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Effect of Anesthetics on Mitochondrial Function

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Knowing the curve of metabolism, reflex irritability and oxygen demand . . . a prognostication of the anesthetic result and the plotting of the anesthetic course become possible and practical.—*Arthur Guedel* [49]

THE ANESTHESIOLOGIST has long been concerned with the effect his drugs exert on cellular respiration and the relationship of metabolic changes to the function of the intact organism. In recent years, increasing sophistication in methodology and interpretation has clarified many earlier observations. It has become apparent that a significant locus of action of anesthetics is the mitochondrion, the subcellular organelle responsible for the major part of energy production and storage. Mitochondrial respiration and oxidative phosphorylation have been the subject of a number of excellent reviews,[18, 20, 60-62, 84, 95] one of which appeared in last year's Symposium.[23] Perusal of the latter article will help the reader greatly in understanding the material which follows.

This paper presents a brief review of the early work which set the stage for modern concepts. It then focuses attention on alterations of mitochondrial function and finally, attempts to assess the significance of these findings.

Early Observations

The setting for our current understanding was elaborated by Quastel and Wheatley four decades ago.[86] They noted that "narcotics exert a profound inhibitory action on the oxidation by the brain of substances important in carbohydrate metabolism, *viz.*, glucose, lactic acid and pyruvic acid—this action being accomplished at concentrations of narcotics which are of the same order as those which give rise to deep narcosis in animals." In examining the effects of a number of oxybarbiturates, as well as urethane, chloretone, paraldehyde, and morphine, they made the important observation that while these compounds inhibit cerebral oxidation of glucose, lactate and pyruvate, they have no depressant action on the oxidation of succinate. These data provoked the statement that "this means that the narcotics can have no injurious effect on oxidations of the brain cell as a whole or on the ability of the cell to activate molecular oxygen." Quastel and Wheatley noted that in each group of sedative or anesthetic drugs, the ability of the compound to inhibit cerebral respiration was directly related to its potency *in vivo*. Finally, they indicated that the inhibition of cerebral respiration by anesthetics was far more pronounced during active respiration (produced by the addition of glucose) than when the tissue was at rest.

These observations were extended by Jowett and Quastel in 1937 through the study of the effects of diethyl ether on slices of rat or guinea pig cortex which were exposed to the drug in the gas phase at 37 C.[58] A dose-related diminution in tissue respiration was produced; however, minimal depression was seen at concentrations of ether which would have resulted in clinical anesthesia. Reversibility of this effect depended upon the concentration and duration of anesthesia. No inhibition of oxygen uptake was observed when succinate was substrate.

Webb and Elliott also demonstrated that oxygen uptake of a suspension of whole brain was diminished by treatment with barbiturates, chloral hydrate, and tribromoethanol.[102] However, even with the highest concentration of drug, 15–20 per cent of respiratory activity remained.

By the end of the 1930's, several facts were evident: 1) anesthetic drugs then in use were capable of diminishing oxygen uptake by cerebral tissue; 2) this effect was dose-related and far more apparent in stimulated than in unstimulated tissue; 3) depression of cerebral respiration was not observed when this tissue

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was oxidizing succinate; 4) there was a relationship between potency *in vivo* and the ability of the drug to diminish respiration *in vitro*. These early observations form a recurring theme in more recent work.

Effects of Barbiturates on Mitochondrial Function

Since it is likely that the actions of barbiturates on tissue respiration are mediated by alterations in mitochondrial function, it is reasonable to devote some attention to two significant areas: 1) What is the site of action in the mitochondrial respiratory chain at which the barbiturates act to depress cellular respiration? 2) Do these drugs have any effect on energy storage by the mitochondrion?

Site of Action

Since the ability of barbiturates to interfere with respiration is absent when succinate is substrate, it would appear that the block produced by this group of drugs is at a site not common to electron transport from both succinate and nicotinamide adenine dinucleotide (NAD)-linked substrates. Greig examined the action of pentobarbital on whole-brain oxygen uptake and demonstrated that inhibition could be reversed by the addition of either methylene blue or ascorbic acid.[47, 48] She concluded that the barbiturate acted by binding to a reduced flavoprotein, preventing its subsequent oxidation by the cytochrome chain. The fact that the affinity of barbiturate for this complex was greater than the affinity for the succinic dehydrogenase–cytochrome b complex explained the lack of inhibition of respiration when succinate was substrate. Other workers disputed these findings, since they were able to measure directly the oxidation of a flavoprotein (d-amino acid oxidase) by both oxygen and cytochrome c in the presence of pentobarbital.[51] They felt that inhibition of cellular oxygen uptake resulted from block of the sulfhydryl groups of the pyruvic oxidase system. They hastened to comment that since the concentration of barbiturate used in this study far exceeded the requirements for anesthesia in the intact organism, this metabolic inhibition was not responsible for the anesthetic state.

Spectrophotometric measurements by Chance and co-workers *in vitro*,[22] as well as in the intact animal,[21] have demonstrated that the inhibition produced by amobarbital (Amytal) results from a block of the transfer of electrons from NADH to flavoprotein. Using a polarographic technique for analysis of oxygen uptake,[19] Pumphrey and Redfearn demonstrated that amobarbital-induced inhibition of oxygen uptake by rat-liver mitochondria was far greater in the presence of NAD-linked substrates than when succinate was substrate.[65] Giuditta and Di Prisco directly measured NADH dehydrogenase activity and the oxidation of NADH by beef-heart mitochondria.[42] Both these functions were inhibited by treatment with oxybarbiturates. Data derived from examination of submitochondrial particles enabled Hatefi also to place the site of inhibition of amobarbital in the area of NADH dehydrogenase.[54]

Ernster and co-workers confirmed these findings and proposed the existence of two pathways capable of reoxidizing NADH.[30-33] They designated the phosphorylating pathway, which is the exclusive oxidative route of mitochondrial dehydrogenase, as the "internal" one. This was capable of being totally blocked by amobarbital when NAD-linked substrates were oxidized. An alternate "external" nonphosphorylating pathway was insensitive to amobarbital. When glutamate was substrate and glutamic dehydrogenase, NAD^+, and cytochrome c were present, some respiration was maintained when rat-liver mitochondria were incubated with high concentrations of amobarbital. These findings might explain the previously mentioned observation that whole-brain oxygen uptake cannot be completely inhibited by barbiturate treatment.[102]

In summary, the great weight of evidence would suggest that barbiturates depress mitochondrial respiration primarily by inhibiting electron transfer in the area of NADH dehydrogenase (see figure 5 of reference 23). Indeed, "Amytal has generally been regarded as a specific agent for blocking electron transport in the region of the NAD^+-linked flavoproteins.[85] Although this finding makes the barbiturates extremely important biochemical tools, it must be stressed that these effects are observed at concentrations which are likely to be far in excess of those required to produce clinical anesthesia.

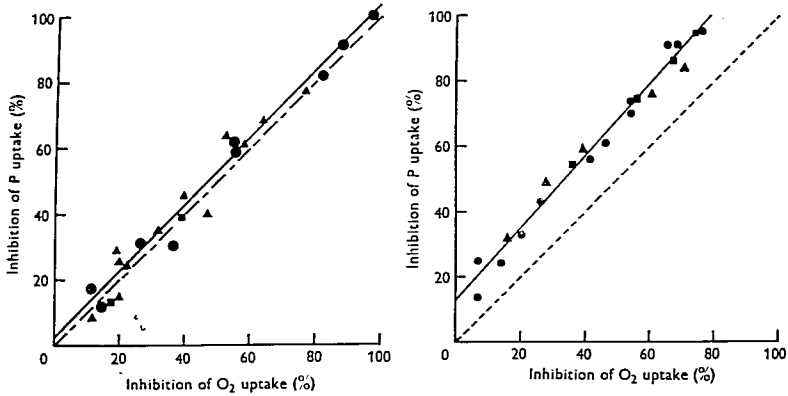

FIG. 1. Effects of oxybarbiturates and thiobarbiturates on oxidative phosphorylation.[1] Left, the effect of oxybarbiturates on phosphorylation by rat-liver mitochondria with pyruvate as substrate. The broken line shows where points should be when the P/O ratio is unchanged; the continuous line is the calculated best straight line. ●, amytal (0.06–0.5 m*M*); ▲, hexobarbital (0.06–1.0 m*M*); ■, phenobarbital (0.5–0.1 m*M*). Right, the effect of thiobarbiturates on oxidative phosphorylation of rat-liver mitochondria with pyruvate as substrate. Broken and continuous lines have same meaning as in the graph on the left. ●, thiopental (0.06–1.0 m*M*); ▲ baytenal (0.2–1.0 m*M*); ■ thialbarbital (0.1–0.4 m*M*).

EFFECTS ON OXIDATIVE PHOSPHORYLATION

The concept that the barbiturates not only interfere with cellular respiration (oxygen uptake) but disturb the mechanism by which mitochondria store energy (oxidative phosphorylation) was proposed by Brody and Bain.[13] These workers studied mitochondria prepared from brain and liver, and analyzed oxygen uptake and phosphate incorporation following treatment with a number of barbiturates. The ratio of phosphate uptake to oxygen uptake (P/O ratio) furnished an index of the efficiency of coupling. When pyruvate, α-ketoglutarate, or oxaloacetate was substrate, treatment with barbiturates resulted in decreased respiration and a *disproportionate* diminution of phosphate incorporation. When succinate was substrate, respiration was unaffected, while phosphate incorporation was markedly diminished. The diminished P/O ratio observed in the presence of both NAD^+-linked substrates and succinate led the authors to conclude that the barbiturates were capable of uncoupling oxidation from phosphorylation. The ability of thiobarbiturates to act as uncouplers was greater than that of the oxybarbiturates. Normal oxidative phosphorylation was resumed following washing in the case of oxybarbiturates. However, the effects of thiopental were reversed in liver only, while those of thioethamyl and thiamylal were irreversible in both preparations. Of significance was the observation that 1,3-dimethyl-butyl-ethyl barbiturate, a convulsant in the intact animal, was also an uncoupling agent of high potency. Furthermore, although comparisons between concentrations found *in vivo* and *in vitro* are difficult when intravenous as opposed to inhalation agents are examined (*vide infra*), the concentrations to which Brody and Bain exposed liver and brain mitochondria ranged up to ten times higher than blood levels observed during clinical anesthesia.

Aldridge and Parker continued this line of investigation.[1] Studying mitochondria prepared from liver and brain, they demonstrated diminished respiration in the presence of all barbiturates when NAD^+-linked substrates were oxidized. Oxidative phosphorylation was uncoupled following treatment with thio-

barbiturates; however, administration of oxybarbiturates was not accompanied by evidence of uncoupling when liver mitochondria were examined (fig. 1). Instability of the preparation of brain mitochondria made it difficult to evaluate changes in oxidative phosphorylation. The authors concluded that three types of metabolic alterations could be demonstrated: 1) no inhibition of respiration accompanying a decreased P/O ratio (the "classic" uncoupler, dinitrophenol); 2) inhibition of respiration with an unchanged P/O ratio (oxybarbiturates); 3) inhibition of respiration with a lowered P/O ratio (thiobarbiturates).

Effects of Inhalation Anesthetics on Mitochondrial Function

Recently, attention has focused on changes in mitochondrial function produced by exposure to the inhalation anesthetics. Snodgrass and Piras used manometric techniques to study rat-liver mitochondria incubated in medium to which liquid halothane had been added.[93] Glutamate oxidation was inhibited to a greater extent than that of succinate. Uncoupling of oxidative phosphorylation was demonstrated at concentrations of halothane at least tenfold higher than exists during clinical anesthesia. Oxidation of NADH was not inhibited. Indeed, when NADH was substrate, respiration was markedly increased by halothane, while amobarbital produced its expected block (figure 4 in reference 93). The authors concluded that halothane was a true uncoupler of oxidative phosphorylation and was capable of inhibiting mitochondrial respiration at a site distinct from that of amobarbital.

Cohen and colleagues used a polarographic technique[19] to measure the effects of anesthetics on respiration of rat-liver mitochondria.[24] Examination of mitochondrial respiration was made in the presence (state 3) and absence (state 4) of adenosine diphosphate (ADP).[20] The rate at which state 3 respiration proceeds is limited by the capacity of the respiratory chain. Thus, any effect of anesthetics on this rate can be related to respiratory-chain function. State 4 respiration (unstimulated by the presence of ADP) is normally quite slow. The ability of mitochondria to maintain slow respiration in the absence of ADP is known as "respiratory control" and is a sensitive index of mitochondrial integrity. Increased state 4 respiration (in the presence of a normally functioning respiratory chain) reflects loss of respiratory control but not necessarily uncoupling of oxidative phosphorylation. Mitochondrial suspensions were equilibrated with a gas phase consisting of halothane vaporized in air. Since the inhalation anesthetics reach equilibrium in tissues both *in vivo* and *in vitro*, it is possible to relate partial pressures to which mitochondria were exposed to those existing in clinical anesthesia.† State 3 oxidation of glutamate was inhibited by halothane in a dose-related fashion (fig. 2). Small changes could be observed following treatment with less than 1 per cent halothane. Complete reversibility of the inhibition occurred provided the concentration to which mitochondria had been exposed was less than 2 per cent. State 4 respiration was unaffected by halothane when glutamate was substrate (fig. 2). When succinate was oxidized, state 3 respiration was not inhibited following treatment with high concentrations of halothane. Exposure to more than 2 per cent halothane increased state 4 respiration when succinate was substrate. This indicated that high concentrations of halothane were capable of producing loss of respiratory control. However, since measurements of oxidative phosphorylation were not attempted, these studies furnish no conclusions concerning the role of halothane in altering oxidative phosphorylation.

The demonstration[93] that halothane is capable of increasing respiration when NADH is substrate is of some interest. Intact mitochondria are unable to oxidize exogenous NADH. Since mitochondrial permeability to NADH might be affected by treatment with halothane, the data of Snodgrass and Piras could reflect the interaction of partial inhibition of NADH oxidation and increased delivery of the substrate to the oxidative site. A more valid study would examine a preparation in which there was no barrier to substrate accessibility. Accordingly, submitochondrial particles capa-

† However, because of temperature differences between *in-vivo* and *in-vitro* conditions, the *concentrations* of anesthetics may differ when partial pressures are the same.

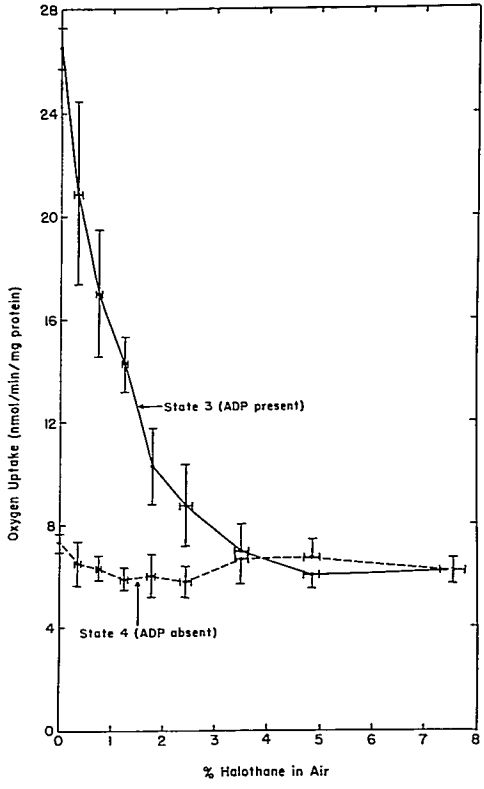

FIG. 2. Effects of halothane on oxygen uptake of rat-liver mitochondria.[25] Mitochondria are suspended in 0.187 *M* mannitol, 0.062 *M* sucrose, 0.010 *M* Tris buffer, 0.010 *M* KH_2PO_4, and 0.010 *M* glutamate at *p*H 7.2. Values are means ± SE.

ble of oxidizing NADH and respiration were examined following equilibration with halothane.[25] These studies demonstrated a concentration-dependent *decrease* of respiration. It would thus appear that the site of action of this anesthetic is similar to that of amobarbital and that the block is in the area of NADH dehydrogenase. Studies of halothane's effect on the isolated perfused liver have led to a similar conclusion.[7] Cohen *et al.*[25] have demonstrated that the respiration remaining following treatment with high concentrations of halothane is mediated via the amobarbital-insensitive "external" respiratory pathway previously mentioned.[30-33]

Miller and Hunter confirmed these data and showed that exposure to 0.5–2 per cent halothane was capable of reversibly inhibiting state 3 respiration when NAD-linked substrates were used.[73] Again, oxidation was not altered by these concentrations of halothane when succinate was substrate. Halothane in concentrations ranging from 5 to 10 per cent altered membrane permeability of mitochondria, resulting in energy-independent swelling.

Examination of mitochondria from livers ob-

tained from halothane-anesthetized rats showed no diminution of oxygen uptake or loss of respiratory control.[88] However, since halothane was undoubtedly lost from the mitochondria during preparation, one cannot conclude that effects were absent during administration of the anesthetic (see Letters to the Editor, ANESTHESIOLOGY 36:625–627, 1972).

The ability of diethyl ether, methoxyflurane, enflurane, fluroxene, and isoflurane also to diminish state 3 respiration when NAD+-linked substrates are oxidized has been demonstrated.[26, 77] Further evaluation when NADH or succinate was substrate indicates that the loci of inhibition of mitochondrial respiration are similar for enflurane, diethyl ether, methoxyflurane, and halothane.[26]

EFFECTS OF INHALATION ANESTHETICS ON OXIDATIVE PHOSPHORYLATION

As already mentioned, Snodgrass and Piras suggested that halothane could function as a true uncoupler of oxidative phosphorylation.[93] This might be expected to be accompanied by increased heat production. Wilson and coworkers demonstrated that dogs anesthetized with halothane had more pronounced hyperthermic responses to 2,4-dinitrophenol (DNP) than animals anesthetized with pentobarbital.[105] This observation was confirmed by Gatz *et al.*, who also produced antagonism of this effect with haloperidol.[39, 41] The aerobic nature of this heat production was suggested by the observation that the DNP-halothane interaction was greater in dogs breathing oxygen than in dogs breathing air.[40, 42] Denborough and colleagues postulated a deficiency in ATP production in Landrace pigs susceptible to malignant hyperthermia.[25] On the basis of these data, it has been suggested that the rare occurrence of malignant hyperthermia could be explained by postulating that halothane served as a "triggering substance" uncoupling oxidative phosphorylation in the patient at risk.[38]

Not all authors have been able to demonstrate anesthetic-induced uncoupling of oxidative phosphorylation. Levy showed 80 per cent xenon or nitrous oxide to be without effect on guinea-pig cerebral respiration and oxidative phosphorylation.[63] Hulme examined the effect of diethyl ether on brain mitochondrial function and concluded that the drug was capable of producing uncoupling.[57] However, examination of the data discloses that there is considerable scatter in control P/O ratios, which ranged from 1.88 to 2.68 (theoretical value is 3.0). In addition, the highest P/O ratio was produced in the presence of the third greatest concentration of ether. The data are therefore inconclusive. An elegant study was performed by Miller and Hunter,[74] who made use of the fact that mitochondrial respiration furnishes energy not only for ATP synthesis but also for ionic movement. When exogenous ATP is unavailable, but electron transfer is allowed, mitochondrial calcium uptake proceeds only when respiratory chain function is coupled to the ATP-producing mechanism. The authors analyzed calcium accumulated by rat-liver mitochondria in response to electron transfer initiated by addition of substrate. After equilibration with halothane and addition of substrate, analysis indicated that the total amount of calcium accumulated was unaffected. This was true following treatment with concentrations as high as 4 per cent, a finding which indicated that the high-energy intermediates required for calcium uptake were still being formed. Thus, although halothane is capable of diminishing mitochondrial respiratory control, it is likely that anesthetic concentrations are not associated with uncoupling of oxidative phosphorylation.

This finding casts doubt on proposals relating anesthetic-induced abnormalities of mitochondrial function to the syndrome of malignant hyperthermia (MH). Several workers have noted that oxidative metabolism alone could not explain the rapid increases in body temperature observed during episodes of MH.[4, 101] Furthermore, Britt demonstrated that oxidative phosphorylation in mitochondria from skeletal muscle of both normal and susceptible patients behaved in an identical fashion following treatment with halothane.[12] Brucker *et al.* showed that skeletal muscle mitochondria from MH-susceptible patients or Poland China swine were even more tightly coupled than control specimens.[14] Finally, a number of investigators have proposed that MH reflects a defect in the ability of sarcoplasmic reticulum to control calcium flux rather than an abnormality of mitochondrial function.[10, 11, 94] This thesis resulted in the apparently successful treatment of the syndrome with procaine.[52, 53]

Mechanism of Action

Although the means by which anesthetics affect mitochondrial function are uncertain, two recent approaches should be mentioned. In the last few years, a number of workers have developed the thesis that mitochondrial energy production is related to changes in mitochondrial configuration.[46, 50, 51] This concept has as its basis the belief "that the *structural state* of the molecular core of mitochondrial membranes transforms when there is a change in the membrane's *activity state*." [50] Recent work by Taylor *et al.* has demonstrated alterations in mitochondrial structure following treatment with 4 per cent halothane.[96] These workers concluded that halothane interacted with "the proteins and lipids of the cristal membrane to dissipate the energized conformation which is on the pathway to oxidative phosphorylation." Conformational changes induced by halothane were also postulated by Miller *et al.*[75] It remains to be seen whether these observations can be extended to other inhalation anesthetics.

Another anesthetic effect on membrane function was elucidated by Seeman and co-workers.[65, 90-92] They demonstrated that the potencies of a number of anesthetic alcohols, phenols, and clinically used drugs were related to their affinities for erythrocyte membranes. Furthermore, concentrations of halothane, chloroform, diethyl ether, and methoxyflurane used during clinical anesthesia were capable of protecting human erythrocytes from osmotic hemolysis, a phenomenon ascribed to their ability to expand the surface area of the erythrocyte membrane. The anesthetic concentrations of these four agents which reduced hemolysis by 8 per cent were approximately equal to their MAC values. The authors conceived that both anesthesia and protection from hemolysis are related to membrane expansion. It is possible, therefore, that the changes in mitochondrial function which have been described and the anesthetic state both result from revesible alterations in membrane structure and function.

Significance of these Findings

Numerous investigators have demonstrated that anesthetics inhibit oxygen uptake by brain slices,[17, 56, 64, 66, 82] liver slices,[5, 55] and the isolated perfused liver.[5, 70] Similar findings were obtained *in vivo* when oxygen consumption of muscle,[95, 99] brain [2, 67] and the whole body [97, 100] were evaluated. That anesthetics are able to affect respiration of mitochondria, tissue, and organs raises a number of significant questions: 1) Is the block of mitochondrial respiration capable of depleting the cell of energy reserves? 2) Does diminished cellular metabolism protect the cell when hypoxia is present? 3) Is the anesthetic state related to alterations in mitochondrial function?

Effect on Energy Reserves

In making the transition from findings *in vitro* to the situation pertaining in the intact organism, it is well to remember that "measurement of oxygen consumption in tissue slices with and without electrical stimulation indicates *capacity* rather than *in vivo performance*." [37] Profound changes in mitochondrial respiratory-chain function observed *in vitro* may be of no biological significance. A decrease in organ oxygen uptake may reflect decreased metabolic demands (resulting from decreased function, *e.g.*, decreased myocardial contractility and work during anesthesia) rather than direct inhibition of mitochondrial respiration. In this event, decreased oxygen uptake will be accompanied by decreased utilization of cellular energy reserves. If energy production and utilization are diminished to the same extent, the concentration of energy reserves will remain constant. If demands on energy reserves decrease to a greater extent than production, the concentration will increase. Obviously, if demand remains constant and ability to produce energy reserves decreases (as in the case of anoxia or cyanide inhibition of respiratory-chain function), the concentration of energy reserves will diminish.

Folbergrová *et al.* evaluated the effects of phenobarbital on substrate levels in mouse cerebral cortex.[36] Increased levels of energy stores consisting of glycogen, glucose, and phosphocreatine were noted. The rate of energy utilization (evaluated by measuring changes in phosphocreatine, ATP, glucose, and glycogen following decapitation) was significantly diminished by the barbiturate. Catfield demonstrated that phenobarbital increased the concentrations of glucose, glycogen, and

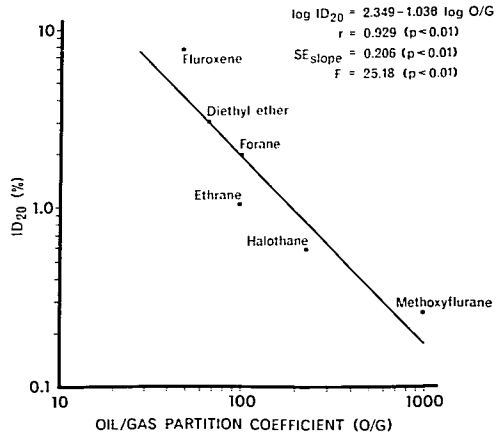

FIG. 3. Relation between *in-vitro* potency and lipid solubility. There is an excellent correlation between the concentration necessary to depress state 3 respiration of rat-liver mitochondria consuming glutamate and the oil/gas partition coefficient.

phosphocreatine in brain, while lactate was decreased.[37] ATP levels did not diminish while the rate of energy reserve utilization was decreased. Biebuyck has shown that halothane anesthesia is accompanied by increased levels of ATP in rat brain.[6] Brunner evaluated the effects of diethyl ether, halothane, methoxyflurane, and enflurane on cerebral metabolism and energy stores.[15, 16] With all agents studied, energy reserves were increased 30–70 per cent, while the rate of energy utilization was approximately half normal. Other workers have demonstrated that anesthesia produced by N_2O, halothane, diethyl ether, cyclopropane, fentanyl, or barbiturates does not produce a decrement in cerebral high-energy phosphate stores.[45, 79, 80]

Whether the diminished energy requirements observed during anesthesia are capable of protecting the organism during hypoxia is problematical. The question has been examined by a number of workers,[3, 9, 44, 65, 69, 89, 104] and was reviewed by the author last year.[23]

Relation of Alterations in Mitochondrial Function to the Anesthetic State

Diminution of state 3 respiration of rat-liver mitochondria was produced by administration of halothane, enflurane, methoxyflurane, isoflurane, diethyl ether, or fluroxene when glutamate was substrate.[77] It was possible to calculate the concentration of each anesthetic that would inhibit mitochondrial respiration by 20 per cent. This value (ID_{20}) correlated exceedingly well with the lipid solubility of these agents (fig. 3). Saidman and co-workers, using MAC as an index of anesthetic potency, found an excellent correlation between potency and the oil/gas partition coefficient.[87] The marked similarity in the relationships of ID_{20} and MAC to lipid solubility is apparent when the slopes of the two regression lines are compared. The slope obtained in the *in-vitro* study is 1.036, while analysis of Saidman's data yields a slope of 0.969.[77]

Caroxin D ‡ and Caroxin F ‡ are fluorocarbons of high lipid solubility but devoid of anesthetic properties. Unpublished observations made in the author's laboratory indicate that these substances are also unable to inhibit mitochondrial respiration.

Administration of .5 MAC of either ethylene or xenon plus .5 MAC of halothane to man produced the same anesthetic effect as 1 MAC of halothane alone.[27, 76] An *in-vitro* analog of this finding exists. A mixture of nitrous oxide capable of inhibiting state 3 glutamate oxidation by 8.5 per cent plus sufficient halo-

‡ Trademark of Allied Chemical Company, Morristown, N. J.

thane to produce 8.5 per cent inhibition depressed state 3 respiration of rat-liver mitochondria by 18 per cent, a figure not statistically different from the expected value were the effects of the two agents strictly additive.[73]

Other workers have proposed correlations among potency, lipid solubility, and depression of oxygen uptake. Miller and co-workers examined a number of gaseous anesthetics, some of which produce their effects only under hyperbaric conditions. The pressures necessary to abolish the righting reflex of mice ranged from 0.4 to 35 atmospheres and showed excellent correlation with lipid solubilities.[71, 72] Fink and colleagues have shown that both growth and oxygen uptake by monolayer cell cultures are depressed by a number of volatile anesthetics.[34, 35] The concentrations of methoxyflurane, halothane, chloroform, diethyl ether, and fluroxene that inhibited oxygen uptake by 50 per cent were quite similar to those producing clinical anesthesia in man. The potency of barbiturates was ten times less *in vitro* than *in vivo*.[35]

Although it is tempting to relate the anesthetic state directly to changes in mitochondrial function, one must be careful in interpreting these data. Anesthetics exert their desired effects in the intact central nervous system; many biochemical data have been obtained in isolated mitochondria suspended in an "unphysiologic" medium. Many biochemical studies are performed at temperatures which are abnormal in the intact organism. It has already been pointed out that the decreased oxygen uptake observed *in vivo* may reflect decreased organ function and metabolic demands as much as direct inhibition of mitochondrial respiration. Finally, it may be that the results observed *in vitro* are due to nonspecific actions of the anesthetics totally unrelated to the anesthetic state. In considering this possibility, it is well to note the recent observation that anesthetic potency in man is related to the drug's ability to produce swelling of a natural rubber membrane.[103]

Thus, at the present time one ought not suggest that the anesthetic state results from depression of mitochondrial function. However, it is likely that these alternations and anesthesia have a common basis. Further examination of the effects of anesthetics on subcellular constituents will help to increase our knowledge of the molecular basis of narcosis.

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