Anesthesiology
V 42, No 3, Mar 1975

Porcine Malignant Hyperthermia:

Effects of Halothane on Mitochondrial Respiration and Calcium Accumulation

Beverley A. Britt, M.D., F.R.C.P. (C), Laszlo Endrenyi, Dipl. Eng., Ph.D.,† Donna L. Cadman, B.Sc.,‡ Ho Man Fan, B.Sc. (Eng.),‡ Harry Y-K. Fung‡*

Vastus lateralis muscle was excised from normal pigs and from pigs susceptible to malignant hyperthermia. Anesthesia consisted of diazepam, $N_2O:O_2$, and a lidocaine field block. In the susceptible (MHS) pigs, respiratory control in mitochondria of excised muscle was normal, while electron transport was accelerated. Glutamate log Q_3's and log Q_4's were in inverse linear relationship to porcine weights. In the presence of glutamate, state 3 respiration was 3.5 times, and state 4 respiration 3.2 times, higher in MHS than in normal mitochondria (independent of weight or halothane dose). Thus, the MHS mitochondria were able to produce ATP more efficiently than normal mitochondria. *In vitro*, halothane inhibited glutamate Q_3's and R.C.I.'s, slightly increased succinate Q_3's and R.C.I.'s, had no significant effect on glutamate or succinate Q_4's, and moderately lowered glutamate and succinate P/O ratios. These changes were similar to those observed in normal pigs. Calcium uptake into MHS mitochondria was markedly less than normal but was not significantly altered by *in-vitro* halothane. These results suggest a mitochondrial membrane component for the defect of porcine malignant hyperthermia, since calcium is normally associated with the formation of the phospholipid lattice of this membrane. (Key words: Hyperthermia, malignant; Metabolism, mitochondrial; Muscle, skeletal, mitochondria; Anesthetics, volatile, halothane; Ions, calcium.)

A FEW YEARS AGO, several investigators[1-3] proposed that the primary defect of malignant hyperthermia (MH) lay in the mitochondrion. They postulated that triggering agents such as halothane and succinylcholine uncoupled oxidative phosphorylation from electron transport, thus accelerating heat, carbon dioxide, and lactate formation but reducing adenosine triphosphate (ATP) production.

We,[4,5] however, found that mitochondria isolated from skeletal muscle of malignant-hyperthermia-susceptible (MHS)§ human patients were normally coupled and that the addition of halothane *in vitro* inhibited electron transport, but caused only slight uncoupling when glutamate was the substrate. These effects were similar to those observed in normal mitochondria, and *in vivo* they would be expected to lower heat, carbon dioxide, and lactate formation.

Data on oxidative phosphorylation in skeletal muscle of MHS pigs are scanty, incomplete, and contradictory. Denborough *et al.*[6] reported that mitochondria of skeletal

§ MHS—a human being or pig known to carry an MH gene, whether fully recovered from an MH crisis or not yet anesthetized with triggering agents. Pain relief for muscle biopsies in such subjects is carried out with nontriggering agents such as local field block or epidural anesthesia, or with general anesthesia with nitrous oxide, thiopental, diazepam or neuroleptanalgesia.

ABBREVIATIONS

MH	= malignant hyperthermia
MHS	= malignant-hyperthermia susceptible
SR	= sarcoplasmic reticulum
ATP	= adenosine 5'-triphosphate
NAD	= β-nicotinamide adenine dinucleotide
DNP	= 2,4-dinitrophenol
ADP	= adenosine 5'-diphosphate
EDTA	= ethylenediamine-tetraacetic acid
EGTA	= ethylene glycol-bis (β-amino ethyl ether)-N,N'-tetraacetic acid
Q_3	= state 3 respiration
Q_4	= state 4 respiration
R.C.I.	= respiratory control index
P/O ratio	= ratio of moles of ADP added to atoms of oxygen consumed

* Assistant Professor, Department of Anaesthesia.
† Associate Professor, Department of Pharmacology.
‡ Department of Anesthesia.
Received from the Faculty of Medicine, University of Toronto, Toronto, Ontario M5S 1A8, Canada. Accepted for publication July 15, 1974.

TABLE 1. Respiratory Values at Various Doses of Halothane in Malignant Hyperthermic Pigs and Control Pigs

	Additives	Glutamate				Succinate			
		Halothane 0 Vol Per Cent	Halothane 0.5 Vol Per Cent	Halothane 1.8 Vol Per Cent	Halothane 5.0 Vol Per Cent	Halothane 0 Vol Per Cent	Halothane 0.5 Vol Per Cent	Halothane 1.8 Vol Per Cent	Halothane 5.0 Vol Per Cent
Q_3*									
Normal	—	0.117	0.094	0.073	0.031	0.130	0.150	0.155	0.164
	NAD	0.109	0.097	0.071	0.030	0.127	0.153	0.162	0.166
	NAD + DNP	0.111	0.094	0.075	0.031	0.127	0.154	0.158	0.167
Malignant hyperthermia	—	0.164	0.119	0.094	0.047	0.153	0.198	0.198	0.194
	NAD	0.157	0.116	0.091	0.041	0.147	0.200	0.200	0.191
	NAD + DNP	0.152	0.117	0.093	0.044	0.153	0.196	0.193	0.190
Q_4*									
Normal	—	0.0112	0.0120	0.0124	0.0121	0.0204	0.0224	0.0201	0.0213
	NAD	0.0101	0.0119	0.0121	0.0117	0.0193	0.0217	0.0200	0.0215
Malignant hyperthermia	—	0.0127	0.0152	0.0147	0.0145	0.0244	0.0246	0.0228	0.0234
	NAD	0.0123	0.0154	0.0146	0.0131	0.0246	0.0240	0.0241	0.0223
R.C.I. (Q_3/Q_4)									
Normal	—	10.71	7.97	5.90	2.60	6.36	6.66	7.62	7.71
	NAD	10.91	8.23	6.05	2.59	6.49	7.05	8.10	7.76
Malignant hyperthermia	—	12.89	7.93	6.37	3.21	6.48	8.15	8.31	8.23
	NAD	12.75	7.79	6.27	3.13	6.05	8.35	8.26	8.54
P/O									
Normal	—	3.03	2.60	2.37	2.04	1.90	1.78	1.69	1.53
	NAD	2.89	2.53	2.37	2.07	1.84	1.76	1.64	1.52
Malignant hyperthermia	—	2.79	2.53	2.31	1.98	1.88	1.75	1.74	1.69
	NAD	2.80	2.51	2.35	1.97	1.89	1.72	1.68	1.62

* Geometric averages uncorrected for weight dependence.

Q_3 = respiration of mitochondria in the presence of oxygen and exogenous substrate, phosphate and ADP in μ atoms of oxygen consumed per mg of protein per minute.

Q_4 = respiration of mitochondria in the presence of oxygen and exogenous substrate and phosphate and the absence of exogenous ADP in μ atoms of oxygen consumed per mg of protein per minute.

TABLE 2. Statistical Tests Evaluating the Effects of Malignant Hyperthermia on Respiration in Pigs

		Glutamate		Succinate	
	Additives	Effect of Malignant Hyperthermia*	Effect of MH × Halothane Dose (Interaction)†	Effect of Malignant Hyperthermia*	Effect of MH × Halothane Dose (Interaction)†
Log Q_3	—	7.62¶	0.84, N.S.	4.82¶	0.40, N.S.
	NAD	6.11¶	0.73, N.S.	4.04¶	0.45, N.S.
	NAD + DNP	6.49¶	0.60, N.S.	4.30¶	0.25, N.S.
Log Q_4	—	4.29¶	0.33, N.S.	2.58‡	0.20, N.S.
	NAD	4.37¶	0.45, N.S.	2.71‡	0.76, N.S.
Log R.C.I.	—	3.01§	1.69, N.S.	1.84, N.S.	0.54, N.S.
	NAD	1.89, N.S.	1.71, N.S.	0.94, N.S.	0.82, N.S.
P/O	—	1.50, N.S.	0.46, N.S.	1.23, N.S.	1.73, N.S.
	NAD	1.67, N.S.	0.43, N.S.	1.11, N.S.	0.83, N.S.

Q_3 = respiration of mitochondria in the presence of oxygen and exogenous substrate, phosphate and ADP in μ atoms of oxygen consumed per mg of protein per minute.

Q_4 = respiration of mitochondria in the presence of oxygen and exogenous substrate and phosphate and the absence of exogenous ADP in μ atoms of oxygen consumed per mg of protein per minute.

R.C.I. = Q_3/Q_4.

P/O ratio = μ moles of ADP added/μ atoms of oxygen consumed.

* t statistic with 15 degrees of freedom evaluating the statistical significance of the difference between averages (or geometric averages), taken over all halothane doses, of the hyperthermic pigs and the control pigs. (N.S. = not significant, $P \geq 0.05$).

† F statistic with 3 and 45 degrees of freedom. It determines whether changes of the responses to different doses of halothane are differently affected by the presence and the absence of halothane. (N.S. = not significant, $P \geq 0.05$.)

‡ $P < 0.05$.

§ $P < 0.01$.

¶ $P < 0.001$.

muscle from both MHS and normal Landrace pigs were more susceptible to halothane *in vivo* in terms of lowering the glutamate P/O ratio than were those from other breeds of pigs. Berman and Kench,[7] on the other hand, observed that mitochondria obtained from Landrace pigs undergoing hyperthermic rigor showed effects similar to those of normal rat liver mitochondria, namely, no uncoupling, but inhibition of glutamate respiration and unimpaired succinate respiration.

Even if the mitochondria of MHS porcine muscle should be normally coupled, one cannot assume that this evidence entirely rules out any mitochondrial abnormality. Mitochondria have functions other than the production of ATP. For example, they store large quantities of calcium, mostly as calcium phosphate crystals.[8] Mitochondria apparently serve as long-term storage sites for excess calcium that is temporarily beyond the capacity of the sarcoplasmic reticulum (SR) to accumulate.[8] It may be that calcium binding by MH mitochondria is in some way impaired in the presence of triggering agents, thus causing an increase in myoplasmic calcium concentration to above the threshold required for activation of various catabolic heat-producing reactions. We were especially desirous of studying this aspect of mitochondrial function in MHS skeletal muscle since other workers have demonstrated that calcium uptake into the sarcoplasmic reticulum of MHS swine is normal,[6] or even greater than normal.[7,9]

The purpose of this paper is to describe our investigations of oxidative phosphorylation of, and calcium uptake into, the sarcosomes of MHS swine in the absence and in

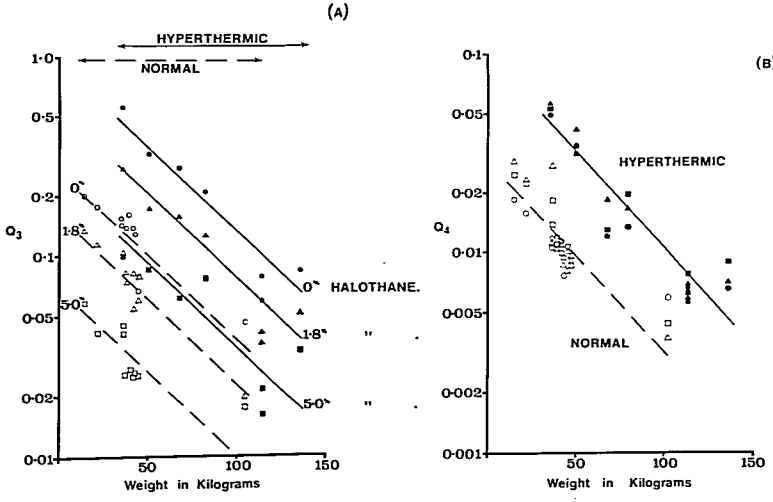

FIG. 1. The relations between porcine weights and (A) log of state 3 or (B) log of state 4 mitochondrial respiration in the presence of glutamate. The horizontal axis represents weight in kilograms. The vertical axis represents μatoms of oxygen consumed by the mitochondria per mg of protein per minute.

Observations made in the absence of halothane are represented by circles, those obtained in the presence of 1.8 and 5.0 per cent halothane are illustrated by triangles and squares, respectively. For the sake of clarity, measurements made with 0.5 per cent halothane are not shown. Nevertheless, they conformed to the trends shown in the diagram and they are part of the statistical calculations, including the fitting of the least-square lines.

Observations obtained for MHS and normal mitochondria are indicated by full and open symbols. The corresponding best lines are continuous and dashed, respectively. The Q_3 lines are drawn by assuming (on the basis of statistical analysis presented in table 3) identical separation among parallel lines corresponding to the same halothane doses in MHS and normal pigs. Thus, apart from a constant logarithmic shift, the Q_3 responses of MHS and normal pigs can be, at all weights and halothane concentrations, superimposed. Stated differently, the effect of MHS, resulting in a 3.5-fold increase in Q_3, is independent of weight or halothane dose.

The Q_4 response can be similarly characterized, except that the effect of halothane is negligible and MHS is associated with a 3.2-fold increase in respiratory rate.

Almost identical results were obtained in the presence of NAD or DNP.

the presence of various concentrations of halothane *in vitro*.

Methods

Pig Colony

The MHS swine were either purebred Poland-China or crossbred Poland-China/York pigs. They were all descendents of two purebred MHS Poland-China hogs obtained from the colony maintained by Jones and Nelson (personal communication, 1971) at Oklahoma State University. The normal pigs were York hogs.

Anesthetic and Surgical Techniques

Premedication consisted of diazepam (Valium),¶ 4 mg/kg, im. Anesthesia was induced by a further 2 mg/kg, iv, diazepam and

¶ Donated by Hoffmann-La Roche of Canada, Limited.

$N_2O:O_2$ (10:4 l/min). Anesthesia was maintained by nitrous oxide and iv diazepam as needed. Lidocaine was infiltrated around but not into the surgical site. The use of these three agents (diazepam, nitrous oxide and lidocaine) *in vivo* was found to have no significant effect in our *in-vitro* experiments. The muscle biopsied was the vastus lateralis. All grossly visible fascia and fat were removed from the muscle prior to severing its blood supply.

Biochemical Techniques

Mitochondria for oxidative phosphorylation studies were isolated from 10 g of muscle excised from ten normal and seven MHS pigs. These mitochondria were equilibrated with 0, 0.5, 2.0, or 5.0 vol per cent halothane and measured in our usual way.[5] The substrates were glutamate and succinate. Efficiency of oxidative phosphorylation was also determined in the presence and absence of nicotinamide adenine dinucleotide (NAD) and in the presence and absence of 2, 4-dinitrophenol (DNP).

Mitochondria for measurement of $Ca^{45}Cl_2$ uptake were isolated from eight normal and eight MHS pigs by the method employed for the mitochondrial oxidative phosphorylation study,[5] except that the final suspension medium was: sucrose 0.3 M and imidazole 0.01 M at *p*H 7.4. These mitochondria were equilibrated with radioactive calcium and ATP in a manner similar to that utilized by us in a previous study of SR,[5] except that the time of equilibration with halothane (0, 0.5, 2.0, and 5.0 vol per cent) was 10 minutes (instead of 5 minutes) and the reaction medium (KCl 0.1 M, imidazole 0.02 M, $MgCl_2$ 0.005 M, diNaSuccinate 0.002 M, diNaATP 0.003 M, $Ca^{45}Cl_2$ 0.0002 M) was at *p*H 7.4.

Protein was measured by the biuret method.[10] Purity of mitochondrial and SR preparations was checked by electron microscopy.

Results

Average respiratory values obtained at various halothane concentrations, in the presence of glutamate or succinate, with or without the addition of NAD or DNP, are presented in table 1.

State 3 respiration (Q_3)** and state 4 respi-

** State 3 respiration (Q_3) = respiration of mitochondria in the presence of oxygen and exogenous substrate, phosphate and ADP.

TABLE 3. Effect of Weight in the Presence of Glutamate on Respiration

	Additives	Average Slope For Dependence on Weight (/1,000 kg)	MH Slope cs Normal Slope (t, d.f. = 13)	MH Intercept cs Normal Intercept* (t, d.f. = 13)	Identity of Separations among Intercepts in MH and Normal Pigs†
Log Q_3	—	−8.64 ± 0.60¶	0.66, N.S.	12.92¶	0.41, N.S.
	NAD	−8.99 ± 0.64¶	0.48, N.S.	12.03¶	0.32, N.S.
	NAD + DNP	−8.93 ± 0.62¶	0.16, N.S.	12.46¶	0.38, N.S.
Log Q_4	—	−9.68 ± 0.58¶	2.13, N.S.	13.22¶	0.09, N.S.
	NAD	−9.94 ± 0.57¶	1.80, N.S.	13.48¶	0.27, N.S.
Log R.C.I.	—	1.06 ± 0.57, N.S.	1.54, N.S.	0.10, N.S.	0.64, N.S.
	NAD	0.95 ± 0.59, N.S.	1.27, N.S.	0.21, N.S.	0.82, N.S.

* At the same halothane dose. Identical separation of the intercepts has been assumed, due to different halothane doses, in the MHS and normal pigs. The t statistic with 13 degrees of freedom measures the effect of malignant hyperthermia (*i.e.*, the difference between intercepts, taken over all halothane concentrations, for the hyperthermic pigs and control pigs). It is similar to the statistics given in table 2 except that now adjustment has been made for weight dependence. (N.S. = not significant, $P \geq 0.05$.)

† The separate lines are related to different halothane doses. The F statistic with 3 and 42 degrees of freedom tests for interaction between the effects of malignant hyperthermia and halothane concentration, *i.e.*, it determines whether the difference between respiratory values obtained in MHS and normal pigs changes by varying the halothane dose. It is similar to the statistic given in table 2 except that now adjustment has been made for weight dependence. (N.S. = not significant, $P \geq 0.05$).

¶ $P < 0.001$.

TABLE 4A. Comparison of Slopes of $Log_{10}Q_3$, $Log_{10}Q_4$, Log_{10}R.C.I., and P/O against Halothane Concentration in the Presence of Glutamate

	Malignant Hyperthermia	Normal	t Values for Group Comparisons Hyperthermia cs Normal
$Log_{10}Q_3$			
Without NAD	−0.103 ± 0.006¶	−0.108 ± 0.007¶	0.48, N.S.
With NAD	−0.112 ± 0.008¶	−0.114 ± 0.006¶	0.20, N.S.
With NAD + DNP	−0.104 ± 0.006¶	−0.108 ± 0.007¶	0.42, N.S.
$Log_{10}Q_4$			
Without NAD	0.004 ± 0.007, N.S.	0.002 ± 0.007, N.S.	0.20, N.S.
With NAD	−0.001 ± 0.009, N.S.	0.003 ± 0.007, N.S.	0.35, N.S.
Log_{10}R.C.I.			
Without NAD	−0.108 ± 0.008¶	−0.113 ± 0.006¶	0.47, N.S.
With NAD	−0.112 ± 0.010¶	−0.118 ± 0.007¶	0.53, N.S.
P/O			
Without NAD	−0.145 ± 0.016¶	−0.171 ± 0.029¶	0.63, N.S.
With NAD	−0.159 ± 0.018¶	−0.144 ± 0.015¶	0.61, N.S.

N.S. = not significant.
¶ $P < 0.001$.

TABLE 4B. Comparison of Slopes of $Log_{10}Q_3$, $Log_{10}Q_4$, Log_{10}R.C.I., and P/O Ratios Against Halothane Concentration in the Presence of Succinate

	Malignant Hyperthermia	Normal	t Values for Group Comparisons Hyperthermia cs. Normal
$Log_{10}Q_3$			
Without NAD	0.012 ± 0.009, N.S.	0.014 ± 0.006‡	0.22, N.S.
With NAD	0.013 ± 0.010, N.S.	0.014 ± 0.007, N.S.	0.09, N.S.
With NAD + DNP	0.015 ± 0.010, N.S.	0.014 ± 0.007, N.S.	0.03, N.S.
$Log_{10}Q_4$			
Without NAD	−0.004 ± 0.012, N.S.	−0.003 ± 0.005, N.S.	0.03, N.S.
With NAD	0.002 ± 0.013, N.S.	−0.002 ± 0.006, N.S.	0.33, N.S.
Log_{10}R.C.I.			
Without NAD	0.014 ± 0.010, N.S.	0.018 ± 0.006§	0.33, N.S.
With NAD	0.012 ± 0.012, N.S.	0.017 ± 0.008‡	0.36, N.S.
P/O			
Without NAD	−0.038 ± 0.011§	−0.072 ± 0.007¶	2.62‡
With NAD	−0.042 ± 0.019‡	−0.061 ± 0.008¶	1.07, N.S.

N.S. = not significant.
‡ $P < 0.05$.
§ $P < 0.01$.
¶ $P < 0.001$.

ration (Q_4)†† were significantly higher (in the presence of either glutamate or succinate and in the presence or absence of NAD of DNP) in MHS than in normal mitochondria (tables 1 and 2). As indicated by the absence of interactions (table 2), none of these substantial MHS effects was affected by addition of halothane. The malignant-hyperthermia-susceptible R.C.I.'s (respiratory control indexes)‡‡ and P/O ratios††† did not differ sig-

†† State 4 respiration (Q_4) = respiration of mitochondria in the presence of oxygen and exogenous substrate and phosphate and the absence of ADP.

‡‡ Respiratory control index (R.C.I.) = Q_3/Q_4.

††† P/O ratio = μ moles ADP added/μ atoms oxygen consumed.

nificantly (with the exception of glutamate R.C.I. in the absence of NAD) from the normal R.C.I.'s and P/O ratios.

The dependence in the presence of glutamate of Q_3 and Q_4 on weights of the pigs is illustrated in figure 1. There was an inverse relationship between the log of the Q_3's and Q_4's, on the one hand, and porcine weights on the other. The weight dependencies for both Q_3's and Q_4's were identical in MHS and normal mitochondria. Statistical evaluation by analysis of covariance (table 3) indicates that the weight effect is highly significant and that (not shown in the table) this effect, as represented by the slope, is not altered by addition of halothane. Adjustment for weights (in the presence or absence of NAD or DNP) did not affect glutamate R.C.I.'s or P/O ratios or any of the respiratory variables observed when succinate was the substrate.

Halothane diminished (table 4*A*) the glutamate Q_3's (fig. 1*A*), R.C.I.'s and P/O ratios, but not the glutamate Q_4's (fig. 1*B*). Halothane (table 4*B*) lowered the succinate P/O ratio but (with marginal statistical significance) elevated in normal pigs the succinate Q_3's and R.C.I.'s while leaving the succinate Q_4's unchanged. Susceptibility to malignant hyperthermia or the addition of NAD or DNP did not alter the halothane slopes, except for a slight diminution in the succinate P/O slope in MHS pigs.

Calcium accumulation by MHS mitochondria was significantly less than normal (fig. 2) (t 12.2, d.f. 56, $P < 0.001$). Halothane appeared to enhance somewhat calcium uptake in both MHS and normal mitochondria, but this increase was statistically not significant. The MHS effect was independent of the halothane concentration.

Discussion

The normal R.C.I.'s and P/O ratios observed for the MHS muscle in the absence of halothane show that mitochondria are in a normal functional state with regard to their ability to produce ATP with a minimum expenditure of time, ATP, oxygen, and heat.[11] These findings are in agreement with those previously observed by us in human MHS mitochondria.

The elevated glutamate Q_3's and Q_4's seen in the MHS mitochondria are even more striking when relationships to porcine weights are taken into account. Thus, when this adjustment is made, MHS state 3 respiration is found to be 3.5 times, and MHS state 4 respiration 3.2 times, higher than normal (fig. 1, *A* and *B*). The weights of pigs may be considered to be related to their ages. The absence of earlier effective considerations of age influences may account for the failure, until now, to detect the MHS effects.

The changes produced by halothane—namely, inhibition of glutamate electron transport in the region of NAD (*i.e.*, complex I) plus a slight uncoupling of oxidative phosphorylation, are similar in direction and extent in normal and MHS mitochondria. These changes are essentially like alterations seen in the presence of halothane in normal and MHS human skeletal-muscle mitochondria[4,5] and also in normal rat skeletal-muscle[12] and liver[13–17] mitochondria. The net result is lessened oxygen consumption and heat, lactate, and carbon dioxide production. The effects of halothane on the electron-transport chains of MHS porcine mitochondria, therefore, do not appear to account for the clinical features of MH. We cannot, however, rule out the possibility that an abnormality of *in-vivo* respiratory assemblies may be masked by the artificial experimental conditions existing during our *in-vitro* experiment. For instance, it has been suggested§§ that use of calcium-chelating agents such as EDTA or EGTA in the bathing medium may mask differences between normal and MHS mitochondria.

MHS porcine mitochondria appear to be unable to accumulate normal amounts of calcium under *in-vitro* conditions. The mechanism by which this insufficiency occurs and its possible relationship to the elevated Q_3's and Q_4's of these MHS mitochondria are not elucidated by our experiments. Popinigis,§§ however, has pointed out that calcium is involved in the formation of the phospholipid lattice of the mitochondrial membrane.[18] Perhaps the impaired calcium uptake is related to an ab-

§§ Popinigis J, Warsaw, Poland: Personal communication, 1973.

normality in the structure of this phospholipid membrane. Furthermore, reduced calcium uptake by the mitochondrion would be consistent with a more efficient production of ATP within the mitochondrion,[19,20] such as has occurred in the MHS mitochondria.

Inability of MHS mitochondria adequately to take up calcium prior to anesthesia would mean that other membranes such as the SR would have to accumulate the excess calcium in order to maintain myoplasmic calcium concentrations within the physiologic range. Some reported experimental evidence suggests that, indeed, this might occur, *i.e.*, that the sarcoplasmic reticulum, apparently in the face of prolonged excessive demand, seems to have developed the ability to amass greater than normal quantities of calcium.[7,9] Accelerated uptake of calcium into the SR is not incompatible with Kalow's observation (unpublished data, 1974) that caffeine induces greater than normal contracture of isometric muscle obtained from MHS swine. This is because caffeine makes muscle contract not only by inhibiting calcium uptake into the SR, but also by causing release of calcium from the SR.[21]

The failure of halothane *in vitro* to alter calcium uptake into either normal or MHS mitochondria significantly is in contrast to data reported by Rosenberg and Haugaard.[22] The concentrations of halothane they used were, however, considerably higher than those employed by us. Moreover, both the tissue and the species were different.

The low calcium-accumulating capacity of the MHS mitochondria cannot by itself explain the derangements observed during an MH crisis, since halothane, a known triggering agent, induced no further impairment of uptake. It may be, however, that halothane damages MHS muscle organelles in such a way as to accelerate the release of calcium from them to the myoplasm. Thus, it may ultimately be shown that the myoplasmic calcium concentration increases during an MH reaction because of an enhanced rate of release of calcium ions from some intracellular calcium storage site, rather than because of impaired calcium accumulation by such an organelle.

The data we have described do not rule out a concomitant sarcolemmal defect. In fact, reports of increased serum CPK levels in MHS swine prior to anesthesia[23-25] indicate that the integrity of the sarcolemma is less than ideal. The increase in serum calcium that immediately follows commencement of hyperthermic rigor in pigs may be due to release of calcium from defective sarcolemma.

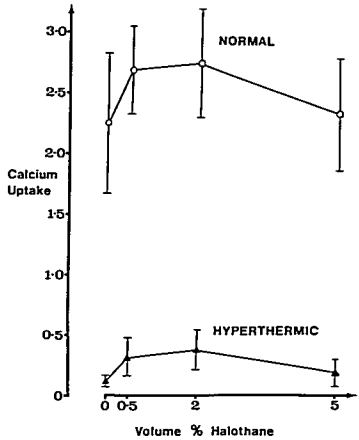

FIG. 2. Calcium uptake by normal and MHS mitochondria. The horizontal axis represents concentration of halothane expressed as vol per cent added to a homogenate of porcine mitochondria. The vertical axis represents uptake into mitochondria of $Ca^{45}Cl_2$ in μM/mg protein during 10 minutes.

For their helpful contributions to this study the authors thank Dr. D. Mihic, Dr. J. Cummings, Mr. F. H. Chung, and Mrs. S. F. Lee. The study was supported by grants from the Atkinson Charitable Foundation and Ayerst Laboratories.

References

1. Wilson RD, Nichols RJ Jr, Dent TE, et al: Disturbances of the oxidative-phosphorylation mechanism as a possible etiological factor in sudden unexplained hyperthermia occurring during anesthesia (abstr). ANESTHESIOLOGY 27:231–232, 1966
2. Gatz EE, Jones JR: Effects of six general anesthetics upon mitochondrial oxidative phosphorylation: Possible site and mecha-

nism of malignant hyperpyrexia. Fed Proc 28:356, 1969
3. Gatz EE: The mechanism of induction of malignant hyperpyrexia based on *in vitro* to *in vivo* correlative studies, International Symposium on Malignant Hyperthermia. Edited by RA Gordon, BA Britt, W. Kalow. Springfield, Ill., Charles C Thomas, 1973, pp. 399–408
4. Britt BA, Kalow W, Endrenyi L: Malignant hyperthermia and the mitochondria in human patients, International Symposium on Malignant Hyperthermia. Edited by RA Gordon, BA Britt, W Kalow. Springfield, Ill., Charles C Thomas, 1973, pp 387–398
5. Britt BA, Kalow W, Gordon A, et al: Malignant hyperthermia: An investigation of five patients. Can Anaesth Soc J 20:431–467, 1973
6. Denborough MA, Hird FJR, King JO, et al: Mitochondrial and other studies in Australian Landrace pigs affected with malignant hyperthermia, International Symposium on Malignant Hyperthermia. Edited by RA Gordon, BA Britt, W Kalow. Springfield, Ill., Charles C Thomas, 1973, pp 229–237
7. Berman MC, Kench JE: Biochemical features of malignant hyperthermia in landrace pigs, International Symposium on Malignant Hyperthermia. Edited by RA Gordon, BA Britt, W Kalow. Springfield, Ill., Charles C Thomas, 1973, pp 287–297
8. Lehninger AL: Mitochondria and calcium ion transport. Biochem J 119:129–138, 1970
9. Nelson TE, Jones EW, Venable JH, et al: Malignant hyperthermia of Poland China swine. Studies of a myogenic etiology. ANESTHESIOLOGY 36:52–56, 1972
10. Layne E: Spectrophotometric and turbidimetric methods for measuring proteins. Methods Enzymol 3:447–454, 1957
11. Lehninger AL: Biochemistry. Part II. New York, Worth Publishers, Inc., 1970
12. Britt BA, Kalow W, Endrenyi L: The effects of halothane and methoxyflurane on rat skeletal muscle mitochondria. Biochem Pharmacol 21:1159–1169, 1972
13. Harris RA, Munroe J, Farmer B, et al: Action of halothane upon mitochondrial respiration. Arch Biochem Biophys 142:435–444, 1971
14. Cohen PJ, Marshall BE, Harris JE, et al: Halothane-induced changes in mitochondrial oxygen uptake and respiratory control. Fed Proc 27:705, 1968
15. Cohen PJ, McIntyre R: The effects of general anesthesia on respiratory control and oxygen consumption of rat liver mitochondria, Cellular Biology and Toxicity of Anaesthetics Edited by BR Fink. Baltimore, Williams and Wilkins, 1968
16. Nahrwold ML, Cohen PJ: Additive effect of nitrous oxide and halothane on mitochondrial function. ANESTHESIOLOGY 39:534–536, 1973
17. Hall GM, Kirtland, SJ, Baum H: The inhibition of mitochondrial respiration by inhalational anaesthetic agents. Br J Anaesth 45:1005–1009, 1973
18. Rand RP, Sengupta S: Cardiolipin forms hexagonal structures with divalent cations. Biochim Biophys Acta 255:484–492, 1972
19. Lehninger AL: Physiology of mitochondria, Enzymes, Units of Biological Structure and Function. Edited by OH Gaebler. New York, Academic Press, 1956
20. Lehninger AL: Biochemistry. Chapter 18. New York, Worth Publishers, Inc., 1970
21. Batra S: The effect of drugs on calcium uptake and calcium release by mitochondria and sarcoplasmic reticulum of frog skeletal muscle. Biochem Pharmacol 23:89–101, 1974
22. Rosenberg H, Haugaard N: The effects of halothane on metabolism and calcium uptake in mitochondria of the rat liver and brain. ANESTHESIOLOGY 39:44–53, 1973
23. Allen WM, Berrett S, Harding JDJ, et al: Plasma levels of muscle enzymes in the pietrain pig in relation to the acute stress syndrome. Vet Rec 87:410–411, 1970
24. Woolf N, Hall L, Thorne C, et al: Serum creatine phosphokinase levels in pigs reacting abnormally to halogenated anesthetics. Br Med J 3:386, 1970
25. Jones EW, Kerr DD, Nelson TE: Malignant hyperthermia—observations in Poland China pigs, International Symposium on Malignant Hyperthermia. Edited by RA Gordon, BA Britt, W Kalow. Springfield, Ill., Charles C Thomas, 1973, pp 198–207