no effect of bilirubin levels on later I.Q. Stewart and his colleagues make the point that subtle changes in visual perceptual motor integration in their series were related to low cord haemoglobin levels rather than high serum bilirubin concentrations. Somewhat similar findings were shown by Odell *et al.* (1970), who in 32 jaundiced neonates recorded not only serum bilirubin levels but also the saturation index of the serum proteins with bilirubin. Though they found no correlation between serum bilirubin levels and I.Q. scores there was a highly significant one between the serum saturation index and abnormal cognitive findings on psychological testing at 4-7 years.

While there seems to be no statistical correlation between serum bilirubin levels *per se* (up to 20 mg. per 100 ml.) and subsequent I.Q., individual examples of frank kernicterus occurring at quite low levels of serum bilirubin have been described (Stern and Denton, 1965). Clearly serum bilirubin itself is not a precise measure of the risk of brain damage. Other factors such as anaemia (Stewart *et al.*, 1970), hypoxia (Lucey *et al.*, 1964), acidaemia (Windle, 1969), and hypoglycaemia operating in the first few days of life may affect the situation in at least three ways. (1) They may cause brain damage in their own right. (2) They may interfere with protein binding of bilirubin in the plasma and thus cause kernicterus at unexpectedly low bilirubin levels. (3) They may combine with bilirubin toxicity at cellular level to interfere with respiration, giving rise to complex neurological damage requiring subtle testing for detection and evaluation. There is thus need for a more complete evaluation of the neonate to be recorded together with new methods such as the protein saturation index (Odell *et al.*, 1970) and the reserve albumin binding capacity (Porter and Waters, 1966) to be taken into account. This will need careful psychological assessment at around 5 years of age. Until these methods have been properly assessed the paediatrician must interpret the serum bilirubin level along with the other criteria, not forgetting the clinical state of the baby. Meanwhile, there is no evidence of a significant iceberg of lowered intelligence occurring at

bilirubin levels below that at which kernicterus is to be expected, and current indications for exchange transfusion and other treatment remain valid.

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Preliminary Communications

Serum Creatine Phosphokinase Levels in Pigs Reacting Abnormally to Halogenated Anaesthetics

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Summary: Serum levels of creatine phosphokinase (CPK) were estimated in pigs. Among those known to react abnormally either to halothane alone or to halothane and suxamethonium most had very high levels of CPK. Twenty-four hours after five minutes' anaesthesia with halothane and oxygen a sustained, pronounced rise in the serum CPK occurred in some pigs—principally but not exclusively in the abnormal reactors. Estimation of the serum level of CPK may be a useful screening test for an abnormal hyperpyrexial response to halothane.

INTRODUCTION

There are now many reports of an abnormal response, both in humans and in animals, to halogenated anaesthetic agents either alone or in combination with suxamethonium chloride (Denborough and Lovell, 1960; Britt and Gordon, 1969). This response is characterized by the development of generalized muscle rigidity, a severe and sustained rise in body temperature, hyperkalaemia, and metabolic acidosis. Many of the human cases have had a familial distribution, and death occurs in a high proportion of them.

Hall *et al.* (1966) in Britain and Harrison *et al.* (1969) in South Africa have discovered strains of pig which, when exposed to these anaesthetic agents, develop a syndrome clinically similar to that found in humans, and thus, by good fortune, seem to have identified an experimental model for the investigation of this baffling and potentially lethal sequence of events. Most of the affected animals identified by Hall and his colleagues have been very closely related, though a few sporadic cases in unrelated animals have been noted. The data obtained from breeding experiments suggest that the abnormal reaction pattern is inherited as an autosomal dominant of variable penetrance and variable expressivity.

Our clinical findings and those of Harrison *et al.* suggest that the primary abnormality is to be found within the voluntary muscle of the affected animals. We have therefore attempted to find a biochemical "marker" which could support this suggestion and which might serve to identify experimental animals or human patients who are at risk.

Raised serum creatine phosphokinase (CPK) levels have been reported in a number of myopathies both in humans and in experimental animals (Eppenberger *et al.*, 1964; Wilson *et al.*, 1965) and there are also reports of raised serum CPK levels in relatives of patients who have reacted abnormally to halothane and suxamethonium (Denborough *et al.*, 1970; Isaacs and Barlow, 1970). We decided, therefore, to study the serum levels of this enzyme in our strain of abnormally reacting pigs.

PRESENT INVESTIGATION

The CPK levels in the pig serum were determined with the "CPK activated" test kits of Boehringer Mannheim. The assay consists in coupling the CPK catalysed phosphorylation of adenosine diphosphate by creatine phosphate to hexokinase and subsequently glucose 6-phosphate dehydrogenase, the absorption of the NADPH₂ produced being measured spectrophotometrically.

With this method a two-part study was undertaken. In the first of these the serum CPK levels in *unanaesthetized* pigs belonging to the following groups were determined: (1) pigs known to react abnormally either to halothane alone or to halothane and suxamethonium (24 animals); (2) litter mates of the group 1 animals which did not react abnormally to the anaesthetic (10 animals); and (3) unrelated pigs of the same age purchased from a different breeder (9 animals).

RESULTS

A wide range of "resting" serum CPK levels was found in the abnormal reactors (from 79 to 13,700 i.u./l.). Levels of more than 750 i.u./l. were found in 9 of the 24 abnormally reacting animals. None of the related negative reactors or of the unrelated pigs had CPK levels in excess of 750 i.u./l. The highest level found in the related negative reactors was 590 i.u./l. and in the unrelated pigs was 486 i.u./l. Similarly 14 out of the 24 abnormal reactors had serum CPK levels in excess of 500 i.u./l. while only 3 out of the 10 of their negatively reacting litter mates and none of the unrelated animals had CPK levels greater than 500 i.u./l. These results are given in full in Table I; the differences between the groups are statistically significant ($P < 0.01$).

TABLE I

CPK Levels (i.u./l.)	Positive Reactors. 24 Animals	Negative Reactors	
		Litter Mates. 10 Animals	Unrelated. 9 Animals
≥ 2,000	4	0	0
≥ 1,000	2	0	0
≥ 750	3	0	0
≥ 500	5	3	0
≥ 250	6	2	1
< 250	4	5	8

The second set of experiments was directed towards investigating any possible effects of halothane on serum CPK levels and hence, by implication, on muscle membranes. Three abnormal reactors, two negatively reacting litter mates, and four unrelated pigs of the same age had blood samples taken for CPK determination. They were then anaesthetized for five minutes with a mixture of halothane and oxygen and further blood samples were taken within 15 minutes of recovery from anaesthesia. A final sample for the determination of the serum CPK levels was taken 24 hours after recovery from the anaesthetic. Most of the animals showed a transient rise in serum CPK immediately after the anaesthetic, and this was sometimes of quite a high order. What was much more striking, however, was evidence of a sustained and sometimes pronounced rise in the serum levels of the enzyme 24 hours later. This was seen principally but not exclusively in the abnormal reactors. One such animal had a "resting" serum CPK level of 204 i.u./l. Halothane anaesthesia produced a rise to 1,560 i.u./l., and after 24 hours it was 45,600 i.u./l. As the starting levels of CPK were different in all the animals the effects of the halothane "challenge" on CPK levels found 24 hours after anaesthesia were expressed for each animal as a multiple of its own starting level. These results, given in Table II, show that the greatest rises occurred in the abnormal reactors.

TABLE II.—Animals Challenged with Halothane. Rise in CPK Levels 24 Hours after Halothane Anaesthesia. Expressed as a Multiple of the Rise from Pre-anaesthetic Levels in Each Animal

	Positive Reactors	Negative Reactors	
		Litter Mates	Unrelated
235 ..		11	2.5
54 ..			2.5
			0.8
30 ..		4	0.7

These results indicate that in our strain of pigs raised serum CPK levels in the unanaesthetized animal have a fair predictive value for the development of abnormal muscle contracture following induction with halothane and the use of suxamethonium. Of the 34 closely related animals studied, 25 had serum CPK levels in excess of 250 i.u./l. and 20 of these were abnormal reactors. Clearly the finding in an animal of a serum CPK level of less than 250 i.u./l. does not rule out the possibility of an abnormal reaction, but the chances of this happening are much lower.

Of equal interest are the data obtained thus far relating to the effect of halothane alone on serum CPK levels. In the three abnormal reactors tested in this way large rises in enzyme levels were noted 24 hours after recovery from anaesthesia. It is interesting that the "resting" levels in these animals were not particularly high (204, 151, and 388 i.u./l.). The same challenge in four unrelated pigs failed to produce any rise at all in two animals and only small rises in the other two, suggesting that in susceptible animals halothane alters the permeability of muscle membranes and promotes the escape of CPK from the muscle fibre into the plasma. We cannot say whether this altered permeability is an intrinsic part of the muscle abnormality which leads to contracture and hyperpyrexia or merely a "marker" for the presence of some other abnormality. The two reports of an association between cases of malignant hyperpyrexia in humans and raised serum CPK levels in their relatives suggest that the former is the case.

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