The Effects of Halogenated Anesthetics on Mitochondrial Function

To the Editor:—We are concerned with clinical implications of basic research on subcellular effects of anesthetics. In the article by Schumer *et al.* (ANESTHESIOLOGY 35: 253–255, 1971), the investigators attempted to answer one intriguing question: what are the effects of halogenated anesthetics on mitochondrial function? From their report, several questions of fundamental importance are apparent.

1) The isolation of mitochondria in 0.18 M KCl, 10 mM EDTA, and 10 mM Tris-Cl, *p*H 7.2, has been used by many investigators. However, suspension of mitochondria in high-ionic-strength solutions (KCl) renders them less stable to subsequent changes in osmolarity, and may result in spontaneous swelling and other changes. For this reason, most workers prefer either sucrose or sucrose–mannitol solutions. It has been found that sucrose stabilization of mitochondria against gramicidin-induced swelling is abolished by only one wash in KCl medium.[1] Sucrose also inhibits swelling induced by iron and thyroxine.[2, 3] In brief, sucrose or mannitol–sucrose might have been chosen for the adjustment of tonicity in preference to KCl. An additional substance which enhances respiratory control is bovine plasma albumin, which adsorbs free fatty acids (common contaminants during the isolation of mitochondria). The use of 10 mM EDTA is not only adequate but excessive during isolation of mitochondria. Studies by Settlemeier[4] showed that EDTA removed excessive amounts of Mg^{++}, which is necessary for optimal mitochondrial oxidative phosphorylation.

2) The assay system contained "2.3 ml of a solution of 0.25 M sucrose, 5 mM tri succinate, and 0.7 ml of mitochondrial suspension." This medium is inadequate for the observation of respiratory control as defined by Chance and Williams.[5] The reaction $ADP + P_i \rightarrow ATP$ requires that ADP, mitochondria, and phosphate be present in addition to substrate and oxygen. No phosphate concentration is given in the article. Indeed, no buffer at all is described. In addition, the *p*H at which the reactions were carried out is not specified. Chance and Williams, in their original article, and others in subsequent publications have described optimum *p*H for observation of respiratory control as somewhere between 6.8 and 7.4. I wonder as well about the efficacy of studying respiratory control at a temperature of 37 C. Isolated mitochondria are markedly unstable at this temperature.

3) Respiratory control ratio is one acceptable index of mitochondrial functional integrity. However, a respiratory control ratio of 2 for control rat-liver mitochondria is considered an indication of a preparation unacceptable for study.[6] It has been established that succinate-dependent mitochondrial oxygen uptake is almost unaltered during exposure to clinically relevant concentrations of halothane[6, 7] and methoxyflurane, as well as diethyl ether.

4) In the authors' discussion and conclusion, a very brief statement regarding the "basis for resuscitating mitochondria which were depressed *in situ*" is made. It has been demonstrated and discussed in considerable detail[6] that this phenomenon occurs rapidly, and loss of halothane from the mitochondria would occur upon dilution in the homogenate and with the washings described in this article.

5) Currently-available information about the effect of halothane on mitochondrial oxidative phosphorylation has shown specifically that the area of electron transfer or phosphorylation affected is NADH dehydrogenase. Therefore, it would seem that the more logical study would include experiments on NAD-linked substrate oxidative metabolism.

I do not believe that the conclusions in the final paragraph can be drawn from the data in this study. I suggest that this type of study could yield valuable information regarding the effects of anesthetics on subcellular function, but this particular study is deficient as described, and probably should be repeated with

suitable modification of the experimental design.

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To the Editor:—Dr. Miller's major criticism appears to center on his contention that unless reasonably intact functional mitochondria can be isolated, any conclusion as to their functional state following halothane anesthesia is inapplicable. Since the respiratory control ratios (RCR) for our control preparations utilizing succinate were between 2.0 and 2.4, Dr. Miller believed that alternative preparatory and assay procedures would have yielded more suitable reference material.

In reply, we should like to state that rat-liver mitochondria isolated in a medium of 0.18 M KCl, 10 mM EDTA, and 10 mM tris buffer, and assayed at 25 C, produced RCR for succinate respiration in the range of 3.0 to 4.0. Such ratios have been reported by other investigators who used either sucrose–mannitol or sucrose media.[1,2] We attribute the RCR values of 2.0 to 2.4 to the assay temperature of 37 C. Dr. Miller indeed questions the feasibility of assaying mitochondrial function at this temperature because of the instability which ensues. We should like to emphasize that our intent was to determine whether halothane anesthesia had an effect on mitochondria subsequently isolated from rat livers. Preliminary tests in which assays were conducted at 25 C using succinate as respiratory substrate indicated that the control and experimental mitochondria had similar respiratory rates. However, at 30 and 37 C, mitochondria obtained from halothane-anesthetized rats had increasing rates of succinate respiration relative to controls. Based on these results, we conducted the experiments as reported.

As to the suggestions that only preparative and assay conditions which provide mitochondria of high functional integrity should be employed, may we point out that differences between control and experimental preparations could be lost under conditions which may have a pronounced restorative effect on altered or defective mitochondria. For example, we have reported that mitochondria adversely altered by prolonged endotoxemia can be significantly improved by the presence of bovine serum albumin in the assay.[3]

The enhanced rates of succinate respiration which we observed following administration of 3 or 5 per cent halothane and air mixtures for 60 minutes are comparable to the overshoot in glutamate state 4 respiration reported by Cohen and Marshall, who added air to previously-anesthetized suspensions.[4] The fact that these workers observed the same effect for succinate respiration suggests that other components of mitochondrial function besides the NADH–dehydrogenase system may be altered by exposure to halogenated anesthetics.

Once again, our purpose was to determine whether halothane alters the performance of mitochondria isolated after anesthesia. We had no intention of studying the action of halothane directly on isolated mitochondria, as several groups, including Dr. Miller's, are doing. In our study, an alteration in mito-