the peptide for visual estimation. The linear part covers about 75% of the total reflectance/transmission change and corresponds to a hormone concentration range of 1–30. The composite line of six individual reflectometric log dose-response curves for α-MSH is shown in figure 2. A statistical analysis of the lines showed that the mean precision (λ)[16] of the reflectometric assay was 0.11 which corresponds well with the values obtained with other melanophore assays, such as the method using tail-fin pieces of *Xenopus* larvae[7], or the frog skin assay[4]. Visual estimation of skin darkening is less precise than reflectometric determination since not more than seven intermediate steps between minimal and maximal response can be distinguished. It is particularly unfavorable to use a set of noncalibrated skin pieces from different animals because this would introduce a significant error (fig. 2). If on the other hand the skin pieces for standard and unknowns originate from the same (calibrated) dorsal skin, the 95% confidence interval is not larger than that for the microscopic *Xenopus* assay. The precision of the assay reaches 0.2 which is sufficiently accurate for time-response curves in photoaffinity labeling studies.

UV-irradiation of *Anolis* skin in the presence of photoreactive p-azidophenylalanine[13]-α-MSH produces irreversible pigment dispersion[15]. Visual and reflectometric recording of this long-lasting stimulation elicits an almost identical time-response curve, except for a slight difference during the irradiation phase (fig. 3): as melanophores are UV-sensitive, there is a tendency for their pigment to aggregate slightly during exposure to UV-light. In most cases this can only be observed reflectometrically; moreover it is always reversible and does not affect the further course of the time-response curve. Visual and reflectometric recording of the nonirradiated controls hardly differ either (fig. 3) thus demonstrating the usefulness and validity of the visual method using calibrated skin pieces.

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2 Shizume, K., Lerner, A.B., and Fitzpatrick, T.B., Endocrinology *54* (1954) 553.
3 Geschwind, I.I., and Huseby, R.A., Endocrinology *79* (1966) 97.
4 Eberle, A.N., and Schwyzer, R., Helv. chim. Acta *58* (1975) 1528.
5 Hogben, L.T., and Slome D., Proc. R. Soc., B *108* (1931) 10.
6 Landgrebe, F.W., and Waring, H., in: Methods in hormone research, vol. 2, p. 517. Ed. R.I. Dorfman. Academic Press, New York 1962.
7 De Graan, P.N.E., Molenaar, R., and van de Veerdonk, F.C.G., Molec. cell. Endocr. *32* (1983) 271.
8 Burgers, A.C.J., Ann. N.Y. Acad. Sci. *100* (1963) 669.
9 Tilders, F.J.H., van Delft, A.M.L., and Smelik, P.G., J. Endocr. *66* (1975) 165.
10 Hadley, M.E., and Goldman, J.M., Am. Zool. *9* (1969) 489.
11 Björklund, A., Meurling, P., Nilsson, G., and Nobin, A., J. Endocr. *53* (1972) 161.
12 Carter, R.J., and Shuster, S., J. invest. Derm. *71* (1978) 229.
13 Waring, H., Color change mechanisms of cold-blooded vertebrates. Academic Press, New York 1963.
14 Eberle, A.N., de Graan, P.N.E., and Hübscher, W., Helv. chim. Acta *64* (1981) 2645.
15 Eberle, A.N., J. Receptor Res. *4* (1984), in press.
16 Bliss, C.I., and White, C., in: The Vitamins, p. 21. Eds P. György and W.N. Pearson. Academic Press, New York 1967.

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Malignant hyperthermia: Molecular defects in membrane permeability

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Summary. Malignant hyperthermia (MH), a genetically inherited disorder of skeletal muscle, is due to molecular defect in membrane permeability. The alteration in membrane permeability is suggested to be due to enhanced phospholipase A_2 activity which is responsible for the increased level in sarcoplasmic Ca^{2+}. The excess Ca^{2+} is responsible for muscle hyper-rigidity and enhanced rate of glycolysis, resulting in a rapid rate of lactic acid production and a low pH in MH muscle.
Key words. Malignant hyperthermia; membrane permeability; sarcoplasmic Ca^{2+}; phospholipase A_2 activity; calmodulin.

Malignant hyperthermia, a genetically inherited disorder, affects primarily the skeletal muscle of humans and pigs. The syndrome can be induced in apparently healthy humans[1–8] and in stress-susceptible pigs[9–16] by halothane[1–16], a fluorinated hydrocarbon anesthetic, and suxamethonium[3, 17–23], and particularly in pigs, by either environmental or physiological stress triggered by changes in temperature and excitement[10, 23–25]. Once initiated, the classical symptoms of the syndrome are characterized by gross muscular rigidity, rapid rise in body temperature, hyperventilation, severe metabolic acidosis and elevated levels of serum metabolites[26–28]. The body temperature may increase at a rate of 1 °C per 5 min[7], and the metabolic rate can exceed a 17-fold increase over normal[25], if uncontrolled, death occurs[11, 24, 29]. In humans, malignant hyperthermia can occasionally occur without any sign of muscle rigidity[2, 7], with 70% of malignant hyperthermia patients showing skeletal muscle rigidity[2]. The occurrence of anesthetic deaths in apparently healthy patients is about 1 in 15,000 anesthesia[2]. In healthy pigs, the incidence of malignant hyperthermia is dependent on the breed and in highly stress-susceptible breeds, it can be as high as 88%[30]. Malignant hyperthermia susceptible pigs are responsible for a substantial economic loss in the pig industry through transportation deaths[23, 24] and in the production of a commercially undesirable pale, soft and exudative meat[10, 23, 25, 31]. The latter condition is principally due to the denaturation of sarcoplasmic and myofibrillar proteins[32–34] caused by a combination of low muscle pH due to rapid glycolysis and high temperature immediately after death[11, 31, 33, 35]. The syndrome in humans and pigs[36] shows striking similarities, and it is generally assumed that the human and porcine malignant hyperthermia are identical. Thus, genetically selected pigs are frequently used as experimental models for the investigation of human malignant hyperthermia, particularly on the biochemical and physiological aspects of the syndrome.

In humans, the genetic inheritance appears to be autosomal dominant[1, 37–40] with reduced penetrance although multifactorial

inheritance[41] has also been suggested. In pigs, the inheritance is probably recessive[42-45] rather than dominant[46, 47] with incomplete[42] or complete[44] penetrance.

The development of muscle hyper-rigidity in human and porcine malignant hyperthermia is believed to be due to an increased release of calcium into the sarcoplasm. A significantly higher than normal sarcoplasmic calcium was indeed observed in malignant hyperthermia susceptible pigs early post-mortem[31].

This article discusses the current understanding of the molecular mechanism of malignant hyperthermia. It reviews pertinent findings on the calcium regulating organelles, particularly the intensively studied mitochondria and sarcoplasmic reticulum, and to a certain extent the sarcolemma, and discusses the factors most likely to be responsible for the increased membrane permeability in these organelles towards calcium.

Mitochondria. Conflicting results on respiratory functions were reported for mitochondria isolated from post-mortem and biopsy skeletal muscles of malignant hyperthermia susceptible humans and pigs. The majority of investigators[48-52] found no abnormality in these mitochondria from humans and pigs with respect to respiration, oxidative phosphorylation and respiratory contol. The abnormalities reported[11, 53] are mainly from differences in the quality of the isolated skeletal muscle mitochondria. The hypothesis of uncoupling of oxidative phosphorylation[11] and of the existence of an abberant mitochondrial respiratory chain system[53] in malignant hyperthermia were not substantiated since intact and tightly-coupled mitochondria isolated from skeletal muscles immediately post-mortem[48-50] and from biopsy samples[49, 51, 52, 54] exhibited normal respiratory functions.

The calcium transport of skeletal muscle mitochondria has also been investigated in vitro at various temperatures. Experimental data obtained at 37–40 °C are more meaningful than those at 25 °C since the in situ temperature is at least 37 °C and increases to 43 °C during malignant hyperthermia[7]. With the exception of a single observation reporting a reduction in calcium uptake[55], normal calcium uptake by malignant hyperthermia skeletal muscle mitochondria was observed at 25 °C[56-58]. A decrease in calcium uptake was however detected at 37 °C[59] and at 40 °C[50, 57] and also during malignant hyperthermia syndrome[60]. Halothane was found to depress calcium uptake with glutamate plus malate[59] but either to stimulate[55] or to have no effect[59] on calcium uptake with succinate at 37 °C.

Isolated skeletal muscle mitochondria of malignant hyperthermia susceptible pigs, like skeletal muscle mitochondria of dystrophic hamsters[61] and mice[62] contained about twice the normal amount of endogenous calcium[50, 58]. Mitochondria can accumulate calcium at the expense of ATP synthesis[63] and can play a role in regulating intracellular calcium by acting as an efficient store[64] to prevent calcium causing ultrastructural damage in muscle[65]. In frog muscle, electron probe analysis showed that the mitochondrial Ca^{2+} content was low and remained constant in tetanized muscle[66]. This interesting observation probably applies to normal skeletal muscle but not to muscles from malignant hyperthermia susceptible humans or pigs. Frogs are not known to develop malignant hyperthermia syndrome, and these findings might not be applicable to the syndrome. A significantly higher than normal amount of endogenous calcium was indeed observed in vivo by x-ray microanalysis in the skeletal muscle mitochondria of malignant hyperthermia-susceptible pigs[67]. The increase in the mitochondrial calcium content in the skeletal muscle of malignant hyperthermia susceptible pigs[50, 58] and perhaps that of dystrophic hamsters[61] and mice[62] could be a consequence of an increase in the sarcolemmal permeability to calcium. This excess calcium entry into the muscle cell could then be taken up by the mitochondria in order to prevent the cation from causing damage to the muscle[65]. The increase in endogenous mitochondrial calcium in malignant hyperthermia susceptible pigs via the sarcolemma could be due to the action of phospholipase A_2 (see below). Skeletal muscle mitochondria of malignant hyperthermia susceptible pigs released their endogenous calcium significantly faster than normal in the presence of an uncoupling agent[50, 58] and at the onset of anaerobiosis[31, 50, 57]. Skeletal muscle of malignant hyperthermia humans also showed faster calcium release than those of normal humans[68]. At 40 °C, mitochondria from malignant hyperthermia susceptible pigs could accumulate only half the normal amount of exogenous calcium during succinate oxidation; a further increase of exogenous calcium in the medium resulted in large amplitude swelling of mitochondria and this was accompanied by a loss in Ca^{2+}/O ratio and respiratory control[50, 57]. These induced changes in mitochondrial behavior appeared to be specific for calcium since neither large amplitude swelling nor loss of respiratory control was observed with exogenous ADP[50]. Large amplitude swelling of mitochondria was also observed in situ following incubation at 40 °C of skeletal muscle of malignant hyperthermia susceptible pigs but not of mitochondria of similar muscles from normal pigs (Cheah and Cheah, unpublished data). Gross mitochondrial swelling was also observed in biopsy skeletal muscle samples of malignant hyperthermia susceptible pigs during muscle rigidity but prior to temperature increase[27] and at the onset of porcine malignant hyperthermia syndrome[67], and in biopsy samples of human malignant hyperthermia skeletal muscle[69-73]. Thus, in human and porcine malignant hyperthermia the skeletal muscle mitochondria tend to exhibit large amplitude swelling with disrupted cristae but without showing any apparent impairment in their capacity to carry out oxidative phosphorylation[48-52, 54, 59, 67].

Skeletal muscle mitochondria of malignant hyperthermia susceptible pigs showed a transition temperature 9 °C higher than normal in the Arrhenius plots of calcium-stimulated respiration but no difference was observed with ADP[50]. The difference in the transition temperature was attributed to the action of mitochondrial phospholipase A_2 on the mitochondrial membranes since the transition temperature was restored to normal in the presence of a phospholipase A_2 inhibitor[50]. ADP is incapable of activating phospholipase A_2[74] and thus no difference was observed in the transition temperature between the two types of mitochondria during ADP-stimulated respiration. All the features reported for the skeletal muscle mitochondria of malignant hyperthermia susceptible pigs such as the gross amplitude swelling accompanied by enhanced calcium release, the decrease in the rate of calcium uptake at high temperature and the significant difference in the transition temperature are due to enhanced calcium-activated phospholipase A_2[50].

Sarcoplasmic reticulum. Conflicting observations of the calcium uptake by malignant hyperthermia sarcoplasmic reticulum preparations were reported for both humans and pigs. Analogous to the findings with mitochondria this was partly due to the quality of the isolated sarcoplasmic reticulum and partly due to differences in assay temperature. Malignant hyperthermia sarcoplasmic reticulum is difficult to isolate unmodified because of the fast rate of glycolysis in the skeletal muscles. Lower muscle pH,[35] increased free fatty acids[75], or a combination of both factors during the isolation procedure can alter its activity. Recent evidence on the interaction of isolated mitochondria and sarcoplasmic reticulum shows that fatty acids released from the phospholipids of mitochondrial membranes by phospholipase A_2 can inhibit the calcium transport of sarcoplasmic reticulum[75]. When precautions were taken to maintain the pH of muscle homogenate preparations at 7.2 normal calcium uptake was observed in both malignant hyperthermia humans[76] and pigs (Cheah and Cheah, unpublished data). Skinned fiber preparations from biopsy muscle of a malignant hyperthermia human showed normal sarcoplasmic reticulum calcium uptake[77]. The effect of halothane on calcium uptake by sarcoplasmic reticulum has also been investigated and has been shown to inhibit[27, 51, 78-80], to stimulate[14, 81, 82] or to have no effect[26]. These conflicting observations are most likely to be due to differences in the quality of the sarcoplasmic reticulum preparations as discussed above.

Calcium release from sarcoplasmic reticulum can be induced for example by the addition of either EDTA[83] or halothane[76, 84] to sarcoplasmic reticulum preloaded with exogenous calcium. With whole muscle homogenate preparations of human malignant hyperthermia patients, calcium release of sarcoplasmic reticulum by halothane was shown to be normal[76], but in malignant hyperthermia susceptible pigs calcium release from purified preparations was either greater[83, 84] or similar[85] to normal. The significantly greater than normal calcium release by porcine malignant hyperthermia sarcoplasmic reticulum[83, 84] was probably due to instability[35, 75] of the preparations rather than due to a true intrinsic difference. In both normal and malignant hyperthermia porcine sarcoplasmic reticulum preparations calcium release was stimulated by halothane[84].

Calcium can trigger calcium release from sarcoplasmic reticulum in vitro in the presence of caffeine or low concentrations of free magnesium[86], commonly referred as 'calcium-induced calcium release'. Recent reports on one human[77] and on three pigs[78] showed that the calcium-induced calcium release mechanism of malignant hyperthermia sarcoplasmic reticulum in skinned fibers[77] and in purified preparations[78] was significantly more sensitive to calcium than normal. Halothane accelerated the calcium-induced calcium release to a similar extent in both malignant hyperthermia and normal sarcoplasmic reticulum[77, 78]. The possibility that the difference observed in the sensitivity of the calcium-induced calcium release could be an artefact was not excluded[78]. In humans, the authors[77] found the difference in the calcium released by this mechanism between malignant hyperthermia and normal patients was smaller than expected. However, they tentatively postulated it to be sufficient to account quantitatively for the difference in the muscle contracture induced by halothane in normal and malignant hyperthermia humans.

The endogenous calcium content in the isolated sarcoplasmic reticulum was similar in malignant hyperthermia nnd normal pigs[78, 83]. However, the values of 52.0±4.0 nmoles calcium/mg protein for malignant hyperthermia sarcoplasmic reticulum and 52.7±6.4 nmoles calcium/mg protein for normal sarcoplasmic reticulum for *semitendinosus*[78] muscles were higher than the values of 20–40 and 10–30 nmoles calcium/mg protein respectively for *longissimus dorsi*[83], and this could be due to different types of skeletal muscle or to the different methods of muscle sampling. *Semitendinosus* muscle was obtained by biopsy[78] and *longissimus dorsi* was obtained immediately post-mortem[83], and under the latter conditions some calcium was probably released from the sarcoplasmic reticulum prior to its isolation.

The calcium-binding capacity of porcine malignant hyperthermia sarcoplasmic reticulum decreased significantly at high temperature[84, 87], and showed a transition temperature 8 °C higher than normal[87]. The transition temperature of the calcium-dependent ATPase (EC 3.6.1.3) was also abnormal, and its energy of activation within the temperature range of 31–45 °C was significantly lower than for normal sarcoplasmic reticulum[83]. The calcium-dependent ATPase activity of purified sarcoplasmic reticulum was either similar[75–83] or significantly higher[52, 56, 75] than normal. The increase in the calcium-dependent ATPase activity can be attributed to instability of the malignant hyperthermia sarcoplasmic reticulum caused by fatty acids[75] or by a combination of fatty acids and phospholipase A_2 activity[75]. Phospholipase A_2 can cleave the β-ester of membrane phospholipids in normal sarcoplasmic reticulum[88] and sarcolemma[89] and this results in an increase in membrane permeability for calcium.

Abnormalities in membrane permeability in other organelles. In addition to mitochondria and sarcoplasmic reticulum other membrane abnormalities in malignant hyperthermia were observed. Erythrocytes prepared from porcine blood taken with[90] or without[91] anesthesia, and samples obtained immediately post-mortem[58] were significantly more fragile than normal when subjected to osmotic shock. The sarcolemma also appeared to be more permeable since higher than normal activity of creatine phosphokinase, a skeletal muscle enzyme, was observed in the serum of both malignant hyperthermia humans[38, 92–95] and pigs[28, 96–99], and a greater than normal increase in the enzyme activity also occurred during drug-induced malignant hyperthermia[28, 96–99]. Adenylate cyclase activity and cyclic AMP content were also considerably higher than normal in muscle homogenates of malignant hyperthermia susceptible humans[100], indicating an abnormality in the sarcolemma. Clinical concentrations of halothane were shown to depolarize plasma membrane potential in malignant hyperthermia susceptible pigs by 5–15 mV but to have no apparent effect on the normal porcine plasma membrane potential[101]. Porcine malignant hyperthermia muscle was also demonstrated to have a lower potassium-induced contracture threshold than normal[102]. Freeze-fracture electron microscopic studies on the plasma membrane of biopsy muscle fibers from a human malignant hyperthermia patient with elevated creatine phosphokinase revealed possible membrane abnormalities[103]. Unfortunately no studies have yet been reported about the calcium permeability of the sarcolemmal membrane in malignant hyperthermia susceptible humans or pigs. It is however conceivable that in both cases, the muscle cells could easily be overloaded with calcium along an out-in-gradient in malignant hyperthermia. Membrane abnormalities[7, 8] were also observed in the pancreas and platelets. All these membrane abnormalities reported for malignant hyperthermia humans and pigs could be due to phospholipase A_2 activity (see below).

Phospholipase A_2. Significantly higher than normal endogenous phospholipase A_2 activity was observed in the skeletal muscle mitochondria of malignant hyperthermia pigs[75], and the enhanced enzyme activity was inhibited 75% with 1.0 mM spermine, a phospholipase A_2 inhibitor[104]. The phospholipase A_2 activity was shown to be responsible for the difference in the mitochondrial membrane fluidity between malignant hyperthermia and normal pigs since the difference in the transition temperature was abolished by a phospholipase A_2 inhibitor[50]. The calcium-activated phospholipase A_2 was also responsible for the calcium-induced uncoupling and large amplitude swelling of porcine malignant hyperthermia at high temperature as both of these abnormal features were prevented by bovine serum albumin, a binder of free fatty acids[105–108], and by the phospholipase A_2 inhibitors, spermine and tetracaine[109, 110]. Under these conditions, skeletal muscle mitochondria of malignant hyperthermia susceptible pigs could accumulate an amount of exogenous calcium similar to normal mitochondria without showing any sign of becoming uncoupled at 40 °C by calcium[50, 111]. The greater than normal phospholipase A_2 activity in skeletal muscle mitochondria of malignant hyperthermia susceptible pigs were attributed to significantly higher concentrations of endogenous activators such as calcium[50, 57], fatty acids[75] and calmodulin[112]. The phospholipase A_2 activity of malignant hyperthermia skeletal muscle mitochondria was calmodulin-dependent as both the calcium-induced uncoupling and large amplitude swelling could be prevented by low concentrations of trifluoperazine[113], an inhibitor of calmodulin-dependent enzyme[114–117].

The abnormality in membrane permeability in malignant hyperthermia erythrocytes[58, 90, 91], platelets[8, 118], pancreas[8] and plasma membrane[101] could also be due to a higher than normal phospholipase A_2 activity since this enzyme is known to be present in erythrocytes[119, 120], platelets[116, 121, 122], pancreas[123] and plasma membrane[124, 125]. An increase in erythrocyte fragility[126] is indeed linked with enhanced phospholipase A_2 activity[127], and in Duchenne muscular dystrophy both an increase in the enzyme activity[127] and a greater than normal fragility of erythrocytes[128] were indeed observed.

Molecular mechanism. It is universally established and accepted that skeletal muscle is the primary disorder in malignant hyperthermia, and that the principal features of the syndrome are muscle hyper-rigidity, increase in temperature and enhanced rate of glycolysis in association with an enhancement in membrane permeability. The increase in muscle rigidity in malignant

hyperthermia is apparently due to elevated sarcoplasmic calcium[12, 26, 28, 31] as normal muscle contraction is dependent on the concentration of free calcium in the sarcoplasm[129, 130]. In both humans and pigs, the enhanced release of calcium in malignant hyperthermia is temperature-dependent, in that halothane induced significantly greater than normal contractures in malignant hyperthermia muscle at 37 °C[5, 19, 131, 132], but with no contractures occurring at 25 °C[4, 133]. The enhanced rate of glycolysis[7, 12, 15, 33, 134] in malignant hyperthermia syndrome could be explained by the increase in the concentration of sarcoplasmic calcium stimulating glycolysis through activating the phosphorylase kinase (EC 2.7.1.38)[135, 136] and the myofibrillar ATPase (EC 3.6.1.3)[137, 138]. The enormous increase in temperature to 42 °C or higher[7, 12] is due to a combination of several events. Heat production in the initial 10 min in porcine malignant hyperthermia could be accounted for by an increase in aerobic metabolism[22] and in subsequent periods mainly by increased lactate production[12, 22, 26]. Catecholamines, which increase significantly during procine malignant hyperthermia[16, 21, 25, 139] probably exacerbate the syndrome by increasing peripheral vasoconstriction. This would result in a reduction of peripheral heat loss, accompanied by a further increase in body temperature[7, 8].

The generally accepted theory for human and porcine malignant hyperthermia is that there is a loss of control of calcium concentration in skeletal muscle. This has been suggested by various groups of investigators to be due to an alteration of membrane permeability[7, 8, 19, 90, 101, 103]. It has been postulated that this is caused by an enhancement of calcium-activated phospholipase A_2 activity[50, 75, 112] and that the skeletal muscle mitochondria[50, 75, 111–113, 134, 140] are involved in the syndrome. The calcium-activated phospholipase A_2, involved in enhancing calcium release from skeletal muscle mitochondria of malignant hyperthermia susceptible pigs[50, 75], was also demonstrated to operate in the sodium-induced calcium release from mitochondria isolated from various tissues[141]. The increase in free calcium in the sarcoplasm in the malignant hyperthermia syndrome could be accounted for by either one or a combination of the following events. Firstly, the release of all the endogenous calcium for example from mitochondria of *longissimus dorsi* muscle[50], assuming a reduction in sequestering of calcium by the sarcoplasmic reticulum (see explanation below), would be sufficient to raise the concentration of the sarcoplasmic calcium to at least 0.1 mM (the mitochondria from this skeletal muscle of malignant hyperthermia susceptible pigs contain 83±11 (n = 9) nmoles calcium per mg protein and between 6 and 8 mg mitochondria per g wet wt muscle). Secondly, the mitochondrial calcium-activated phospholipase A_2 activity alone[88] or in combination with the fatty acids released from the mitochondria could also alter the membrane permeability of the sarcoplasmic reticulum[72, 142], causing further release of calcium into the sarcoplasm. Thirdly, the calcium transport of the sarcolemma could be inactivated by either the phospholipase A_2 activity[94] or by fatty acids, or by a combination of both of these factors. Phospholipase A_2 is present in the sarcolemma[124, 125]. This enzyme can increase the membrane permeability of the sarcolemma[89], thus probably making the sarcolemma of malignant hyperthermia susceptible pigs more easily depolarized than normal[101] and could also increase the entry of calcium into the muscle cells. Recent data on porcine skeletal muscle mitochondria show that a significantly higher amount of endogenous calmodulin could be responsible for the enhanced endogenous phospholipase A_2 activity in malignant hyperthermia[112] and that calmodulin could be intimately involved in the development of malignant hyperthermia syndrome.

The enhanced release of catecholamines[16, 21, 25, 139, 143] has been suggested[139, 143] to be responsible for the development of the porcine malignant hyperthermia syndrome, but they appear to play only a secondary role in the syndrome[7, 8, 48, 144]. The release of catecholamines is probably stimulated by the increase of either calcium[145], high temperature[25, 146–149] or anoxia[150] or by metabolic acidosis[48, 148, 151, 152] or by a combination of these factors. In normal pigs, the increase in in vivo rectal temperature from 39.5° to 43.0 °C induced by exposure to high environmental temperature resulted in a 12-fold increase in the level of plasma catecholamines, but no development of malignant hyperthermia was observed[146, 147]. In malignant hyperthermia susceptible pigs, initiation of the syndrome was accompanied by an increase in the plasma catecholamines of at least 5-fold greater than normal[139] and a muscle temperature of about 40 °C[12, 22]. Total spinal blockade, which completely inhibited the increase of circulating catecholamines, did not prevent the onset of drug-induced porcine malignant hyperthermia syndrome[48], and halothane caused metabolic changes associated with the syndrome prior to the increase in catecholamines[16]. Furthermore, halothane has recently been shown to inhibit norepinephrine release[153].

The development of porcine malignant hyperthermia induced by stress[10, 23–25] is most likely to be due to a reduction in the oxygen supply to skeletal muscles. This in turn will result in an enhanced calcium release through activation of phospholipase A_2 as previously discussed. *M. longissimus dorsi* of anesthetized hyperthermia susceptible pigs was found to be more sensitive to anoxia induced by administration of pure nitrogen or by exsanguination than similar muscle of normal pigs[154]. Lactic acid levels were also significantly higher in biopsy samples of malignant hyperthermia susceptible pigs breathing oxygen than similar muscle of normal pigs[154]. Pigs anesthetized with halothane also showed a reduction of 84% and 90% in blood flow to skeletal muscles and skin respectively[155], and this would result in a loss of oxygen to these tissues.

The excitation-contraction coupling mechanism in malignant hyperthermia susceptible humans and pigs might also be defective. In malignant hyperthermia susceptible pigs, the threshold potential for activation of the contractile process was shifted towards more negative values and, provided the threshold for in activation was not shifted to the same extent, activation of the muscle occurred following a slight depolarization[156]. This shift of the inactivation curve to more negative potentials was apparently due to lack of calcium ions[157, 158], and this probably accounted for the observation that caffeine and halothane contractures were diminished in a calcium-free medium[159].

The evidence reported for human and porcine malignant hyperthermia thus suggests that an abnormality in the excitation-contraction coupling and an abnormal increase in the membrane permeability to calcium are undoubtedly important attributes to the development of malignant hyperthermia syndrome. The increase in membrane permeability resulting in an increased level of calcium is probably the principal lesion, and an enhanced endogenous phospholipase A_2 activity and calmodulin could be intimately involved in the syndrome.

1 Denborough, M.A., Forster, J.F.A., Lovell, R.R.H. Maplestone, P.A., and Villiers, J.D., Br. J. Anaesth. *34* (1962) 395.
2 Britt, B.A., and Kalow, W., Can. Anaesth. Soc. J. *17* (1970) 293.
3 Britt, B.A., and Kalow, W., Ann. N.Y. Acad. Sci. *151* (1968) 947.
4 Kalow, W., Britt, B.A., Terreau, M.E., and Haist, C., Lancet *2* (1970) 895.
5 Ellis, F.R., Harriman, D.G.F., Keaney, N.P., Kyei-Mensah, K., and Tyrell, J.H., Br. J. Anaesth. *43* (1971) 721.
6 Harriman, D.G.F., Ellis, F.R., Franks, A.J., and Summer, D.W., in: Second International Symposium on Malignant Hyperthermia, p. 67. Eds J.A. Aldrete and B.A. Britt. Grune and Stratton, Inc., New York 1978.
7 Gronert, G.A., Anesthesiology *53* (1980) 395.
8 Denborough, M.A., Pharmac. Ther. *9* (1980) 357.
9 Harrison, G.G., Biebuyck, J.F., Terblanche, J., Dent, D.M., Hickman, R., and Saunders, J.J., Br. Med. J. *3* (1968) 594.
10 Topel, D.G., Bicknell, E.J., Preston, K.S., Christian, L.L. and Matsushima, C.Y., Mod. vet. Pract. *49* (1968) 40.
11 Sybesma, W., and Eikelenboom, G., Neth. J. vet. Sci. *2* (1969) 155.
12 Berman, M.C., Harrison, G.G., Bull, A.A., and Kench, J.E., Nature *225* (1970) 653.

13 Hall, L.W., Trim, C.W., and Woolf, N., Br. Med. J. *2* (1972) 145.
14 Nelson, T.E., Jones, E.W., Venable, J.H., and Kerr, D.D., Anesthesiology *36* (1972) 52.
15 Clarke, M.G., Williams, C.H., Pfeifer, W.F., Bloxham, D.P., Holland, P.C., Taylor, C.A., and Lardy, H.A., Nature *245* (1973) 99.
16 Gronert, G.A., and Theye, R.A., Anesthesiology *44* (1976) 36.
17 Thut, W.H., and Davenport, H.T., Can. Anaesth. Soc. J. *13* (1966) 425.
18 Ryan, J.F., Anesthesiology *32* (1970) 196.
19 Moulds, R.F.W., and Denborough, M.A., Br. Med. J. *4* (1974) 241.
20 Hall, L.W., Woolf, N., Bradley, J.W.P., and Jolly, D.W., Br. Med. J. *4* (1966) 1305.
21 Gronert, G.A., and Theye, R.A., Br. J. Anaesth. *48* (1976) 513.
22 Hall, G.M., Bendall, J.R., Lucke, J.N., and Lister, D., Br. J. Anaesth. *48* (1976) 305.
23 Allen, W.M., Berrett, S., Harding, J.D.J., and Patterson, D.S.P., Vet. Rec. *87* (1970) 64.
24 Allen, W.M., Hebert, C.N., and Smith, L.P., Vet. Rec. *94* (1974) 212.
25 Williams, C.H., Houchins, C., and Shanklin, M.D., Br. Med. J. *3* (1975) 411.
26 Berman, M.C., and Kench, J.E., in: International Symposium on Malignant Hyperthermia, p.287. Eds R.A. Gordon, B.A. Britt and W. Kalow. Charles C. Thomas, Springfield 1973.
27 Brucker, R.F., Williams, C.H., Popinigis, J., Galvez, T.L., Vail, W.J., and Taylor, C.A., in: International Symposium on Malignant Hyperthermia, p.238. Eds R.A. Gordon, B.A. Britt and W. Kalow. Charles C. Thomas, Springfield 1973.
28 van den Hende, C., Lister, D., Muylle, E., Ooms, L., and Oyaert, W., Br. J. Anaesth. *48* (1976) 821.
29 Williams, C.H., Shanklin, M.D., and Houchins, C., in: Malignant Hyperthermia: Current Concepts, p.149. Ed. E.O. Henschel. Appleton-Century-Crofts, New York 1977.
30 Webb, A.J., Anim. Prod. *31* (1980) 101.
31 Cheah, K.S., Cheah, A.M., Crosland, A.R., Casey, J.C., and Webb, A.J., Meat Sci. *10* (1984) 117.
32 Wismer-Pedersen, J., Fd Res. *24* (1959) 711.
33 Bendall, J.R., and Wismer-Pedersen, J., J. Fd Sci. *27* (1962) 144.
34 Penny, I.F., J. Sci. Fd Agric. *28* (1977) 329.
35 Greaser, M.L., Cassens, R.G., Briskey, E.J., and Hoestra, W.G., J. Fd Sci. *34* (1969) 120.
36 Wingard, D.W., and Gatz, E.E., in: Second International Symposium on Malignant Hyperthermia, p.363. Eds J.A. Aldrete and B.A. Britt. Grune and Stratton, Inc., New York 1978.
37 Britt, B.A., Locher, W.G., and Kalow, W., Can. Anaesth. Soc. J. *16* (1969) 89.
38 Isaacs, H., and Barlow, M.B., Br. J. Anaesth. *42* (1970) 1077.
39 King, J.O., Denborough, M.A., and Zapf, P.W., Lancet *1* (1972) 365.
40 Parikh, R.K., and Thomson, W.H.S., Br. J. Anaesth. *44* (1972) 742.
41 Ellis, F.R., Cain, P.A., and Harriman, D.G.F., in: Second International Symposium on Malignant Hyperthermia, p.329. Eds J.A. Aldrete and B.A. Britt. Grune and Stratton, Inc., New York 1978.
42 Ollivier, L., Sellier, P., and Monin, G., Ann. genet. Sel. Anim. *7* (1975) 159.
43 Smith, C., and Bamptom, P.R., Genet. Res., Camb. *29* (1977) 287.
44 Eikelenboom, G., Minkema, D., van Eldik, P., and Sybesma, W., in: Second International Symposium on Malignant Hyperthermia, p.141. Eds J.A. Aldrete and B.A. Britt. Grune and Stratton, Inc., New York 1978.
45 Webb, A.J., and Jordan, C.H.C., Anim. Prod. *26* (1978) 157.
46 Jones, E.W., Nelson, T.E., Anderson, L.L., Kerr, D.D., and Burnap, T.K., Anesthesiology *36* (1972) 42.
47 Williams, C.H., and Lasley, J.H., in: Malignant Hyperthermia: Current Concepts, p.141. Ed. E.O. Henschel. Appleton-Century-Crofts, New York 1977.
48 Cheah, K.S., J. Sci. Fd Agric. *24* (1973) 51.
49 Brooks, G.A., and Cassens, R.G., J. Anim. Sci. *37* (1973) 688.
50 Cheah, K.S., and Cheah, A.M., Biochim. biophys. Acta *634* (1981) 70.
51 Denborough, M.A., Hird, F.J.R., King, J.O., Marginson, M.A., Mitchelson, K.R., Nayler, W.G., Rex, M.A., Zapf, P., and Condron, R.J., in: International Symposium on Malignant Hyperthermia, p.229. Eds R.A. Gordon, B.A. Britt and W. Kalow. Charles C. Thomas, Springfield 1973.
52 Campion, D.R., Olson, J.C., Topel, D.G., Christian, L.L., and Kuhlers, D.L., J. Anim. Sci. *41* (1975) 1314.
53 Eikelenboom, G., and van den Bergh, S.G., J. Anim. Sci. *37* (1973) 692.
54 Britt, B.A., Kalow, W., and Endrenyi, L., in: International Symposium on Malignant Hyperthermia, p.387. Eds R.A. Gordon, B.A. Britt and W. Kalow, Charles C. Thomas, Springfield 1973.
55 Britt, B.A., Endrenyi, L., Cadman, D.L., Fan, H.M., and Fung, H.Y.K., Anesthesiology *42* (1975) 292.
56 Greaser, M.L., Cassens, R.G., Briskey, E.J., and Hoekstra, W.G., J. Fd Sci. *34* (1969) 125.
57 Cheah, K.S., and Cheah, A.M., FEBS Lett. *95* (1978) 307.
58 Cheah, K.S., and Cheah, A.M., FEBS Lett. *107* (1979) 265.
59 Gronert, G.A., and Heffron, J.J.A., Anesth. Analg. *58* (1979) 76.
60 Somers, C.J., and McLoughlin, J.V., J. comp. Path. *92* (1982) 191.
61 Mezon, B.J., Wrogemann, K., and Blanchaer, M.C., Can. J. Biochem. *52* (1974) 1024.
62 Nylen, E.G., and Wrogemann, K., Exp. Neurol. *80* (1983) 69.
63 Lehninger, A.L., Carafoli, E., and Rossi, C.S., Adv. Enzymol. *29* (1967) 259.
64 Borle, A.B., Fedn Proc. *32* (1973) 1944.
65 Publicover, S.J., Duncan, C.J., and Smith, J.L., J. Neuropath. exp. Neurol. *37* (1978) 544.
66 Somlyo, A.V., Gonzalez-Serratos, H., Shuman, H., McClellan, G., and Somlyo, A.P., J. Cell Biol. *90* (1981) 577.
67 Stadhouders, A.M., Viering, W.A.L., Verburg, M.P., Ruitenbeek, W., and Sengers, R.C.A., Acta anaesth. scand. *28* (1984) 14.
68 Heffron, J.J.A., Biochem. Soc. Trans. *12* (1984) 360.
69 Carpenter, G.C., Soc. Paediat. Res. *175* (1966) 29.
70 Schiller, H.H., and Mair, W.G.P., J. neurol. Sci. *21* (1974) 93.
71 Reske-Nielsen, E., Haase, J., and Kelstrup, J., Acta path. microbiol. scand. Sect. A *83* (1975) 651.
72 Isaacs, M., in Second International Symposium on Malignant Hyperthermia, p.89. Eds J.A. Aldrete and B.A. Britt. Grune and Stratton, Inc., New York 1978.
73 Hull, M.T., Muller, J., and Albrecht, W.H. Anesthesiology *48* (1978) 223.
74 Waite, M., Scherphof, G.L., Boshouwers, F.M.G., and van Deenen, L.L.M., J. Lipid Res. *10* (1969) 411.
75 Cheah, K.S., and Cheah, A.M., Biochim. biophys. Acta *638* (1981) 40.
76 Blanck, T.J.J., Gruener, R., Suffecool, S.L., and Thompson, M., Anesth. Analg. *60* (1981) 492.
77 Endo, M., Yagi, S., Ishizuka, T., Horiuti, K., Koga, Y., and Amaha, K., Biomed. Res. *4* (1983) 83.
78 Ohnishi, S.T., Taylor, S., and Gronert, G.A., FEBS Lett. *161* (1983) 193.
79 Dhalla, N.S., Sulakhe, P.V., Clinch, N.F., Wade, J.G., and Naimark, A., Biochem. Med. *6* (1972) 333.
80 Britt, B.A., Kalow, W., Gordon, A., Humphrey, J.G., and Rewcastle, N.B., Can. Anaesth. Soc. J. *20* (1973) 431.
81 Steward, D.J., and Thomas, T.A., in: International Symposium on Malignant Hyperthermia, p.409. Eds R.A. Gordon, B.A. Britt and W. Kalow. Charles C. Thomas, Springfield 1973.
82 Britt, B.A., Endrenyi, L., and Cadman, D.L., Br. J. Anaesth. *47* (1975) 650.
83 McIntosh, D.B., Berman, M.C., and Kench, J.E., Biochem. J. *166* (1977) 387.
84 Gronert, G.A., Heffron, J.J.A., and Taylor, S.R., Eur. J. Pharmac. *58* (1979) 179.
85 White, M.D., Collins, J.G., and Denborough, M.A., Biochem. J. *212* (1983) 399.
86 Endo, M., Physiol. Rev. *57* (1977) 71.
87 Nelson, T.E., and Bee, D.E., J. clin. Invest. *64* (1979) 895.
88 Fiehn, W., and Hasselbach, W., Eur. J. Biochem. *13* (1970) 510.
89 Sulakhe, P.V., Drummond, G.I., and Ng, D.C., J. biol. Chem. *248* (1973) 4150.
90 Harrison, G.G., and Verburg, C., Br. J. Anaesth. *45* (1973) 131.
91 King, W.A., Ollivier, L., and Basrur, P.K., Ann. genet. Sel. Anim. *9* (1976) 537.
92 Denborough, M.A., Ebeling, P., King, J.O., and Zapf, P., Lancet *1* (1970) 1138.
93 Steers, A.J.W., Tallack, J.A., and Thompson, D.E.A., Br. Med. J. *2* (1970) 341.
94 Isaacs, H., and Barlow, M.B., J. Neurol. Neurosurg. Psych. *36* (1973) 228.
95 Tammisto, T., and Airaksinen, M., Br. J. Anaesth. *38* (1966) 510.
96 Woolf, N., Hall, L., Thorne, C., Down, M., and Walker, R., Br. Med. J. *3* (1970) 386.
97 Berman, M.C., Du Toit, P., and Kench, J.E., S. Afr. med. J. *45* (1971) 1208.

98 Nelson, T.E., Jones, E., Henrickson, R., Falk, S., and Kerr, D.D., Am. J. vet. Res. *35* (1974) 347.
99 Heffron, J.J.A., and Mitchell, G., Anesth. Analg. *54* (1975) 536.
100 Willner, J.H., Cerri, C.G., and Wood, D.S., J. clin. Invest. *68* (1981) 95.
101 Gallant, E.M., Godt, R.E., and Gronert, G.A., Muscle and Nerve *2* (1979) 491.
102 Gallant, E.M., Gronert, G.A., and Taylor, S.R., Neurosci. Lett. *28* (1982) 181.
103 Schmalbruch, H., J. Neuropath. exp. Neurol. *38* (1979) 407.
104 Sechi, A.M., Cabrini, L., Landi, L., Pasquali, P., and Lenaz, G., Archs Biochem. Biophys. *186* (1978) 248.
105 Borst, P., Loos, J.A., Christ, E.J., and Slater, E.C., Biochim. biophys. Acta *62* (1962) 509.
106 Vazquez-Colon, L., Ziegler, F.D., and Elliott, W.B., Biochemistry *5* (1966) 1134.
107 Weinbach, E.C., Garbus, J., and Glaggett, C.E., J. biol. Chem. *241* (1966) 3708.
108 Nixon, M., and Chan, S.H.P., Analyt. Biochem. *97* (1979) 403.
109 Seppala, A.J., Saris, N.E.L., and Gauffin, M.L., Biochem. Pharmac. *20* (1971) 305.
110 Scherphof, G.L., Scarpa, A., and van Toorenenbergen, A., Biochim. biophys. Acta *270* (1972) 226.
111 Cheah, K.S., and Cheah, A.M., in: First European Bioenergetic Conference, Bologna, Italy, p. 397. Patron Editore (1980).
112 Cheah, K.S., Biochem. Soc. Trans. *12* (1984) 358.
113 Cheah, K.S., and Waring, J.C., Biochim. biophys. Acta *723* (1983) 45.
114 Levin, R.M., and Weiss, B., Molec. Pharmac. *13* (1977) 690.
115 Levin, R.M., and Weiss, B., J. Pharmac. exp. Ther. *208* (1979) 454.
116 Wong, P.Y.-K., and Cheung, W.Y., Biochem. biophys Res. Commun. *90* (1979) 473.
117 Weiss, B., Prozialeck, W., Cimino, M., Barnette, M.S., and Wallace, T.L., Ann. N.Y. Acad. Sci. *356* (1980) 319.
118 Solomons, C.C., Tan, S., and Aldrete, J.A., in: Second International Symposium on Malignant Hyperthermia, p. 221. Eds J.A. Aldrete and B.A. Britt. Grune and Stratton, Inc., New York 1978.
119 Frei, E., and Zahler, P., Biochim. biophys. Acta *550* (1979) 450.
120 Engelsen, S.J., and Zata, M., Biochim. biophys. Acta *711* (1982) 515.
121 Kannagi, R., and Koizumi, K., Biochim. biophys. Acta *556* (1979) 423.
122 Lagarde, M., Menashi, S., and Crawford, N., FEBS Lett. *124* (1980) 23.
123 Scherphofd, G., and Westenberg, H., Biochim. biophys. Acta *398* (1975) 442.
124 van den Bosch, H., Biochim. biophys. Acta *604* (1980) 191.
125 Franson, R.C., Pang, D.C., Towle, D.W., and Weglicki, W.B., J. molec. Cell Cardiol. *10* (1978) 921.
126 van Deenen, L.L.M., FEBS Lett. *123* (1981) 3.
127 Iyer, S.L., Katyare, S.S., and Howland, J.L., Neurosci. Lett. *2* (1976) 103.
128 Lloyd, S.J., and Nunn, M.G., Br. Med. J. *11* (1978) 252.
129 Ebashi, S., Endo. M., and Ohtsuki, I., Q. Rev. Biophys. *2* (1969) 351.
130 Weber, A., and Murray, J.M., Physiol. Rev. *53* (1973) 612.
131 Gallant, E.M., Godt, R.E., and Gronert, G.A., J. Pharmac. exp. Ther. *213* (1980) 91.
132 Sullivan, J.S., and Denborough, M.A., Br. J. Anaesth. *53* (1981) 1217.
133 Nelson, T.E., Bedell, D.M., and Jones, E.W., Anesthesiology *42* (1975) 301.
134 Cheah, K.S., and Cheah, A.M., Experientia *35* (1979) 1001.
135 Ozawa, E., Hosoi, K., and Ebashi, S., J. Biochem., Tokyo *61* (1967) 531.
136 Heilmeyer, L.M.G. Jr, Meyer, F., Haschke, R.H., and Fischer, E.H., J. biol. Chem. *245* (1970) 6649.
137 Brostrom, C.O., Hunkeler, F.L., and Krebs, E.G., J. biol. Chem. *246* (1971) 1961.
138 Scopes, R.K., Biochem. J. *142* (1974) 79.
139 Lucke, J.N., Hall, G.M., and Lister, D., Br. J. Anesth. *48* (1976) 297.
140 Mitchell, G., and Heffron, J.J.A., S. Afr. J. Sci. *76* (1980) 546.
141 Harris, E.J., and Heffron, J.J.A., Archs Biochem. Biophys. *218* (1982) 531.
142 Cheah, A.M., Biochim. biophys. Acta *648* (1981) 113.
143 Williams, C.H., in Malignant Hyperthermia. Current Concepts, p. 117. Ed. E.O. Henschel. Appleton-Century-Crofts, New York 1977.
144 Gronert, G.A., Milde, J.H., and Taylor, S.R., J. Physiol. *307* (1980) 319.
145 Douglas, W.W., and Rubin, R.P., J. Physiol. *167* (1963) 288.
146 Ingram, D.L., Dauncey, M.L., Barrand, M.A., and Callingham, B.A., in: Catecholamines and Stress. Recent Advances, p. 273. Eds E. Usdin, R. Kvetnansky and I.J. Kopin. Elsevier, North Holland, Inc., Amsterdam 1980.
147 Barrand, M.A., Dauncey, M.L., and Ingram, D.L., J. Physiol. *316* (1981) 139.
148 Hillarp, N.-A., and Nilson, B., Acta physiol. scand. *31* (1954) 79.
149 Hillarp, N.-A., Acta physiol. scand. *43* (1958) 292.
150 Comline, R.S., and Silver, M., Nature *181* (1958) 283.
151 Nahas, G.G., Ligou, J.C., and Mehlman, B., Am. J. Physiol. *198* (1960) 60.
152 Johnson, R.G., Carlson, N.J., and Scarpa, A., J. biol. Chem. *253* (1978) 1512.
153 Rorie, D.K., Tyce, G.M., and Mackenzie, R.A., Anesth. Analg. *63* (1984) 1059.
154 Lister, D., Sair, R.A., Will, J.A., Schmidt, G.R., Cassens, R.G., Hoekstra, W.G., and Briskey, E.J., Am. J. Physiol. *218* (1970) 102.
155 Tranquilli, W.J., Manohar, M., Parks, C.M., Thurmon, J.C., Theodorakis, M.C., and Benson, G.J., Anesthesiology *56* (1982) 369.
156 Bryant, S.H., and Anderson, I.L., Soc. Neurosci. *3* (1977) 213 (abstract).
157 Luttgau, H.Ch., and Spiecker, W., J. Physiol. *296* (1979) 411.
158 Graf, F., and Schatzmann, H.J., J. Physiol. *349* (1984) 1.
159 Moulds, R.F.W., and Denborough, M.A., Clin. exp. Pharmac. Physiol. *1* (1974) 197.

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Hypersensitivity to endotoxin hepatotoxicity in rats with inflammatory lesions

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Summary. The ratio of sinusoidal nonparenchymal cells to hepatocytes in rat liver was significantly increased following induction of inflammation, and decreased after subsequent exposure to endotoxin, particularly in the region around the terminal portal venules. Rats with inflammatory lesions were more sensitive to endotoxin hepatocytotoxicity than normal controls, as judged from the dose-dependent increase in activity of serum transaminases and from the extent of liver tissue injury. In addition, these animals, which were already in a state of depletion of hepatic glycogen, demonstrated marked hyperglycemia 24 h after endotoxin administration in small doses of less than 2 mg/kg.
Key words. Inflammation; nonparenchymal cells; endotoxin; liver injury.

Inflammation causes some functional alterations in rat hepatocytes, such as enhanced glycoprotein synthesis[1], impaired drug metabolism[2], and delayed dye-uptake[3], although histologic lesions are virtually absent. On the other hand, toxic liver injury can be greatly enhanced by ordinarily harmless doses of bacterial endotoxin in animals[4].