PORCINE MALIGNANT HYPERTERMIA. I: METABOLIC AND PHYSIOLOGICAL CHANGES

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SUMMARY

Porcine malignant hyperthermia was studied in seven Pietrain pigs under standardized experimental conditions. In five animals malignant hyperthermia was triggered with suxamethonium and halothane, but in two pigs suxamethonium alone was used. Characteristic metabolic and physiological changes were found and these are discussed with regard to a possible mechanism to explain the malignant nature of the syndrome.

The malignant hyperthermia (MH) syndrome in pigs was first described by Hall and colleagues (1966) and since that time there have been several reports of investigations into this fatal reaction in Landrace (Harrison et al., 1969; Denborough et al., 1973), Poland China (Jones et al., 1972; Nelson et al., 1972) and Pietrain pigs (Sybesma and Eikelenboom, 1969; Allen et al., 1970; Lister, 1973). A variety of methods of inducing MH have been used but there has been no study in which physiological and metabolic data have been collected systematically.

Lister, Hall and Lucke (1974) have shown that the administration of two 50-mg doses of suxamethonium is a reliable method of triggering MH in Pietrain pigs. This paper describes a series of experiments in seven pigs in which this method of inducing MH was used and in five animals in which the response was enhanced by the subsequent administration of halothane. The purpose of this study was to examine and evaluate the course of certain metabolic and physiological changes in porcine MH under standardized experimental conditions.

METHODS

Animal preparation

Five male and two female Pietrain pigs with a mean body weight of 55 kg were selected from three mixed litters. Anaesthesia was induced with thiopentone and, following endotracheal intubation, the pigs were ventilated with 30% oxygen in air by means of a volume-cycled ventilator (Starling “Ideal Pump”, C. F. Palmer). Anaesthesia was maintained with incremental doses of thiopentone and the ventilation was adjusted to achieve an arterial Pco₂ of about 40 mm Hg. Two polyvinylchloride catheters (P.P. 160, Portex Ltd) were introduced via the right and left jugular veins into the right ventricle and anterior vena cava respectively. A superficial branch of the femoral artery in both hind legs was cannulated with a Stille 18G Tefnyl infusion cannula (AB Stille-Werner). Calibrated thermistors were placed in the rectum, 10 cm deep into the lateral aspects of the thigh muscles, and on the skin of the posterior abdomen.

Experimental protocol

After preparation of the pig as described, two arterial and venous blood samples were taken for analysis and a duplicate estimation of the cardiac output was performed. A single collection of mixed expired gas was used to derive the resting oxygen consumption and carbon dioxide production. Following this control period of approximately 30 min, suxamethonium 50 mg (Sux I) was injected i.v. and a second 50-mg dose (Sux II) was given 15 min later. Fifteen minutes after Sux II, 0.5–0.75% halothane was administered to five of the seven pigs studied for a period of 10 min. In the remaining two animals the development of MH was so fulminating that further muscle stimulation was not required. Table I shows the sequence of measurements made after each triggering agent (Sux I, Sux II and halothane) and also repeated 15 min after the last pharmacological challenge (terminal measurements).

Physiological measurements

Temperature measurements were recorded automatically at 1-min intervals using a Digitec thermometer (Digitec Instruments Ltd, Ohio). The e.c.g.
Table I. Sequence of measurements

<table>
<thead>
<tr>
<th>Time of sample</th>
<th>Measurement</th>
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<tr>
<td>Triggering agent + 2 min</td>
<td>Arterial blood-gas analysis</td>
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<tr>
<td>Triggering agent + 3 min</td>
<td>Cardiac output</td>
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<tr>
<td>Triggering agent + 5 min</td>
<td>Plasma and serum biochemical concentrations</td>
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<tr>
<td>Triggering agent + 5 to 8 min</td>
<td>Mixed expired gas collection</td>
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<tr>
<td>Triggering agent + 10 min</td>
<td>Arterial blood-gas analysis</td>
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was monitored throughout the experiment using a dorso-ventral chest lead and a Videograph Mark IIIA recorder (M.I.E. Instruments Ltd). Arterial pressure was measured from a femoral artery cannula by means of a Statham P23 transducer and displayed continuously on a chart recorder (Devices Ltd).

The dye-dilution technique was used for the cardiac output estimations. Indocyanine green was injected into the right ventricle, femoral arterial blood was sampled through a Gilford densitometer 105S and the resulting curve was inscribed on a Rikadenki chart recorder (Rikadenki Kogyo Co. Ltd, Tokyo). The cardiac output was derived by measurement of the area under the dye curve using a computer program written for a Wang 500 desk-top computer (Simons and White, 1976) and checked by planimetry.

Mixed expired gas was collected over a 3-min period by means of a unidirectional collect valve (Sykes, 1969) and a mixing circuit (Sykes, 1968). The expired gas volume was measured with a calibrated dry-gas meter. A Servomex 101A paramagnetic oxygen analyser was used to determine the inspired and mixed expired oxygen concentrations and a Hartmann-Braun URAS 4 infra-red analyser was used for the estimation of the mixed expired carbon dioxide concentrations. Oxygen consumption and carbon dioxide production were derived from standard equations incorporated in a program for the Wang 500 computer (Simons, 1975).

Biochemical measurements

The arterial blood samples were analysed for pH, $P_{O_2}$ and $P_{CO_2}$ using a Radiometer blood-gas system (Radiometer Ltd, Copenhagen). The haematocrit and haemoglobin concentration of each arterial sample were determined also.

Central venous blood samples were used for the following plasma and serum estimations. Serum sodium, potassium, calcium and magnesium concentrations were measured by atomic absorption spectrophotometry (Pye Unicam SP 90A, Series 2), serum chloride by the method of Schales and Schales (1941) and serum inorganic phosphate by the method of Gomori (1942). A plasma sample was separated for lactate (Hohorst, 1962), pyruvate (Czok and Lamprecht, 1970), glucose (Werner, Rey and Wielinger, 1970) and free fatty acid (Duncombe, 1964) estimations. Plasma catecholamines were measured using the semi-automated fluorimetric technique of McCullough (1968) with modifications suggested by Davies and Sheridan (1970). Recovery rates of 70–80% were achieved for combined noradrenaline/adrenaline standards over the wide range found in porcine MH.

The results were expressed as a mean value (+ SEM) for each estimation. A paired Student's t test was used to assess the significance of the metabolic and physiological changes in the hyperthermic response relative to the second control value.

![FIG. 1. Mean values (+ SEM) of arterial pH (units), $P_{CO_2}$ (mm Hg) and $P_{O_2}$ (mm Hg). Suxamethonium 50 mg was administered at times S1 and SII and halothane was commenced at time H.](image-url)
RESULTS

Blood-gas analysis
The results of arterial blood-gas analysis are shown in figure 1. Two minutes after Sux I there was a significant decrease in arterial pH to 7.27 (P<0.001), in PaO₂ to 81 mm Hg (P<0.001) and an increase in PaCO₂ to 58 mm Hg (P<0.05). Arterial pH continued to decrease throughout the experiment and reached a value of 6.71 units in the terminal stage. PaO₂ recovered slightly from its lowest value of 81 mm Hg recorded after Sux I so that PaO₂ was maintained at about 100 mm Hg for the duration of the experiment with an inspired oxygen concentration of 30%. In four animals mixed venous PO₂ was 40 mm Hg or more until the terminal stages of the response, when values as low as 12-15 mm Hg were recorded. PaCO₂ increased during the experiment and reached a maximum value of 92 mm Hg. In the terminal stage there was a characteristic reduction in PaCO₂ although 57 mm Hg was still significantly greater than the control value (P<0.01).

Gaseous exchange
The changes in oxygen consumption and carbon dioxide production during the hyperthermic response are shown in figure 2. There was a large increase in oxygen consumption and carbon dioxide production from control levels (P<0.001) following Sux I, and, at the end of the halothane challenge when the hyperthermic response was well developed, an oxygen consumption rate of 815 ml/min and carbon dioxide production rate of 935 ml/min were recorded.

Metabolic changes
The onset of MH following Sux I was associated with a seven-fold increase in the plasma lactate concentration to 10.7 m mol/litre (P<0.001) (fig. 3). In the terminal stages a concentration of 20.2 m mol/litre was recorded. Plasma pyruvate values increased significantly (P<0.05) for the duration of the hyperthermic response but decreased to control values in the terminal stages (fig. 3).

A significant increase in plasma glucose (P<0.05) was recorded after Sux I (fig. 4) and the plasma glucose continued to increase to a terminal value of 323 mg/100 ml (P<0.001). There was a steady decline in plasma FFA throughout the development of MH and this became statistically significant (P<0.05) after the administration of Sux II.
In figure 5 the mean serum electrolyte concentrations and the haematocrit and haemoglobin concentration are shown as a percentage increase above the control value. Serum potassium, magnesium and phosphate concentrations increased more than might be expected from the degree of haemocoagulation observed. The serum calcium increased in proportion to the haemocoagulation whereas the increases in serum sodium and chloride were less than would have been expected.

There was an increase in plasma catecholamines from 1.6 µg/litre in the control period to 7.4 µg/litre after Sux I ($P < 0.001$) (fig. 6) and the concentration had increased to 44.6 µg/litre shortly before death. In the early stages of the response the predominant amine was noradrenaline, but in the later stages considerable quantities of adrenaline were also detected.

Cardiovascular responses

The mean control cardiac output in the seven pigs studied was 5.2 litre/min increasing to 7.5 litre/min after Sux I ($P < 0.01$) (fig. 7). The cardiac output reached 9.6 litre/min ($P < 0.05$) following halothane stimulation, but decreased to 6.5 litre/min in the terminal stage ($P < 0.05$). An increase in heart rate was observed 3 min after Sux I and the tachycardia increased as the hyperthermic response progressed. Arrhythmias often occurred immediately following the administration of the triggering agents. The most common arrhythmias were ventricular extrasystoles and a supraventricular tachycardia. Arterial pressure was well maintained until the later stages of the development of MH. The typical arterial pressure changes are shown for one pig in figure 8.

Temperature measurement

The observed changes in temperature are shown in figure 9. The first significant increase in temperature above the control values was seen in the muscle 5 min after Sux I ($P < 0.05$). The increase in rectal temperature measured in muscle and subcutaneous tissues rose from control values of 36.9°C and 36.7°C respectively, to a peak of 40.6°C and 39.3°C respectively.
METABOLIC AND PHYSIOLOGICAL CHANGES IN PORCINE MH

Fig. 6. Mean values (±SEM) for plasma catecholamine concentrations (on log scale). Suxamethonium was administered at SI and SII and halothane commenced at H.

Fig. 7. Changes in the mean values (±SEM) of the cardiac output. Suxamethonium was administered at times SI and SII and halothane commenced at H.

The rapidly progressive metabolic and physiological changes associated with MH did not permit the accurate measurement of oxygen consumption and carbon dioxide production under “steady-state” conditions. Thus any small changes in gaseous exchange would be difficult to evaluate. However, the gross changes in oxygen consumption and carbon dioxide production (fig. 2) indicated a massive stimulation, primarily of aerobic metabolism. A similar large increase in gas exchange was observed by Berman and colleagues (1970) in hyperthermic Landrace pigs receiving halothane. A respiratory quotient (RQ) greater than 1.0 was a constant feature of the established response and was probably caused by the buffering of the lactic acidosis by the plasma bicarbonate.

Despite the large increase in oxygen consumption and the maintenance of a mean arterial P\textsubscript{O}\textsubscript{2} greater than 80 mm Hg, anaerobic glycolysis and lactate production were early features of porcine MH. The seven-fold increase in the plasma lactate concentration which followed the severe muscle stimulation of the first dose of suxamethonium did not change appreciably thereafter until the terminal stages of the syndrome. The relative contributions of aerobic and anaerobic muscle metabolism to the heat production in porcine MH are discussed in a further paper (Hall et al., 1976).

Serum potassium (P<0.05), magnesium (P<0.01) and phosphate (P<0.01) had increased significantly 5 min after Sux I (fig. 5). The results indicated a shift of these ions from the intracellular to the extracellular fluid, which may be a result of an increase in the permeability of the sarcolemmal membrane. The largest change in the electrolyte concentration was the 150% increase in the inorganic phosphate concentration shortly before death, which was probably a result of the increased rate of hydrolysis of the intracellular organic phosphates. A similar increase in phosphate in porcine MH was noted by Berman and colleagues (1970), Nelson and colleagues (1972) and Denborough and colleagues (1973).

The large increase in circulating catecholamines during the hyperthermic response (fig. 6), which has
not been reported previously, was associated with tachycardia, arrhythmia and an increase in the cardiac output (fig. 7). Britt (1974) suggested that the myopathy described in human MH may also involve cardiac muscle. The results of the cardiac output estimations in this study indicate that an underlying cardiomyopathy is unlikely to be present in Pietrain pigs. Indeed the ability of the animal to increase its cardiac output to meet the metabolic demand in the presence of severe acidosis, hyperkalaemia and dehydration was an impressive feature of the porcine syndrome. In the later stages of the response the cardiac output decreased but still remained above the control value and arterial hypotension was not observed until the terminal stages (fig. 8).

The progressive hyperglycaemia (fig. 4) may be a result partly of the stimulatory effects of the circulating catecholamines on hepatic glycogenolysis. Other factors likely to contribute to this increase include gluconeogenesis from the lactic acidosis and the inhibition of insulin secretion by the high circulating concentrations of noradrenaline (Imura et al., 1971; Porte and Robertson, 1973). The inhibition of lipolysis and the consequent decrease in plasma FFA in the presence of high concentrations of plasma catecholamines (fig. 4) seemed to be an anomalous response. However, Boyd and colleagues (1974) have shown in man that catecholamine-stimulated lipolysis was inhibited at a plasma lactate concentration greater than 8 m mol/litre, a finding which readily explains the inhibition of lipolysis in our hyperthermic pigs.

The cause of the fulminating course of MH has yet to be elucidated. Once muscle metabolism has been stimulated sufficiently, withdrawal of the

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![Arterial pressure response of one pig recorded during an episode of MH](image)

**Fig. 8.** Arterial pressure response of one pig recorded during an episode of MH. Suxamethonium was administered at times SI and SII and halothane commenced at H.

![Mean (±SEM) muscle and rectal temperatures](image)

**Fig. 9.** Mean (±SEM) muscle and rectal temperatures. Suxamethonium was administered at times SI and SII and halothane commenced at H.
triggering agent does not prevent the development of the hyperthermic response. Hall, Lucke and Lister (1975), Moulds (1975) and Lister, Hall and Lucke (1975) have suggested that a metabolic product of the initial muscle stimulation may cause catecholamine secretion which stimulates muscle metabolism further. Nahas, Ligou and Mehlman (1960) demonstrated that a metabolic acidosis stimulated catecholamine secretion in the dog. In view of the importance of anaerobic glycolysis in MH we considered that an association between the plasma lactate and catecholamine concentrations might exist. We found a highly significant correlation \((r = 0.846; P < 0.001)\) between the logarithm of the total plasma catecholamine concentration and the plasma lactate concentration (fig. 10). The correlation between the individual catecholamines and the lactate values was greater for noradrenaline \((r = 0.822; P < 0.001)\) than for adrenaline \((r = 0.656; P < 0.001)\). The log-linear nature of this relationship provides supporting evidence for our contention that the catecholamine increase is associated with, if not directly attributable to, a product of metabolism.

We cannot exclude the possibility that suxamethonium itself may stimulate catecholamine secretion for, as Mori, Iwabuchi and Fujita (1973) have shown in man, suxamethonium can cause an increase in sympathetic neuronal activity. In our control Large White pigs, which do not develop MH, we have observed a small increase in circulating catecholamines following suxamethonium, without any change in arterial pH (Hall, Lucke and Lister, unpublished results). Therefore the contribution of suxamethonium to the overall catecholamine stimulation in porcine MH is likely to be small.

The metabolic and physiological changes observed in porcine MH are essentially similar to those found after any severe muscle stimulation such as exhaustive exercise (Pernow and Saltin, 1971). In the case of MH the problem arises from the subject’s inability to control the severe catabolic (metabolic) state and enter a recovery phase. It is likely that the solution of this problem will provide the necessary insight into the aetiology of MH and a rational basis for therapy.

**ACKNOWLEDGEMENTS**

G. M. Hall received generous financial support from the Wellcome Trust and the Muscular Dystrophy Group of Great Britain. Mr Roger Lovell of the Meat Research Institute and Mr Alan Tait and Mrs Yvette White of the Royal Postgraduate Medical School provided skilled technical assistance. G. M. Hall and J. N. Lucke are visiting research workers at the A.R.C. Meat Research Institute and are most grateful to the Director, Professor Norris, for the use of the Institute’s facilities. Miss Karen Gorman provided skilled secretarial assistance.

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HYPERTERMIE MALIGNE DES PORCS

I. VARIATIONS METABOLIQUES ET PHYSIOLOGIQUES

RESUME

L'hypothermie maligne des porcs a été étudiée sur sept porcs Pietrain dans des conditions expérimentales normalisées. Sur cinq animaux, l'hypothermie maligne a été déclenchée par le suxaméthonium et l'halothane, mais sur deux porcs on n'a utilisé que le suxaméthonium seul. On a trouvée des changements metaboliques et physiologiques caracteristiques et on les discute dans ce document en vue de trouver un mécanisme susceptible d'expliquer la nature maligne du syndrome.

MALIGNE HYPERTERMIE BEIM SCHWEIN. I: METABOLISCHE UND PHYSIOLOGISCHE VERÄNDERUNGEN

ZUSAMMENFASSUNG

Bei 7 Pietrain Schweinen wurde die maligne Hyperthermie unter standadisierten experimentellen Zuständen erforscht. Bei 5 Tieren wurde die maligne Hyperthermie durch Suxamethonium und Halothan ausgelöst, während bei zwei Schweinen ausschliesslich Suxamethonium verabreicht wurde. Es ergaben sich charakteristische metabolische und physiologische Veränderungen. Die letzteren werden in Bezug auf die Möglichkeit, eines malignen, das Syndrom auslösenden Faktoren besprochen.

HIPERTERMIA PORCINA MALIGNA. I: CAMBIOS METABOLICOS Y FISIOLOGICOS

SUMARIO

Se estudió en siete cerdos Pietrain la hipertermia porcina maligna con arreglo a condiciones experimentales normales. En cinco animales la hipertermia maligna se accionó con suxametonio y halotano, pero en dos de los cerdos se usó sólo suxametonio. Se encontraron cambios metabólicos y fisiológicos característicos, los cuales se discuten en relación con un posible mecanismo para explicar la naturaleza maligna del síndrome.