Biochemical Pharmacology, Vol. 21, pp. 1159–1169. Pergamon Press, 1972. Printed in Great Britain.

EFFECTS OF HALOTHANE AND METHOXYFLURANE ON RAT SKELETAL MUSCLE MITOCHONDRIA

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(*Received* 2 *June* 1971; *accepted* 15 *October* 1971)

Abstract—Halothane and methoxyflurane, at clinical concentrations, both inhibit oxygen consumption of rat skeletal muscle mitochondria. The locus of action is in complex I of the electron transport chain. The mode of action appears to be chiefly inhibition of electron transport and only slightly uncoupling of oxidative phosphorylation. At higher than clinical concentrations, methoxyflurane appears to cause moderate uncoupling of oxidative phosphorylation. Statistical analysis of the data suggests that mitochondrial responses are characterized by the logarithm of oxygen uptake and not by the uptake itself, as is customarily assumed. The biochemical observations are not identical with, but essentially similar to, data described in the literature on mitochondria from sources other than muscle. Thus the actions of halothane and methoxyflurane at clinical concentrations upon normal skeletal muscle mitochondria do not provide a basis to explain the enormous acceleration of oxygen consumption and heat production observed in malignant hyperthermia.

HALOTHANE and methoxyflurane are two of the most commonly used potent inhalational anesthetic agents. The effects of these anesthetics on oxidative phosphorylation are of interest in view of the problems posed by malignant hyperthermia with rigidity, a pharmacogenetic disease produced by clinical concentrations of potent inhalational general anesthetics and muscle relaxants.[1–3] The mode of inheritance is autosomal dominant with reduced penetrance and variable expressivity.[4] In addition to fever and skeletal muscle rigidity, it is characterized by release of myoglobin, potassium and muscle enzymes from the cells, and increased oxygen consumption and carbon dioxide and hydrogen ion production by the cells.[5] Since the combination of fever and rigidity may also occur in dinitrophenol poisoning, uncoupling of oxidative phosphorylation has been held to be a cause of malignant hyperthermia.[6–8]

However, the literature reports regarding the effects of halothane on oxidative phosphorylation and respiration of mitochondria from normal tissues are not uniform. Although all available evidence indicates that the occurrence of malignant hyperthermia with rigidity is based on an unusual vulnerability of skeletal muscle,[1–3] none of the published investigations deals with metabolic effects of halothane or methoxyflurane on either normal or malignant hyperthermic skeletal muscle. Most of the studies which have been done were performed on mitochondria from rat liver and brain, and most of the observed differences seem to be due to intrinsic differences between the mitochondria from these tissues.

Relevant information in the literature may be summarized as follows. Hoech *et al.*[9] found that halothane depressed oxygen consumption of rat brain, liver and heart slices. Theye[10] showed in dogs that halothane reduced both total and myocardial oxygen consumption.

Some years ago Snodgrass and Piras[7] claimed that halothane was a true uncoupler of oxidative phosphorylation in the normal rat liver. However, they employed concentrations of halothane far in excess of those expected *in vivo* during normal anesthesia.

Gatz and Jones[11] using normal rat brain mitochondria found that with nicotinamide adenine dinucleotide (NAD)-linked substrates, methoxyflurane and halothane inhibited oxygen uptake and oxidative phosphorylation, while with succinate, halothane but not methoxyflurane evoked a 2,4-dinitrophenol (DNP)-like effect, even at clinical concentrations. The concentrations of the anesthetic agents employed were not specified.

More recently, Miller and Hunter[12] found that halothane, in concentrations akin to those used clinically, i.e. 0·5–2·0 vol./100 ml, inhibited NAD-linked substrate oxidation of normal rat liver mitochondria but not succinate oxidation. This inhibition was not relieved by DNP. At higher than clinical concentrations, halothane had an uncoupling effect.

Cohen *et al.*[13,14] in similar types of studies on normal rat liver mitochondria reported essentially the same results.

Harris *et al.*,[15] on the basis of studies on rat liver and beef heart mitochondria, concluded that halothane depressed respiration by inhibiting the enzyme activity in the vicinity of the rotenone-sensitive site of complex I of the electron transfer chain.

Popinigis and Williams[16] observed that halothane, at all concentrations, accelerated succinate oxidation of liver mitochondria isolated from swine with malignant hyperthermia. This effect was seen in normal pig mitochondria only at low halothane concentrations, while high concentrations of halothane actually inhibited respiration of normal swine mitochondria.

Thus in rat liver and brain mitochondria, halothane at clinical concentrations appears to act on the mitochondria by inhibiting electron transport in the region of complex I. Uncoupling of oxidative phosphorylation occurs beyond coenzyme Q at clinical concentrations in rat brain mitochondria, but only at higher than clinical concentrations in rat liver mitochondria.

The purpose of this investigation is to study the actions of halothane and of methoxyflurane in clinical concentrations on normal rat muscle mitochondria to see if these agents cause mainly an inhibition of electron transport and therefore a decreased heat production, or if uncoupling of oxidative phosphorylation with associated increased heat production is the most notable effect. These experiments will, therefore, serve as a baseline study for future experiments which will be performed on human skeletal muscle removed from normal human controls and from patients with malignant hyperthermia during and after acute reactions.

While one cannot hope to establish the cause of a rare pathological reaction in human muscle by studying the muscle of healthy rats, any abnormal drug effect can be recognized only against the background knowledge of normal effects. Furthermore, an abnormal reaction may be merely an exaggeration of a normal event.

METHODS

The proteolytic enzyme, Nagarse, was obtained from the Enzyme Development Corp., 64 Wall St., New York. Calcium-free adenosine triphosphate (ATP), adenosine diphosphate (ADP), NAD, glutamate, malate, succinate and Cohen's fraction V

bovine albumin were purchased from the Sigma Chemical Company. Halothane was donated by Ayerst Laboratories. Methoxyflurane was bought from Abbott Laboratories Ltd. All other reagents were obtained commercially from Fisher Scientific Company.

Preparation of mitochondria. The mitochondria were prepared at 0–4° using a method developed from accepted standard techniques.[17–22] The techniques used were as follows. Male Wistar rats weighing approximately 200 g were sacrificed. Skeletal muscle from the back and legs was excised, weighed, minced, rinsed and suspended in 2 vol. of a modified Chappel–Perry medium[23] (Table 1). After the addition of 20 mg Nagarse, the mince was rapidly homogenized by hand with a TRI-R glass and Teflon pestle and further diluted with an equal volume of the medium. The preparation was left on ice for 10 min and was then rehomogenized and further diluted with the above medium to 200 ml.

TABLE 1. PREPARATION AND REACTION MEDIA

	Low speed preparation medium (modified Chapell–Perry medium) (M)	High speed preparation medium (M)	Reaction medium (modified Dow's medium) (M)
Tris–HCl buffer (pH = 7·4)	0·05	0·005	0·025
Phosphate buffer			0·03
Na-ATP	0·001		
$MgSO_4$	0·005		
$MgCl_2$		0·0005	0·0005
KCl	0·10	0·015	0·015
EGTA	0·001		
EDTA		0·00025	0·00025
Sucrose		0·125	0·045
Glucose			0·02
Mannitol			0·01
Cohen's Fraction V bovine albumin	0·5	1·0%	0·2%

The suspension was centrifuged twice for 10 min at 600 *g*. The supernatant was then centrifuged at 8500 *g* for another 10 min. The pellets were rinsed, suspended in a sucrose solution containing high speed preparation medium (Table 1), and given a final 10 min of centrifuging at 13,300 *g*. The pellets were rinsed again and resuspended in the high speed preparation medium ready for use.

Mitochondrial protein was measured by the biuret method[24] and ADP was standardized by the technique described by Estabrook *et al.*[25]

Equilibration of halothane with mitochondria. All of the remaining steps were carried out at 25°. Approximately 500 μg of mitochondrial protein was added to 2 ml of a modified Dow's reaction medium[18] (Table 1). One liter/min of air was passed through either a calibrated[26] Mark III Fluotec halothane vaporizer or a calibrated Mark II Pentec methoxyflurane vaporizer. The vapor then went for 3 min through the tightly stoppered and constantly agitated reaction chamber.

The concentrations of halothane in air were 0, 0·5, 2·75 and 5·0 vol./100 ml and those of methoxyflurane were 0, 0·2, 1·1 and 2·0 vol/100 ml. These concentrations and the time of equilibration were selected so as to give an intramitochondrial concentration range spanning that which is thought to exist *in vivo* during normal anesthesia.[27–29] Separate mitochondrial aliquots were used for each dose of halothane.

Measurement of oxidative phosphorylation. The Qo_2, i.e. the microatoms of oxygen consumed per milligram of mitochondrial protein per minute, was measured by a tightly fitting, modified Clark oxygen electrode (Yellow Springs). The system was thus anaerobic, apart from the oxygen remaining dissolved in solution. The substrates used were 10 mM glutamate and 10 mM succinate. Respiration was measured both in the presence of 0·1 mM ADP (state 3 respiration or Q_3) and after the ADP had become exhausted (state 4 respiration or Q_4). The respiratory control index (RCI) was calculated as the ratio of state 3 to state 4 respiration. The ratio of micromoles of ADP added to microatoms of oxygen consumed, i.e. the P/O ratio, was also determined. In a separate experiment, respirations with and without exogenous 0·5 mM NAD were compared on the same mitochondrial preparations at all dose levels of halothane and methoxyflurane. Still another experiment was performed with and without 0·2 mM DNP in both the absence and the presence of 2·75 per cent halothane and of 1·1 per cent methoxyflurane. Each group of experiments was repeated on a minimum of three rat muscle mitochondrial preparations, each from a different rat, with the order of exposure to different concentrations of halothane or methoxyflurane being varied in each preparation. For each sample the measurement was repeated two or more times.

RESULTS

Q_3, Q_4, RCI and P/O ratios

The ranges of the average observations are presented in Table 2. Table 3 shows the slopes of $\log_{10}$ (response) vs. anesthetic concentration plots. The $\log_{10}Q_3$ vs. anesthetic concentration plot is further detailed in Fig. 1, while the standard deviations for $\log_{10}Q_3$ are given in Fig. 2. These diagrams are analyzed in the next section.

Stastical aspects of the data. The data were subjected to statistical analysis in order to arrive at a simple description of the results. Such analysis led us to conclude that the mitochondrial responses were characterized not by the absolute rate of respiration, as it is customarily assumed, but by the logarithm of the respiration rate. This result was based on the following observations.

The reproducibility of the measurements in terms of variance or standard deviation varied greatly with different tissue samples or anesthetic concentrations. This was especially conspicuous for the glutamate Q_3 measurements, which covered a wide range of values. In these experiments, Bartlett's test[30] indicated strong heterogeneity of the variances ($\chi^2 = 62{\cdot}7$, d.f. $= 15$, $P < 0{\cdot}001$ for halothane; $\chi^2 = 32{\cdot}8$, d.f. $= 14$, $P < 0{\cdot}01$ for methoxyflurane). More specifically, the standard deviations were approximately proportional to the corresponding average values (Fig. 2). This suggested applicability of the logarithmic transformation.[31] Indeed, with logarithmic data, Bartlett's test[30] permitted one to assume homogeneity of the variances ($\chi^2 = 16{\cdot}0$, d.f. $= 15$, $P > 0{\cdot}05$ for halothane; $\chi^2 = 13{\cdot}1$, d.f. $= 14$, $P > 0{\cdot}05$ for methoxyflurane).

TABLE 2. EFFECT OF HALOTHANE AND METHOXYFLURANE ON ELECTRON TRANSPORT AND OXIDATIVE PHOSPHORYLATION OF RAT SKELETAL MUSCLE MITOCHONDRIA*

	Halothane in air (vol./100 ml)				Methoxyflurane in air (vol./100 ml)			
	0·0	0·50	2·75	5·0	0·0	0·20	1·1	2·0
Glutamate (10 mM)								
Q_3	0·127– 0·292	0·098–0·283	0·047–0·091	0·019–0·042	0·094–0·282	0·088–0·281	0·029–0·087	0·021–0·059
Q_4	0·013– 0·024	0·016–0·036	0·013–0·026	0·011–0·027	0·017–0·034	0·020–0·040	0·017–0·044	0·015–0·039
RCI	9·17 –12·64	6·42 –7·85	3·04 –3·98	1·40 –2·00	8·42 –11·76	4·39 –7·18	1·85 –2·37	1·28 –1·58
P/O	2·64 – 2·84	2·37 –2·48	2·22 –2·41	1·83 –2·16	2·58 – 2·65	2·33 –2·78	2·17 –2·62	1·81 –2·14
Succinate (10 mM)								
Q_3	0·096–0·233	0·113–0·226	0·118–0·297	0·143–0·277	0·177–0·224	0·123–0·222	0·165–0·335	0·181–0·400
Q_4	0·031–0·063	0·026–0·057	0·018–0·065	0·028–0·061	0·034–0·086	0·034–0·100	0·037–0·086	0·054–0·096
RCI	3·04 –4·30	3·18 –4·35	4·55 –6·52	4·52 –5·24	2·98 –4·04	2·14 –4·86	3·75 –6·17	2·95 –4·19
P/O	1·61 –1·94	1·64 –1·85	1·54 –1·86	1·55 –1·67	1·53 –1·84	1·46 –1·88	1·46 –1·67	1·13 –1·45

* The data represent ranges of the average replicate observations measured in four mitochondrial preparations. Q_3 = microatoms of oxygen consumed per milligram of mitochondrial protein per minute, in the presence of ADP; Q_4 = microatoms of oxygen consumed per milligram of mitochondrial protein per minute, in the absence of ADP; RCI = Q_3/Q_4; P/O = micromoles of ADP added/microatoms of oxygen consumed.

TABLE 3. SLOPES FOR THE INHIBITION OF MITOCHONDRIAL RESPIRATION

	$Log_{10}Q_3$ vs. dose		$Log_{10}Q_4$ vs. dose		P/O vs. dose	
	Halothane	Methoxyflurane	Halothane	Methoxyflurane	Halothane	Methoxyflurane
Glutamate (10 mM)	−0·165*	−0·745*,†	−0·011 NS‡	−0·026 NS	−0·115*	−0·328*
Succinate (10 mM)	0·030§	0·081§	0·000 NS	0·048‖	−0·039§	−0·180*

* $P < 0·001$.
† Initial slope at low methoxyflurane concentration.
‡ NS = not significant ($P \geq 0·05$).
§ $P < 0·01$.
‖ $P < 0·05$.

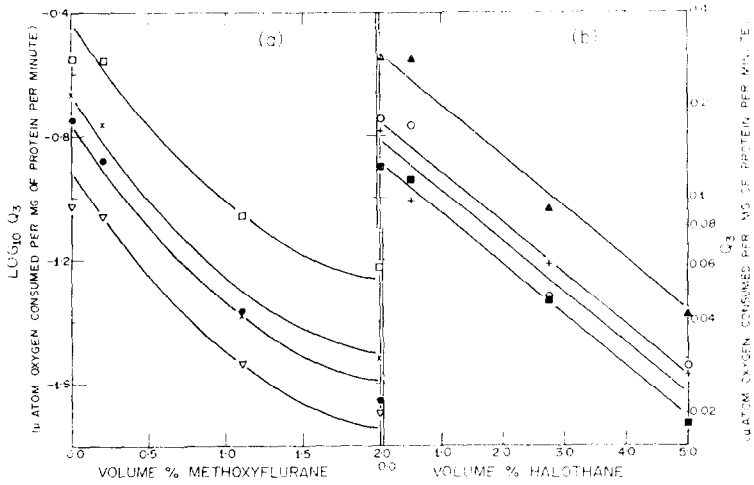

FIG. 1. Dependence of respiration rates of four mitochondrial preparations on anesthetic concentration, in the presence of ADP and glutamate. The various symbols and the corresponding fitted curves or lines refer to the different mitochondrial preparations. In the investigated range of anesthetic concentrations, linear least-squares fit was satisfactory in the presence of glutamate, but a different (empirically parabolic) fit was required for methoxyflurane. In either case, the best curves (lines) were parallel, vertically parallel for the parabolas. This observation led to the suggestion that at any given anesthetic concentration the fractional inhibition is identical in all tissue samples.

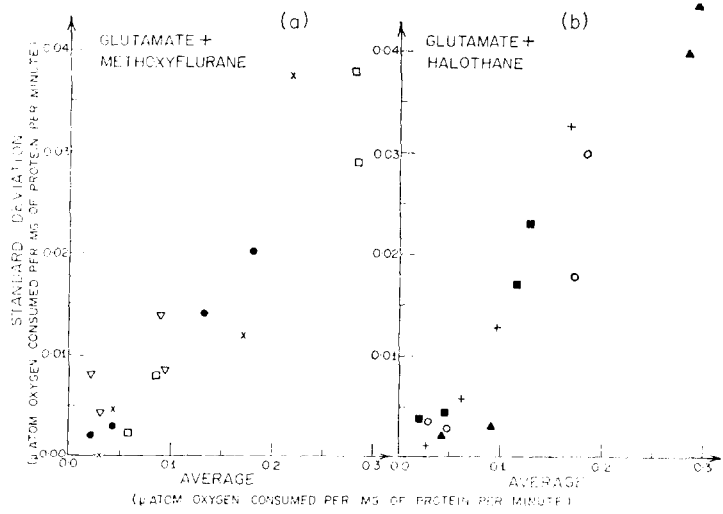

FIG. 2. Relationships between the standard deviations and averages of replicate Q_3 measurements which were carried out at different anesthetic concentrations on four mitochondrial preparations. The various symbols refer to the different mitochondrial preparations. In these scatter diagrams, the linear correlation coefficient, $r = 0{\cdot}909$ ($P < 0{\cdot}001$) for halothane and $r = 0{\cdot}918$ ($P < 0{\cdot}001$) for methoxyflurane.

When the measured respiration rates were plotted against the anesthetic concentrations or their logarithms, the curves or lines for the various samples were not parallel. Indeed the F-ratios which tested deviations from parallelism of lines in plots of Q_3 against anesthetic concentrations were 15·0 (d.f. = 3 and 35, $P < 0·001$) in the presence of halothane and glutamate, and 29·4 (d.f. = 3 and 30, $P < 0·001$) in the presence of methoxyflurane and glutamate.

In contrast, logarithmic oxygen uptake data yielded parallel plots (Fig. 1): the F-ratios for nonparallelism in plots of log Q_3 against anesthetic concentrations were 2·35 (d.f. = 3 and 35, $P > 0·05$) for halothane and glutamate, and 2·16 (d.f. = 6 and 30, $P > 0·05$) for methoxyflurane and glutamate. For the latter plot, the improvement caused by a quadratic fit in comparison with a linear fit ($F = 55·5$, d.f. = 1 and 30, $P < 0·001$) demonstrated its strong nonlinearity. The remarkable vertical parallelism is illustrated by the parabolic curves in the log Q_3 scale shown in Fig. 1. Such parallelism in the logarithmic scale suggested that any given anesthetic concentration generates the same fractional response in each mitochondrial preparation.

The parallel lines or curves in Fig. 1 were distinctly separated from each other (F for the difference among adjusted means is 69·8, d.f. = 3 and 35, $P < 0·001$ for halothane and glutamate; $F = 120·1$, d.f. 3 and 30, $P < 0·001$ for methoxyflurane and glutamate). This was the reason why average respiration rates covering all mitochondrial preparations had to be analyzed. The diversity of the mitochondrial responses was shown to apply also to Q_3 measurements in the presence of succinate and to observations of the Q_4, P/O ratio and RCI. The use of the logarithmic response scale was extended, by analogy, to measurements of Q_4.

Biochemical aspects of the data. Halothane in the presence of glutamate significantly reduced the RCI. The reduction was due to inhibition of state 3 respiration. The dose of halothane was linearly related to decreasing log Q_3. State 4 glutamate respiration remained unchanged. Halothane moderately reduced the glutamate P/O ratio.

Halothane slightly raised the succinate Q_3, but appeared to have no significant effect on succinate Q_4. A small reduction in the succinate P/O ratio was observed.

Methoxyflurane, like halothane, inhibited glutamate state 3 respiration, but as noted above, unlike halothane, the dose–log response plot was curved rather than linear. These differences in the shape of the dose response curves for halothane and methoxyflurane may have been due to differences in effective concentrations of the agents. Again, no change in the glutamate Q_4 was observed in the presence of methoxyflurane. Thus the net effect on the RCI was one of fairly marked inhibition paralleling the reduction in the glutamate Q_3. The glutamate P/O ratio was moderately lowered by the administration of methoxyflurane.

Methoxyflurane caused a small but statistically significant stimulation of state 3 succinate respiration. While the lower doses (0·2 and 1·1 per cent) of methoxyflurane failed to alter the succinate Q_4, 2·0 per cent methoxyflurane did slightly accelerate succinate state 4 respiration. The P/O ratio was moderately lowered by methoxyflurane, the dose being inversely related to the P/O ratio.

The previously mentioned observation that the curves for the different mitochondrial suspensions were distinctly separated from each other may have been due to differing concentrations of oxidation chains in the various preparations. This, together with the saturating substrate concentration present, may have accounted for the constancy of the fractional inhibition in each mitochondrial preparation. The inhibition

by the anesthetics could not be competitive (or, more generally, the relationship between response and anesthetic dose could not be the one describing competitive inhibition), since in this case inhibitory effects could not have been detected at saturating substrate concentrations.

Effect of NAD on Q_3

The weighted ratios of respiration rates measured in the absence and presence of NAD varied slightly and fairly randomly around 1·0 and, with one exception, they did not differ significantly from unity (the significance tests were performed in the logarithmic scale). The statistical significance of one test in 14 may be disregarded, since this frequency is not very different at the 5 per cent level from the theoretical frequency of one "significant" average observation out of 20 randomly selected mean values. The weighted ratios pooled over all anesthetic concentrations also did not differ significantly from unity (Table 4).

TABLE 4. NAD STUDY—AVERAGE RATIOS OF STATE 3 RESPIRATION RATES AND SLOPES OF THEIR LOGARITHMIC PLOTS AGAINST ANESTHETIC CONCENTRATIONS

	Halothane	Methoxyflurane
Glutamate		
$Log_{10}Q_3$ slope (without NAD)	−0·164† ± 0·018	−0·779† ± 0·058‡
$Log_{10}Q_3$ slope (with NAD)	−0·175† ± 0·017	−0·910† ± 0·049‡
$Log_{10}Q_3$ slope (with NAD)/Q_3 slope (without NAD)	−0·004 ± 0·015	0·004 ± 0·010
Pooled weighted average (Q_3 with NAD)/(Q_3 without NAD)	0·959 (×/÷) 1·04	1·010 (×/÷) 1·04
Succinate		
$Log_{10}Q_3$ slope (without NAD)	0·034§ ± 0·014	0·093§ ± 0·038
$Log_{10}Q_3$ slope (with NAD)	0·034§ ± 0·016	0·092§ ± 0·042
$Log_{10}Q_3$ slope (with NAD)/ Q_3 slope (without NAD)	0·006 ± 0·038	−0·001 ± 0·023
Pooled weighted average (Q_3 with NAD/ (Q_3 without NAD)	1·000 (×/÷) 1·02	0·963 (×/÷) 1·02

* Q_3 in microatoms of oxygen consumed per milligram of mitochondrial protein per minute.
† $P < 0·001$.
‡ Initial slope at low methoxyflurane concentration.
§ $P < 0·05$.

The slopes of $log_{10}Q_3$ vs. anesthetic concentration curves are presented in Table 4. The slopes observed in the absence and in the presence of NAD are quite similar. Thus it is not surprising that the corresponding plots of the NAD effects are approximately horizontal. The good agreement between the slopes measured in the absence of NAD in these experiments and those recorded in Table 3 is gratifying.

Effect of DNP on Q_3

In the presence of glutamate, the weighted overall averages of the ratios of respiration rates measured in the absence and presence of DNP, like the NAD ratios, varied slightly and fairly randomly around 1·0 and they did not differ significantly from

unity [the pooled ratio was 0·969 ($\times/\div$) 1·06]. DNP also did not stimulate succinate state 3 respiration, but, if anything, caused a marginal inhibition, the weighted ratios being consistently slightly less than 1·0 with a pooled ratio of 0·887 ($\times/\div$) 1·05 ($P < 0·01$).

DISCUSSION

The present study has shown that both halothane and methoxyflurane inhibit glutamate Q_3 but not succinate Q_3. Thus the site of action of these agents must lie in the region of NAD. There are several possibilities to explain the mechanism of action at this location. Since the addition of NAD did not alter the glutamate Q_3, the fall in the respiratory rate was not likely due to a loss of endogenous NAD from the mitochondria.[12]

Halothane and methoxyflurane could uncouple phosphorylation of ADP from electron transport with regeneration of the free carrier in a manner similar to that of DNP.[32–35] The observed reductions in the P/O ratios and the slight rise in the methoxyflurane succinate Q_4 would agree with this concept. However, the lack of rise of the halothane succinate Q_4 or of the halothane or methoxyflurane glutamate state 4 respiration seems contradictory. Nevertheless, real acceleration of these latter Q_4's may have been obscured by a simultaneous inhibition of electron transport.[12] The small rise in succinate Q_4 that occurred with 2 per cent methoxyflurane does indicate that, at high concentrations, this anesthetic may produce uncoupling, and therefore accelerated heat production.

Another possibility is that halothane and methoxyflurane, in a way analogous to that of oligomycin,[32–35] might slow the regeneration of the free electron transport carrier and so prevent ATP formation. Such an action would inhibit glutamate Q_3 and would not accelerate glutamate Q_4. These changes were indeed observed. However, the fact that DNP failed to relieve the inhibition of glutamate respiration suggests that such an inhibition of phosphorylating respiration is probably not the cause of the observed effects of halothane and methoxyflurane on the mitochondria.

Rather, this inability of DNP to ameliorate the markedly suppressed glutamate Q_3 suggests that the major effect of halothane and methoxyflurane on the mitochondria appears to be inhibition of electron transport in the vicinity of complex I.[15] Such an amytal-like action[32–35] should, by slowing the glutamate Q_3, inhibit oxaloacetate formation, which would therefore not be available to inhibit succinate dehydrogenase.[36–38] Succinate then would not be prevented from transferring its electrons to the respiratory chain via flavin adenine dinucleotide (FAD). Thus the slight acceleration of succinate Q_3 seen with halothane and methoxyflurane could be due to failure of succinate dehydrogenase inhibition. The reduction in heat production and oxygen consumption associated with inhibition of mitochondrial electron transport is in agreement with the known actions *in vivo* of halothane and methoxyflurane on these two processes.[39]

These results at clinical concentrations of the anesthetic agent are similar to those found by Miller and Hunter[12] and by Cohen *et al.*[13] in rat liver mitochondria and by Gatz and Jones[11] in rat brain mitochondria. At higher than clinical concentrations, we did not find an overt DNP-like effect for halothane as did Miller and Hunter[12] and Gatz and Jones;[11] however, we did detect such an effect with methoxyflurane

while Gatz and Jones[11] did not. Perhaps these apparent inconsistencies are simply due to varying intramitochondrial concentrations of halothane and methoxyflurane due to differing techniques of administration.

In conclusion, the actions of clinical concentrations of halothane and methoxyflurane on the mitochondria from normal skeletal muscle of the rat cannot serve as a basis for explanation of the massive heat output and accelerated oxygen consumption observed in malignant hyperthermia. The net effect of these actions on normal muscle is rather to reduce oxygen consumption and heat output. The reason for such discrepancies may be either that there is a fundamentally new effect in the skeletal muscle mitochondria from individuals genetically susceptible to malignant hyperthemia or that the primary defect in patients with malignant hyperthermia is extramitochondrial, as indicated by the work of Kalow *et al.*[2] Thus much further work remains to be done to elucidate the true nature of malignant hyperthermia.

Acknowledgements—We are grateful to Mrs. M. Betlem for the electron micrographs and to Mr. F. H. F. Kwong for his assistance in the calculations. We also wish to thank Dr. G. R. Williams and Dr. P. Seeman for helpful discussions. This work was supported by grants from the Medical Research Council of Canada and from Ayerst Laboratories.

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