THE PORCINE, PALE, SOFT, EXUDATIVE MUSCLE CONDITION – WHAT IS THE BASIC BIOCHEMICAL LESION?

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The porcine stress syndrome occurs with increasing frequency in breeding and fattening pigs of the Piétrain, Landrace, and Poland China breeds (Briskey, 1964; Bickhardt, Giese, Chevalier & Reinhard, 1972; Patterson & Allen, 1972). The syndrome manifests itself as sudden death over a wide range of stressful conditions, such as heat, anoxia, service, parturition, transport, herding, and certain forms of anaesthesia. In slaughtered pigs the syndrome occurs in the form of a pale, soft, exudative musculature (Wismer-Pedersen & Briskay, 1961; Bendall & Lawrie, 1964; McLoughlin & Goldspink, 1964). Such pigs are said to be stress-susceptible in contrast to the stress-resistant Chester White and Large White breeds (Bendall & Lawrie, 1964; Topel, 1969). Stress-susceptibility is associated with a highly accelerated metabolism, particularly glycolysis and phosphate ester hydrolysis, and overproduction of lactic acid in the skeletal muscles. Even under mild to moderate conditions of stress, as much as 100 µmoles of lactate per gram of tissue may accumulate in the muscles of susceptible animals. The accumulation of high levels of lactate near body temperature at the time of death or slaughter results in the development of pale, soft, exudative characteristics in the skeletal muscles. Stress-susceptible pigs accumulate lactate at a faster rate, and develop PSE characteristics in their musculature. Stress-susceptibility in certain breeds of pigs appears to be the result of intensive genetic selection for high total muscularity, high growth rate, and maximal food conversion over many generations.

Bendall (1966) and McLoughlin (1970) postulated that the rapid rate of post-mortem glycolysis and lactate accumulation were due to intense trains of neural stimuli reaching the muscle. The hyperirritability of the muscles of stress-susceptible pigs has been interpreted by Lister (1971) to indicate an increased permeability of the sarcolemma to sodium and potassium ions. This would mean that the membrane potential has an important controlling influence on the rate of muscle metabolism. Stress-resistant pigs show a low rate of glycolysis and lactate accumulation, and do not develop PSE characteristics in their musculature. Stress-susceptibility in certain breeds of pigs appears to be the result of intensive genetic selection for high total muscularity, high growth rate, and maximal food conversion over many generations.

Symptoms of the pale, soft, exudative muscle condition.

The characteristics of the PSE condition are: (a) a pale, muscle colour, noticably less red than normal; (b) the presence of very large amounts of free fluid in the muscle; (c) a very soft, spongy texture. The muscles which are most affected are the longissimus dorsi and the semimembranosus, and to a lesser extent the rectus femoris. The longissimus dorsi is affected to different extents along its length, the most susceptible areas being between the ninth and eleventh thoracic and second and fourth lumbar vertebrae (Lawrie & Gatherum, 1961). In the muscles of pigs which eventually become PSE, the pH falls very rapidly post-mortem, that is, there is an unusually rapid production of lactic acid while the body temperature is still close to 37°C (vide review, McLoughlin, 1971). The high level of acid at body temperature causes denaturation of the sarcoplasmic proteins, precipitation of the latter on the myofibrillar proteins, and a masking of the colour of the muscle pigment myoglobin.
(b) Increased permeability of the sarcolemma.

The hyperirritability of the muscles of stress-susceptible pigs has led to the suggestion that the sarcolemma is more permeable to sodium ions than the sarcolemma of normal muscles. In a study of the resting membrane potential in muscles of stress-susceptible Poland China and stress-resistant Chester White pigs, Schmidt et al. (1972) found that the membrane potential, measured forty-five minutes post-mortem, was 20 - 25 mV lower in the stress-susceptible animals. The decreased membrane potential may reflect an increased sodium permeability, or it may indicate a defect in the sodium-potassium activated adenosine triphosphatase enzyme which is responsible for repolarisation. The findings of the above authors certainly suggest that the sarcolemma may be an important site of abnormality in the muscles of stress-susceptible pigs. Before a definite conclusion can be made as to the nature of the defect in the cell membrane, it will be necessary to measure the in vivo membrane potential of affected muscles, and to follow its rate of decay post-mortem. In the experiments of Van der Kloot (1967), already referred to, depolarisation of the cell membrane of frog muscle caused an increased oxygen consumption apparently mediated by calcium. If this finding is applicable to pig muscle, the released calcium would activate the myofibrillar adenosine triphosphatase and cause contraction. If the membrane potential of the muscles of stress-susceptible pigs is an unstable one, it follows that the muscles will be hyperirritable, and that more energy will be required to maintain the muscle fibres in the polarised state.

(c) Alteration of the relaxing system in the sarcoplasmic reticulum.

Most of the evidence indicating the role of the sarcoplasmic reticulum in the genesis of the malignant hyperthermia syndrome in stress-susceptible pigs has come from the experiments of Harrison, Saunders, Biebuyck, Hickman, Dent, Weaver, & Terblanche (1969), Berman, Harrison, Bull & Kench (1970), and Harrison (1973). The latter author has shown that halothane induces malignant hyperthermia in stress-susceptible pigs even under conditions of neuromuscular block by curare. Furthermore, Harrison found that procaine blocked the initiation of the syndrome by halothane. It has long been known that caffeine causes a persistent contraction similar to rigor in skeletal muscle fibres (Sandow, 1970), and that is persists until the caffeine is removed. The rigor is due to stimulation of calcium release and interference with calcium re-binding by the sarcoplasmic reticulum. The net effect is to increase the sarcoplasmic concentration of calcium, which activates the myofibrillar contractile proteins and maintains contraction. Procaine is known to block the action of caffeine by preventing the efflux of calcium from the sarcoplasmic reticulum. These experiments clearly localise the defective site in the muscle fibre to the sarcoplasmic reticulum. Since halothane initiates malignant hyperthermia in stress-susceptible pigs, but not in stress-resistant animals, there must be an intrinsic functional defect in the sarcoplasmic reticulum of the susceptible animals. Since the PSE condition is observed only in the musculature of stress-susceptible pigs and pigs slaughtered with the captive-bolt and electrical stunning methods, it is most probable that an increased sarcoplasmic calcium concentration is responsible for the very elevated rates of ATP hydrolysis and glycolysis observed in muscles post-mortem, and which eventually exhibit PSE characteristics (Heffron & McLoughlin, 1971; Heffron, 1971; Klingbiel & Naudé, 1972).

The source of heat in malignant hyperthermia has been the subject of much dispute, though the evidence at this stage favours anaerobic metabolism of the form of breakdown of glycogen to lactic acid, neutralisation of hydrogen ions, and hydrolysis of high energy phosphate esters (Berman & Kench, 1973). Gatz (1973) investigated most of the agents commonly associated with the malignant hyperthermia syndrome for their possible effects on the uncoupling of oxidative phosphorylation in dogs and rats. Because halothane, chloroform, thiopentone, and chlorpromazine were found to uncouple oxidative phosphorylation and to enhance 2,4-dinitrophenol-induced hyperpyrexia, the author concluded that most of the heat in the syndrome arises from an uncoupled oxidative phosphorylation. However, these results are in conflict with those of Eikelboom & van den Bergh (1971), already referred to, who showed that halothane inhibited the oxygen consumption of mitochondria from stress-susceptible pigs.

Furthermore it must be borne in mind that although malignant hyperthermia may be induced by many different drugs, it remains to be proven that they all affect the calcium accumulating system of the sarcoplasmic reticulum as halothane does (Steward & Thomas, 1973). The porcine stress syndrome will result in rigidity and hyperthermia without pharmacological challenge, thus indicating the very unstable nature of the relaxing system of these pigs. The evidence thus suggests a defective sarcoplasmic reticulum, with the heat having its origin in the associated, accelerated anaerobic metabolism.

(d) Changes in the myoneural junction.

Very little detailed attention has been given to possible changes in the myoneural junction in the stress-susceptible pigs. Schmidt et al. (1972) found that the cholinesterase activity of muscles from stress-susceptible and stress-resistant pigs was not significantly different, thus ruling out the possibility of accumulation of the transmitter at the myoneural junction being responsible for the observed hyperirritability.

Conclusions.

The capacity of the sarcoplasmic reticulum to bind calcium is similar in stress-susceptible and normal pigs. Although calcium uptake is similar before anaesthesia with halothane, it is decreased by about half in the sarcoplasmic reticulum of stress-susceptible pigs after halothane anaesthesia, thus proving that halothane alters the relaxing system in the reticulum (Vide Denborough, Hird, King, Marginson, Mitchelson, Nayler, Rex, Zapf, & Condron, 1973). These authors showed that the flux of calcium in the sarcoplasmic
It is the denaturation of the muscle proteins that leads to the appearance of abnormally high amounts of free fluid in the tissue, that is, the water-binding capacity of the muscle is reduced by denaturation of the proteins. Unusually low in the tissue, that is, the water-binding capacity of the muscle capillary density than the stress-resistant animals (Merkel, Bickhardt). Isacks and Barlow (1970) consider that the halothane-susceptible pigs are in the range for normal animals, 5.1 - 5.4.

Morphological studies have thus far shown that the ultrastructure of muscles of stress-susceptible pigs is not dramatically different from that of normal animals (Venable, 1973). The muscles of stress-susceptible animals tend to have greater proportions of classical white fibres and a lower capillary density than the stress-resistant animals (Merkel, 1971). The term "dystrophy" has often been used in reference to PSE muscle, but it has little to do with such a pathological state, and there are no clear or definable symptoms associated with PSE in living muscle. Bickhardt (1971) is of the view that there are decreased levels of the high energy phosphates, phosphocreatine and ATP, in the muscles of stress-susceptible animals, most probably due to the primary dependence of the muscle fibres on the glycolytic pathway for ATP synthesis, and to a decreased capillary density. Bickhardt et al. (1972) point out that stress-susceptible pigs may have an exertional myopathy which remains clinically latent but that the myopathic process is activated by bodily exertion, and there occurs an increased efflux of lactate and enzymes (creatine phosphokinase and aldolase) into the extracellular space. In this regard it is interesting that Isaacs and Barlow (1970) consider that the halothane-induced malignant hyperthermia in humans may be due to a subclinical myopathy, and that its pattern of inheritance is autosomally dominant. Clinically the malignant hyperthermia syndrome in man is indistinguishable from that in the pig. The pig exhibits hyperthermia which is usually fatal, and the skeletal muscles become rigid, and as in man, the causative agents are halothane and succinylcholine.

Stress-susceptible pigs develop tachycardia and the respiratory rate increases during the first ten minutes of heat stress (Judge & Marple, 1971). These authors have obtained direct evidence of adrenal insufficiency in stress-susceptible pigs using a sensitive radioimmunoassay for plasma levels of ACTH and a competitive protein binding method for plasma corticosteroids. They calculated the ratio of plasma corticosteroid levels to plasma ACTH levels as an index of adrenocortical responsiveness to endogenous ACTH. Throughout the course of a six week period of imposed environmental stress the corticosteroid:ACTH ratio was two-to-three times higher in the stress-resistant than the stress-susceptible pigs. Adaptation to stress in its manifold forms is accomplished by the sequential action of the hormones adrenaline, cortisol, and 11-deoxycorticosterone. These hormones participate in the provision of energy under conditions of stress and aid in the restoration of cell potassium in the recovery phase. This typical metabolic response to stress has been referred to as the "general adaptation syndrome" by its discoverer, Selye (1950). The circulating levels of corticosteroids participate in a sensitive feedback system with the hypothalamic corticotrophin releasing factor. Stress accommodation is effected by the concerted action of the hypothalamus, the adrenal, and pituitary glands. Adapational disease sets in when the normal homeostatic mechanisms become inadequate. In stress-susceptible pigs, relative adrenal insufficiency results in a severely impaired ability to adapt to stress, and should the intensity and duration of stress become too great death follows quickly.

Theories of the biochemical basis of PSE.

At present there are four main theories of the biochemical basis of PSE, namely: (a) uncoupling of oxidative phosphorylation of muscle mitochondria; (b) increased permeability of the sarcolemma; (c) alteration of the relaxing system of the sarcoplasmic reticulum; (d) changes in the myoneural junction. Discussion of the relevant evidence for each theory will be based on the hypothesis proposed by Harrison (1972) that the PSE state, the porcine stress syndrome, and the malignant hyperthermia syndrome share a common, primary, biochemical lesion.

(a) Uncoupling of oxidative phosphorylation.

A theory of aberrant muscle metabolism based on uncoupling of mitochondrial oxidative phosphorylation in the muscles of stress-susceptible pigs was first advanced by Sybesma & Eikelenboom (1969) to explain the rapid metabolic rate and excessive heat production in these animals. Uncoupling of oxidative phosphorylation would explain the increased rate of heat production observed in stress-susceptible pigs. Gatz (1973) proposed a similar mechanism for the promoting effect of halothane on 2,4-dinitrophenol-induced malignant hyperthermia in dogs. However further work by Eikelenboom & Van den Bergh (1971) showed that mitochondria from stress-susceptible pigs had a lower oxygen consumption than mitochondria from stress-resistant pigs. A lower rate of synthesis of ATP in mitochondria of muscle of stress-susceptible pigs would be of central importance since, ATP, utilised in muscular activity, will be synthesised at a much slower rate during activity and the subsequent recovery period. The resulting lower level of ATP and higher level of ADP will accelerate glycolysis by allosteric activation of the key enzyme of the glycolytic cycle, phosphofructokinase. Thus glycolysis would be stimulated to an extent which compensates for the decreased mitochondrial ATP synthesis. Since glycolysis produces three moles of ATP per mole of glucose (from glycogen) and mitochondrial oxidative phosphorylation produces thirty six moles of ATP, it is clear that compensatory glycolysis must be stimulated several-fold depending on the extent of decreased oxygen consumption of the mitochondria. Muscle lactate will increase accordingly, and will soon appear in the general circulation due to the inadequacy of the mitochondria in oxidising the sarcoplasmic NADH which enters the mitochondria via the glycerol-3-phosphate cycle. The theory of uncoupling of oxidative phosphorylation has been abandoned in favour of a theory of diminished mitochondrial oxygen consumption.

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reticulum of stress-susceptible pigs was doubled. These results and those of Harrison (1973) suggest the following biochemical basis of the porcine stress syndrome: the sarcoplasmic reticulum of stress-susceptible pigs is highly unstable due most probably to a defect in the enzyme responsible for pumping calcium against its concentration gradient, or in the protein responsible for binding calcium within the reticulum; thus an unstable relaxing system produces increased muscle tone and an accelerated ATP hydrolysis in vivo. The hypothesis is consistent with the hereditary nature of the syndrome (Patterson & Allen, 1972) since it only occurs in pigs which have been genetically selected for high growth rate and total muscle mass. It appears to be transmitted as a genetically altered protein defect of the sarcoplasmic reticulum of the so-called stress-susceptible animals.

References


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