cdot}01$; effect of genetic type: ^{c}, ^{d}, $P < 0{\cdot}05$; ^{C}, ^{D}, $P < 0{\cdot}01$.

The biochemical characteristics measured from the muscle *in situ* are reported in Table 3. The phosphocreatine content was significantly higher while the G-6-P and lactate contents were lower in halothane-negative pigs than in halothane-positive pigs. The pH value was higher in halothane-negative pigs. The temperature had no effect on any trait except for the lactate values from halothane-positive pigs. The phosphocreatine and glycogen contents and the pH value decreased during the muscle stimulation, while the contents of lactate, G-6-P and IMP increased. There was a significant interaction between genetic type and temperature ($P < 0{\cdot}05$) for the lactate content (Table 4) which was higher in halothane-positive pigs than in halothane-negative pigs at 40°C.

In excised muscle, the levels of most compounds and the pH values were influenced by the genetic type. the post-excision temperature and the time (Table 5). Again, the *in situ* temperature had no effect on any trait. There was an interaction between genetic type and *in situ* temperature for the lactate content (Table 4). Interactions between genetic type and post-excision temperature were found for IMP ($P < 0{\cdot}01$), and G-6-P ($P < 0{\cdot}05$) (Table 6). The former was more

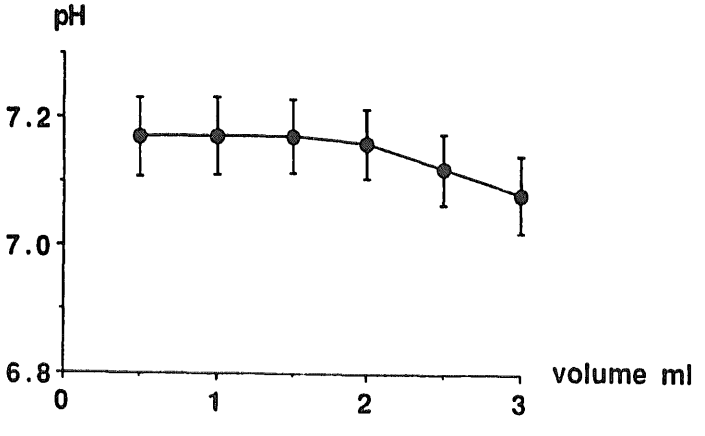

Fig. 2. Changes in pH following dilution of the muscle homogenate. Results are given as means ± S.D. of eight samples. Volume: volume of 0·005 M iodoacetate added to the muscle sample (50–100 mg).

TABLE 3
Effects of Genetic Type, *in situ* Temperature and Time on Biochemical Traits in Pig *Tibialis cranialis in situ*

		*PC**	*ATP*	*IMP*	*Glycogen*	*G-6-P*	*Lactate*	*pH*
Genetic type†	HN	20^{A}	7·9	1·0	45	$0·14^{A}$	11^{A}	$7·05^{A}$
	HP	15^{B}	6·3	1·2	40	$0·66^{B}$	18^{B}	$6·90^{B}$
Temperature (°C)	35	19	7·8	1·0	47	0·52	12	7·00
	40	16	6·5	1·2	38	0·27	16	6·95
Time (min)	0	22^{A}	8·0	$0·8^{A}$	50^{a}	0·44	8^{A}	$7·03^{a}$
	20	13^{B}	6·4	$1·5^{b}$	36^{B}	0·36	21^{B}	$6·96^{b}$

*All compounds are expressed in μmol/g fresh tissue.
†HN: halothane-negative; HP: halothane-positive.
Means with different superscripts in the same source within a column are significantly different: ^{a}, ^{b}, $P < 0·05$; ^{A}, ^{B}, $P < 0·01$.

TABLE 4
Genetic Type × *in situ* Temperature Interactions on Lactate Content in *Tibialis cranialis* Before and After Excision

		Before		*After*	
*Genetic type**		*HN*	*HP*	*HN*	*HP*
Temperature (°C)	35	11	13^{a}	35^{C}	$47^{a,D}$
	40	11^{c}	$23^{b,d}$	32^{C}	$54^{b,D}$

*HN: halothane-negative; HP: halothane-positive.
Means with different superscripts in the same line or the same column are significantly different. Effect of temperature: ^{a}, ^{b}, $P < 0·05$; ^{A}, ^{B}, $P < 0·01$; effect of genetic type: ^{c}, ^{d}, $P < 0·05$; ^{C}, ^{D}, $P < 0·01$.

increased at 40°C in halothane-positive pigs than in halothane-negative pigs. Glucose-6-phosphate was lower at 40°C than at 35°C in halothane-positive pigs and the reverse was observed in halothane-negative pigs.

DISCUSSION

The values found here for the peak tension agreed with those reported by Quinlan *et al.* (1986) for the halothane-negative pigs, but were lower for the halothane-positive pigs. They were lower than those reported by Campion *et al.* (1974) for both stress-resistant and stress-susceptible animals. These variations could result from the differences in the age of the animals among studies, as the metabolic and contractile expression of halothane sensitivity has been reported to vary with age (Kozak-Reiss *et al.*, 1985). The pigs studied by Quinlan *et al.* (1986) were 14 weeks old and the age was not indicated in the work of Campion *et al.* (1974). Both groups of authors found significantly higher tensions in halothane-positive

TABLE 5
Effects of Genetic Type, *in situ* and Post-Excision Temperatures and Time on Biochemical Traits in Excised Pig *Tibialis cranialis*

		*PC**	*ATP*	*IMP*	*Glycogen*	*G-6-P*	*Lactate*	*pH*
Genetic type†	HN	$1{\cdot}8^{A}$	$5{\cdot}2^{A}$	1·4	26	$0{\cdot}49^{A}$	34^{A}	$6{\cdot}36^{A}$
	HP	$0{\cdot}3^{B}$	$1{\cdot}8^{B}$	2·5	21	$0{\cdot}29^{B}$	50^{B}	$6{\cdot}13^{B}$
In situ	35	1·2	3·8	1·9	24	0·38	41	6·26
temperature (°C)	40	0·9	3·4	2·0	23	0·41	42	6·24
Post-excision	35	$1{\cdot}5^{A}$	$4{\cdot}3^{A}$	$1{\cdot}4^{A}$	25	0·38	37^{A}	$6{\cdot}32^{A}$
temperature (°C)	40	$0{\cdot}7^{B}$	$2{\cdot}9^{B}$	$2{\cdot}4^{B}$	22	0·40	46^{B}	$6{\cdot}18^{B}$
Time (min)	60	$1{\cdot}5^{A}$	$4{\cdot}7^{A}$	$1{\cdot}6^{A}$	27^{a}	0·44	34^{A}	$6{\cdot}36^{A}$
	120	$0{\cdot}6^{B}$	$2{\cdot}5^{B}$	$2{\cdot}3^{B}$	19^{b}	0·34	49^{B}	$6{\cdot}14^{B}$

*All compounds are expressed in μmol/g fresh tissue.
†HN: halothane-negative; HP: halothane-positive.
Means with different superscripts in the same source within a column are significantly different: ^{a}, ^{b}, $P < 0{\cdot}05$; ^{A}, ^{B}, $P < 0{\cdot}01$.

TABLE 6
Genetic Type × Post-excision Temperature Interactions on IMP and G-6-P Contents in *Tibialis cranialis*

Compound		*IMP*		*G-6-P*	
*Genetic type**		*HN*	*HP*	*HN*	*HP*
Temperature (°C)	35	$1{\cdot}16^{A,c}$	$1{\cdot}73^{A,d}$	0·42	0·36
	40	$1{\cdot}67^{B,C}$	$3{\cdot}29^{B,D}$	$0{\cdot}57^{C}$	$0{\cdot}24^{D}$

*HN: halothane-negative; HP: halothane-positive.
Means with different superscripts in the same line or the same column are significantly different. Effect of temperature: ^{a}, ^{b}, $P < 0{\cdot}05$; ^{A}, ^{B}, $P < 0{\cdot}01$; effect of genetic type: ^{c}, ^{d}, $P < 0{\cdot}05$; ^{C}, ^{D}, $P < 0{\cdot}01$.

pigs than in halothane-negative pigs, which was not observed here. Campion *et al.* (1974) reported slower contraction and half-relaxation times in halothane-positive pigs than in halothane-negative pigs, while Quinlan *et al.* (1986) found no difference between both types of pigs. The present results agreed with the latter. The fact that the peak tension was more decreased in halothane-positive pigs than in halothane-negative pigs during 1 Hz stimulation indicated that the former were more sensitive to muscle fatigue. The increase in the contraction time in halothane-positive pigs during the 1 Hz stimulation is suggestive of some disturbance in the contraction process, maybe in the intracellular calcium regulation (Campion *et al.*, 1974).

The samples taken for pH determination were not weighed in order to minimize changes between dissecting and crushing. Indeed, the pH decrease can be rather fast in anoxic muscle, and the error elicited by a delay of several tenths of seconds before crushing could have been important relative to the actual pH

variation. Figure 2 shows that an imprecision of 1–4 in the sample weight, which was improbable in practice, would have had a very limited influence on the measured pH value. This resulted from the buffering capacity of the muscle tissue. The slight decrease in pH with increasing dilutions can be attributed to the weakening of the buffering capacity of the homogenate, allowing the pH to shift towards the pH of the iodoacetate solution, which was 5·9.

Although significant, the effect of the genetic type on the biochemical changes following excision was reduced, compared to the differences reported for muscles of slaughtered halothane-negative and halothane-positive pigs (i.e. Eikelenboom and Minkema, 1974; Monin *et al.*, 1980–1981). This may be due to the general anaesthesia, as Klont *et al.* (1993) showed that the *post mortem* changes are much slowed down in halothane-positive pigs slaughtered under anaesthesia.

The increase of muscle temperature from 35°C to 40°C can be considered as very large from a physiological point of view. However, it did not affect significantly the function and the metabolism of the muscle, except that it shortened slightly the contraction time and it increased the lactate content in the halothane-positive animals. This indicates that the metabolic changes observed in muscles of pigs whose body temperature is increased by exposition to stressful conditions are probably not determined mainly by the temperature itself, but are mediated by the physiological changes (hormonal, respiratory. circulatory) associated with the efforts of the animal organism to adapt to the adverse situation. However, it must be kept in mind that the metabolic characteristics vary to a large extent among muscles. To our knowledge, the contractile and metabolic characteristics of the *tibialis cranialis* have not been determined. It would be desirable to confirm the present results on muscles of various types.

Regarding the effect of the post-excision temperature, the present results agreed with those reported by Klont *et al.* (1994) in isolated muscle strips of pig *semitendinosus*. These authors reported that an increase of the temperature of 4°C (from 38°C to 42°C) doubled approximately the rate of *post mortem* biochemical changes. In the present study, the lactate content increased on average from 21 μmol/g to 29 and 39 μmol/g ($P < 0·01$) after 1 h incubation at 35°C and 40°C, respectively, while the ATP content decreased from 6·4 μmol/g to 5·2 and 4·2 μmol/g ($P < 0·05$), respectively. Concerning the pH change, the agreement was less good, as the pH decreased from 6·93 just before excision to 6·43 and 6·28 ($P < 0·01$), respectively. Nevertheless, the present results confirm the conclusion of Klont *et al.* (1994) that the effect of temperature variation, in the upper physiological range, is higher than could be expected from the known relations between temperature and rate of biochemical changes in anoxic muscle.

CONCLUSION

A rise in muscle temperature above the normal resting level in the physiological range had no important effect on the metabolic state of muscle tissue, as long as the blood and nerve supplies were kept. By contrast, it affected markedly the metabolism of excised muscle. Consequently, the effects of the various treatments on the rate of metabolism in the excised muscle were wholly explainable in terms of temperature from the time of excision. The temperature treatments *in situ* might not be responsible for the differences after excision. The present results

suggest that the control of muscle temperature is less important before than after the death of animals from the point of view of meat quality. However, in practice, the muscle temperature of slaughter pigs should be kept as low as possible before killing, as it determines directly the muscle temperature in the immediate *post mortem* period.

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