cytes. The intensity of the staining reaction was reduced and the reaction product confined to the central, perinuclear population of yolk bodies in the vitellogenic oocyte (stage 5) (figure 2D). The enzyme was uniformly distributed throughout the smaller yolk vesicles. With the transformation of the larger yolk vesicles into yolk platelets, acid phosphatase activity was further diminished and restricted in distribution to the noncrystalline portions of these inclusions. The enzyme in the postvitellogenic or stage 6 oocyte was detectable in trace amounts in the yolk platelets in the center of these cells (figure 2D). Again, reactive material was never observed in the cortical granules of stage 5 and stage 6 oocytes.

The present study shows that acid phosphatase is highly localized in previtellogenic, vitellogenic and postvitellogenic oocytes. The same enzyme has been previously detected histochemically in the follicle cells surrounding vitellogenic and postvitellogenic oocytes[24]. The absence of detectable acid phosphatase activity in the cortical granules of the zebrafish egg conforms with observations made on eggs of echinoderms[14] and mammals[17]. Although Schuel et al.[15] found acid phosphatase in cortical granule fractions prepared from centrifuged sea urchin eggs, yolk platelets were contaminants of these fractions. Yolk platelets stain positively for this enzyme (see below). Other studies[16] claiming to have demonstrated the presence of acid phosphatase in cortical granules must be viewed with reservation when staining reactions are not clearly shown. The absence of acid phosphatase in the cortical granules or adjacent cortical cytoplasm in *Brachydanio* strongly suggests that this enzyme plays no role in their exocytosis upon activation. Acid phosphatase in the developing oocyte of the zebrafish appears to be largely localized in yolk vesicles, yolk platelets and their precursors. Yolk platelet acid phosphatase has been identified in the eggs of other animal species[25]. The reduction in acid phosphatase reactivity which accompanies the transformation of the yolk vesicle into the yolk platelet in the zebrafish may be related to the enzyme becoming membrane-bound[25]. We suggest that acid phosphatase is stored in the yolk platelets of *Brachydanio* to subsequently be used to initiate and/or promote the utilization of yolk during embryogenesis.

The sites of acid phosphatase activity in the previtellogenic oocyte (stages 3 and 4) are clearly preyolk bodies, but they cannot be precisely correlated with any subcellular structure on the basis of this study. There appears to be a dual origin for yolk vesicles and yolk platelets in the zebrafish. Yolk platelets have been described as being assembled from modified mitochondria[26] or from the fusion of pinocytotic vesicles[27]. Presumably, the source of most of the yolk proteins of these platelets is the liver. The sites of acid phosphatase activity detected in previtellogenic oocytes appear to topographically overlap the distribution of mitochondria as described in studies by Malone and Hisaoka[23] and Ulrich[27]. Temporally, pinocytotic vesicles do not appear in the developing zebrafish oocyte until stage 5[27] or well after the time when acid phosphatase can be initially localized.

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Mitochondrial calcium efflux and porcine stress-susceptibility

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Summary. Mitochondrial Ca^{2+} efflux rates of *M. longissimus dorsi* correlate very closely with parameters associated with porcine stress-susceptibility. Experimental data support the measurement of mitochondrial Ca^{2+} efflux to be a very sensitive and reliable method for differentiating porcine stress-susceptibility.

Two well known stress syndromes exist in certain breeds of pigs, particularly those developing leaner carcasses. The first is malignant hyperthermia which could be induced by various agents such as halothane[2-7] and suxamethonium[8]. The predominant clinical symptoms for this syndrome are gross muscular rigidity, rapid rise in body temperature, tachycardia, hyperventilation, severe metabolic acidosis and elevated levels of serum metabolites[9,10]. (The manifestations of porcine malignant hyperthermia are also similar to those described for human malignant hyperpyrexia, but it is not clear to what extent the porcine data are applicable to humans. The frequency of occurrence in anaesthetic deaths in apparently healthy patients is about 1 in 15,000 anaesthesia[11]). The 2nd type of porcine stress syndrome is

associated with the formation of pale, soft and exudative (PSE) meat, and this condition is linked with muscles exhibiting rapid glycolysis post-mortem. The ultimate pH of muscles in PSE-prone pigs is reached within 1 h post-mortem while the carcass is still hot, and PSE is formed by the denaturation of some of the myofibrillar and sarcoplasmic proteins as a result of low pH and high temperature conditions[12,13].

In an earlier paper we reported variations in the rate of mitochondrial Ca^{2+} efflux in different breeds of pigs and also suggested that Ca^{2+} efflux was linked with porcine stress-susceptibility[1]. It was also postulated[14] that the Ca^{2+} liberated from mitochondria of stress-susceptible pigs is probably the 'trigger' responsible for rapid glycolysis post-mortem, by the ability of Ca^{2+} to activate the myofibrillar ATPase and the phosphorylase kinase (E.C. 2.7.38) so that more glycogen is converted to lactate. This paper shows that mitochondrial Ca^{2+} efflux rates correlate very closely with parameters associated with PSE and malignant hyperthermia syndromes. It also emphasizes the reliability and sensitivity of the Ca^{2+} efflux measurements as a potential procedure for assessing porcine stress-susceptibility.

Materials and methods. All the halothane-screened pigs (Pietrain/Hampshire and Sire Line[15]) were supplied by Dr A.J. Webb of the Agricultural Research Council, Animal Breeding Research Organisation (ABRO), Edinburgh. The pigs (about 35 kg) were transferred to the Meat Research Institute, Langford, where they were kept until slaughter at 75 kg or 100 kg. Mitochondria were isolated from *M. longissimus dorsi* (LD) immediately post-mortem using *Bacillus subtilis* proteinase[14]. The rates of mitochondrial Ca^{2+} efflux were measured at 540–510 nm with murexide[16] using the Aminco-Chance dual-wavelength/split-beam spectrophotometer operating in the dual-wavelength mode at 20 °C. The reaction medium (pH 7.20) contained 220 mM mannitol, 50 mM sucrose and 15 mM Tris-HCl in the presence of 2.50 nM P_i. Protein was estimated with Folin-phenol reagent[17] using bovine serum albumin as standard. Muscle pH and metabolites were determined as described by Bendall et al[18].

Results and discussion. The table illustrates the rates of Ca^{2+} efflux from LD muscle mitochondria of ABRO halothane-screened pigs, which were clearly differentiated into 2 distinct groups by Ca^{2+} efflux measurements.

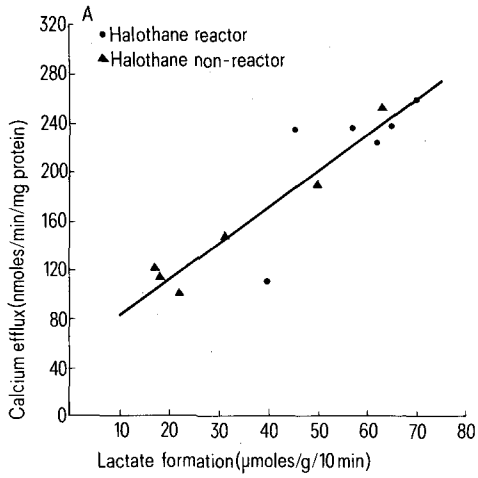
Prediction of halothane sensitivity was made from mitochondrial Ca^{2+} efflux rates prior to knowledge of the results of the halothane test carried out by Dr Webb. The minimum rate of Ca^{2+} efflux for the halothane-reactors was 170 nmoles/min/mg protein, and the maximum rate for the halothane nonreactors was 147 nmoles/min/mg protein. A value of 160 nmoles Ca^{2+}/min/mg protein was suggested to clearly demarcate the halothane reactors from the nonreactors, but this tentative value would have to be established with more pigs. 4 out of the 33 pigs were misclassified (12%) by halothane screening but were correctly classified by Ca^{2+} efflux measurements. The Ca^{2+} efflux rates for the halothane-reactors and nonreactors were highly significantly different ($p < 0.001$). The rates of Ca^{2+} efflux and of glycolysis (measured by the decline in tissue pH post-mortem) were directly related in that high Ca^{2+} efflux rates were only observed in muscles showing rapid glycolysis, and low Ca^{2+} efflux rates with muscles having a slow rate of glycolysis.

Ca^{2+} efflux rates of LD muscle mitochondria were also measured in 6 halothane-reactors and 6 nonreactors of Pietrain pigs in collaboration with Dr Monin of Theix (France). 1 out of the 12 pigs was misclassified (8.3%) by halothane screening in that a halothane-reactor showed an

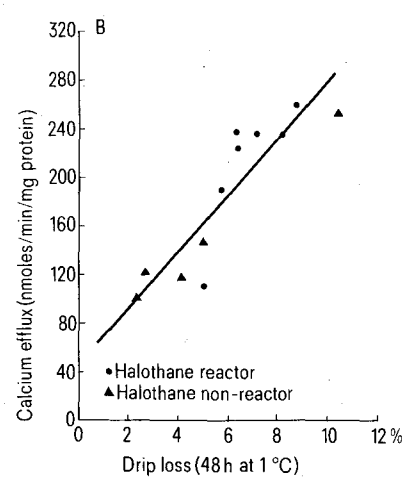
Rates of Ca^{2+} efflux from LD muscle mitochondria of ABRO halothane-screened Pietrain/Hampshire and Sire Line pigs

Ca^{2+} efflux rate (nmole/min/mg protein)	Prediction from Ca^{2+} efflux	Halothane screening (Webb)
216 ± 26 (15)[a] (260 – 170)	+	+
121 ± 19 (14)[b] (147 – 87)	–	–
252 (1)[c]	+	–
189 (1)[c]	+	–
110 (1)[c]	–	+
210 (1)[d]	+	–

The rates of Ca^{2+} efflux estimated in the presence of 2.50 mM P_i, refer to the initial fast rate observed at the onset of anaerobiosis[14]. The rates were measured at 540–510 nm with murexide using the Aminco-Chance dual-wavelength/split-beam spectrophotometer operating in the dual-wavelength mode at 20 °C. With the exception of the 4 misclassified pigs, the results are means ± SD for the numbers of pigs used in parenthesis[a,b]. The values within parenthesis represent the maximum and minimum Ca^{2+} efflux rates for each group. 15 halothane reactors (12 Pietrain/Hampshire and 3 Sire Line pigs)[a] and 14 nonreactors (11 Pietrain/Hampshire and 3 Sire Line pigs)[b] were correctly classified. The 4 misclassified pigs (3 Pietrain/Hampshire[c] and 1 Sire Line[d]) are illustrated individually.



A Relationship between Ca^{2+} efflux and lactate formation. 12 pigs were used (6 halothane-reactors and 6 nonreactors). Data include 2 pigs misclassified by halothane screening.



B Relationship between Ca^{2+} efflux and drip produced postmortem. The data were obtained using the same pigs as described in A.

identical rate of Ca^{2+} efflux and rate of glycolysis to that observed for halothane nonreactors. With the exception of this single misclassified pig, all the Ca^{2+} efflux rates correlated very closely with the halothane test in that high Ca^{2+} efflux rates were only observed with halothane reactors, which also showed rapid glycolysis post-mortem. The values of the high and low Ca^{2+} efflux rates were also highly significantly different ($p < 0.001$)[19].

The rates of Ca^{2+} efflux correlate very closely with parameters associated with porcine stress and malignant hyperthermia syndromes. The figure illustrates the relationship of Ca^{2+} efflux rates to lactate (A) and to drip (B) produced postmortem; the correlation coefficients were 0.91 and 0.88 respectively. The Ca^{2+} efflux rates were also directly related to the rate of hydrolysis of creatine phosphate and of adenosine triphosphate (ATP). High Ca^{2+} efflux rates were only observed in LD muscles showing rapid rates of hydrolysis of both creatine phosphate and ATP, and low efflux rates with slow rates of creatine phosphate and ATP hydrolysis (unpublished data). Ca^{2+} efflux rates also correlated very closely with the quality of the carcasses in that PSE was only observed with LD muscles showing high efflux rates, and normal pork with muscles having low efflux rates.

Various methods have been reported for predicting porcine stress syndromes, based on either serum enzyme analysis[20,21], blood grouping[22,23], muscle biopsy analysis of metabolites[24] or halothane screening[24-27]. The reliability and sensitivity of Ca^{2+} efflux measurements with murexide was stringently tested and shown to correlate very closely with parameters associated with PSE and malignant hyperthermia syndromes. Measurement of Ca^{2+} efflux rates does not have the limitation of misclassification observed with halothane screening, though the latter method is less complicated than Ca^{2+} efflux measurements. However, the biochemical procedure for assessing porcine stress-susceptibility is in its infancy and no doubt could be simplified in the very near future. The results presented in this paper were carried out using post-mortem samples. Preliminary experiments showed that similar Ca^{2+} efflux rates were observed using biopsy and post-mortem samples. If the procedure for obtaining biopsy samples could be simplified, Ca^{2+} efflux measurements offer a very sensitive and reliable method for differentiating porcine stress-susceptibility and could probably be applied in breeding programmes to eliminate the undesirable porcine stress syndromes.

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Evidence for dissociation of ferrihemoglobin by poly-L-lysine

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Summary. Circular dichroism and absorption spectra of ferrihemoglobin were shown to be altered upon binding with poly-L-lysine at alkaline pH. When ferrihemoglobin immobilized to Sepharose gel was treated with poly-L-lysine, hemoglobin subunits were released from the gel. These results suggest that ferrihemoglobin was dissociated into subunits by poly-L-lysine.

Poly-L-lysine (PLL) is a polyelectrolyte which has often been used as a model compound for studies on interaction with proteins or enzymes. We have recently found that PLL inhibited the activity of α-ketoglutarate dehydrogenase complex purified from pig heart[3,4]. The inhibition seemed to be caused by interference with interaction between constituent enzymes[3] and by dissociation into them with an excess amount of PLL[4]. It is interesting to see whether this is a specific reaction occurring only with α-ketoglutarate dehydrogenase, since this seemed, to our knowledge, to be the first example of a polyaminoacid dissociated subunit enzyme. In the present study, another oligomeric protein, hemoglobin, was treated with PLL and it was found that PLL interacted also with hemoglobin at alkaline pH and dissociated it into subunits.

Materials and methods. Human oxyhemoglobin was pre-