

Metabolic Effects of Volatile Anesthetics in Cell Culture

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Monolayer cell cultures constitute a convenient *in vitro* model for the study of biochemical processes associated with narcosis. Chloroform, diethyl ether, fluorene, halothane, methoxyflurane, trichloroethylene and nitrous oxide slow the multiplication rate of mouse heteroploid cells; the effect of halothane and nitrous oxide is not counteracted by excess oxygen. In the presence of halothane, glucose uptake and lactate output increase and oxygen consumption is inhibited, 50 per cent inhibition occurring with 0.9 per cent halothane in the heteroploid strain and with 0.35 per cent halothane in the mouse sarcoma I strain. The magnitude of the effect on the carbohydrate metabolism of the cell cultures is roughly in proportion to the logarithm of the partial pressure of halothane; the effect is reminiscent of the action of barbiturates *in vitro* in many types of mammalian tissues. A variety of biochemical mechanisms implicating the mitochondrial membranes may be involved.

THE DISCOVERY that gaseous general anesthetics interfere with mammalian hemopoiesis¹ and embryonic growth² has stimulated fresh interest in the effects of anesthetics on cellular metabolism. Directly or indirectly, the effects on cell growth probably are related to anesthetic action in other organs or cell systems, so that understanding of the mechanism of action in any one of these systems is likely to throw some light on the molecular events associated with anesthesia in the others, including the nervous system and liver. It appears rational, therefore, to study the general aspects

of the phenomena of narcosis † in as simple a living mammalian model as possible.

On this premise, the mammalian cell culture seemed to us to be an advantageous system on many counts, foremost among which were longevity, controllability, ease of measurement and the facility with which volatile anesthetics could be introduced and removed. We have undertaken a detailed investigation of the biochemical actions of general anesthetics in such a system. The objectives in our initial study were to measure the effects of certain volatile anesthetics on the multiplication rates and the oxidative metabolism of the cells. We report here a well-marked shift to glycolysis, induced by halothane and proportional to the logarithm of the partial pressure of the anesthetic.

Methods

CELL CULTURE METHODS

Cells were propagated serially on glass in minimum essential medium—Eagle (MEM), with Hanks' basic salt solution as diluent—supplemented with 10 per cent horse serum (10HS) for mouse heteroploid and 10 per cent fetal bovine serum for sarcoma I. For experiments concerning glucose consumption the serum supplement was dialyzed, sephadex G-25 gel-filtered fetal bovine serum. The bicarbonate concentration was 5.6 mM, in equilibrium at pH 7.3 with 19 mm. Hg (2.5 per cent) carbon dioxide in the gas phase. With a P_{O_2} of 60 mm. Hg (8 per cent) the pH in mouse heteroploid cell cultures fell to 7.0 after four days of growth. In later experiments we increased the buffer power by doubling HCO_3^- and CO_2 concentrations and including

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† "Narcosis" in this usage encompasses the non-subjective effects of anesthetics on biological systems, both *in vivo* and *in vitro*. "Anesthesia" is a subjective state *in vivo*.

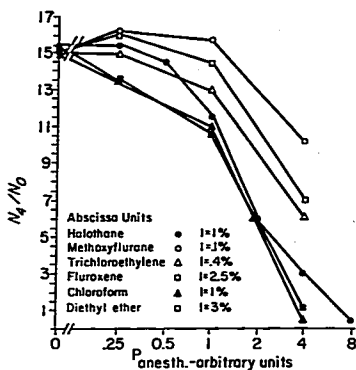


FIG. 1. Four-day growth of mouse heteroploid cells in anesthetic-containing atmospheres. P-anesthetic is in arbitrary units, the absolute value of which is as shown.

TES \bar{p} pH 7.3 at 10 mM. Cells, dispersed with 0.05 per cent trypsin from bottle cultures, were permitted to attach to the Petri dish surface for 24 hours prior to growth experiments. The inoculum was 150,000 cells suspended in 5 ml. medium per 60-mm. Petri dish.

GAS PHASE PREPARATION

The cultures were placed in 200-mm. glass desiccators. The desiccators were evacuated with a high-vacuum pump, flushed several times with high-purity nitrogen and finally filled with test atmospheres. The latter were constructed by admitting oxygen, carbon dioxide, and nitrogen or nitrous oxide from storage cylinders into the desiccators via a manifold. The partial pressure of each gas was measured carefully with a mercury manometer. The desiccators had a volume of 6 liters, large enough to allow a 5-day metabolic exchange without causing significant changes in the composition of the atmosphere. Preliminary observations confirmed that cell growth was not affected by the evacuation. A beaker of water included with the Petri dishes prevented the medium from foaming, as the boiling point of water was reached before that of

the medium. The final pressure was 100 mm. Hg below atmospheric, to provide a seal and a method of checking for leaks when the desiccators were to be opened.

For the most part the experiments were yield experiments, in which cells were cultivated in different desiccators under various sets of conditions. Results in the various sets were compared at the end of four days' growth.

ANALYTICAL METHODS

The supernatants were collected immediately on opening a desiccator. The cells adhering to each replicate dish were harvested by trypsinization and counted with an electronic cell counter. The cell counts in the figures show the mean and scatter of the four replicates. Supernatants were pooled after measurement of pH and preserved by freezing for further analysis. Glucose content of the supernatant was determined enzymatically in duplicate using a glucose oxidase and peroxidase system. \S Lactate was measured with a lactic dehydrogenase system. $^{\circ}$ A calibration curve for glucose was prepared at each session with 1 mM and 5 mM standards, and the accuracy of measurement tested with a 3 mM standard. The 95 per cent level of confidence was ± 0.06 mM. The lactate calibration curve was established with stock standards for each set of determinations. Oxygen consumption was determined polarimetrically at 37 $^{\circ}$ C. in a glass tissue respirometer. \dagger The respirometer (volume 1.81 ml.) was filled with a counted suspension of cells and the oxygen tension recorded continuously during stirring with a magnetic stirrer. At this time, scale calibration was effected with air and by allowing cells to exhaust the oxygen. Next, the suspension was placed in a Petri dish, re-aerated at room temperature and returned to the respirometer. The effect of additions of a solution of halothane in culture medium was then observed. The solution, saturated with halothane at room temperature and therefore containing a known partial pressure of halothane

\S Glucostat Kit, Worthington Biochemical Corporation, Freehold, N. J.

$^{\circ}$ L $^{\circ}$ Lactic Acid Kit, Sigma Chemical Co., 3500 de Kalb St., St. Louis, Miss.

\dagger Instrumentation Laboratories, Inc., Watertown, Mass.

\bar{p} TES = N-tris (hydroxymethyl) methyl-2-aminoethanesulfonic acid. Calbiochem, Los Angeles, California, Box 54282.

vapor, was added in measured quantities from an Agla \dagger micrometer syringe. The halothane vapor concentration in the respirometer was calculated from the dilution factor, taking into account the increase in volume of the chamber, which was accommodated by a plunger. Dilution of the cells was allowed for in calculating the rate of oxygen uptake. After the last addition of halothane the cells were re-aerated in the Petri dish for five minutes and again returned to the respirometer for measurement of the degree of recovery. This exceeded 90 per cent in all cases.

Results

EFFECTS OF ANESTHETICS ON CELL GROWTH

In the first set of experiments reported, the population growth of mouse heteroploid cells was observed in the presence of a series of concentrations of the common volatile anesthetics. Figure 1 shows the increase in cell numbers (day-4 population/day-0 population) at various partial pressures of anesthetic (P-anesthetic). Because of the differing potencies of the anesthetics, the partial pressures are expressed as multiples of an arbitrary unit, defined as the partial pressure estimated to produce moderate depression of the nervous system in man. It is clear from the figure

\dagger Burroughs Wellcome Inc., Tuckahoe, N. Y.

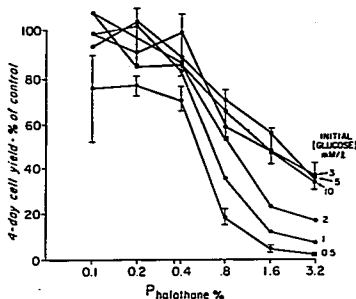


FIG. 2. Four-day yield of mouse heteroploid cells propagated in MEM-10HS-containing 0.5-10 mM glucose. P_{O_2} 60, P_{CO_2} 19 mm. Hg. Three mM glucose suffices for maximal growth, thus growth limitation in the presence of 5 mM glucose and halothane is not due to glucose deficiency.

that most of the anesthetics already depress cell-population growth at unit partial pressure.

EFFECT OF GLUCOSE CONCENTRATION ON GROWTH IN THE PRESENCE OF HALOTHANE

The initial glucose concentration of the medium in the above experiments was 5 mM (25 μ M/dish). Because of the possibility that population limitation might be due to exhaustion of the glucose supply, we compared the

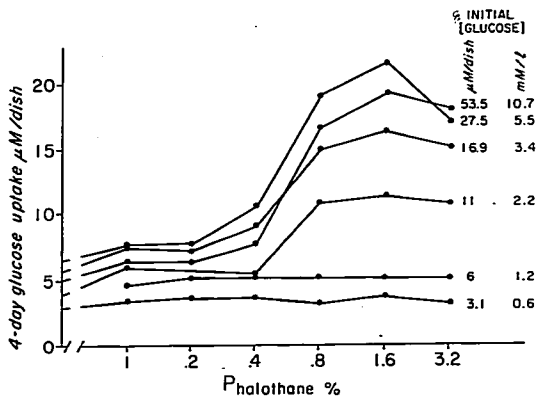


FIG. 3. Four-day glucose uptake of mouse heteroploid cells propagated in MEM-10HS containing 0.5-10 mM glucose. Same experiment as fig. 2. Glucose consumption reaches a maximum in 5 mM glucose.

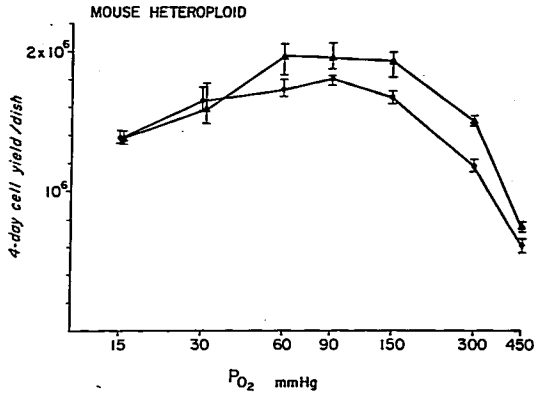


FIG. 4. Four-day yield of mouse heteroploid cells in an atmosphere containing N₂, CO₂ 2.5 per cent and O₂ as shown. ▲ — ▲ no halothane. ● — ● 0.5 per cent halothane. Optimal growth of these cells occurred at P_{O₂} 60–150 mm. Hg. It is also clear that excess oxygen did not counteract the depressant effect of halothane. Medium: MEM-10HS.

4-day cell yields and glucose consumption of mouse heteroploid cells growing in media of initial glucose contents ranging from 0.5 to 10 mM/L (2.5 to 50 μ M/dish (Figs. 2 and 3). In the absence of anesthetic and up to halothane partial pressures of 0.4 per cent, 1 mM glucose was ample for optimum 4-day growth, although 0.5 mM glucose was less satisfactory. In 0.8 per cent halothane vapor, population growth definitely was limited by the availability of glucose: with initial glucose concentrations below 3 mM/liter, i.e., in 2, 1 and 0.5 mM glucose, the lower the initial glucose concentration, the less the four-day growth. It is also clear that with concentrations above 2 mM, glucose was no longer the limiting factor, since the population growths in 3, 5 and 10 mM glucose were essentially identical. Change in pH may have been partly responsible for the limited multiplication, but in a pilot experiment the mouse heteroploid-cell yield was uniform between pH 7.5 and 6.7. The yield declined rapidly when pH fell below 6.7. The lowest pH attained in the present experiments was 6.75.

In 5 mM-glucose-MEM-10HS the glucose uptake (and lactate production) approached a ceiling with P-halothane 0.8 per cent, and showed little additional rise in 1.6 per cent halothane. However, the increased metabolic demand per cell was reflected in the further decrease in cell-number growth observed at

the higher concentration. The glucose uptake (amount of glucose disappearing from the medium) per million cells increased with the glucose concentration, reaching a maximum in 5 mM glucose. Figure 3 shows this trend in the control and at all halothane partial pressures. It can be inferred that equivalent population growth at the lower glucose concentration was due to the utilization of larger amounts of non-glucose substrates.

EFFECT OF OXYGEN TENSION ON GROWTH

In the preceding experiments (as in most of these studies) the partial pressure of oxygen was initially 60 mm. Hg (8 per cent) and remained essentially constant, since the four-day metabolism consumed less than one-hundredth of the oxygen in a desiccator. The choice of this partial pressure was based on experiments like that represented in figure 4. In this experiment optimum population growth of the mouse heteroploid strain occurred at oxygen tensions ranging from 60 to 150 mm. Hg. Outside this range the growth in numbers tended to fall off logarithmically. The lower curve shows that similar relations prevailed in the presence of 0.5 per cent halothane, and demonstrates that excess of oxygen did not counteract the retarding effect of the anesthetic.

The ability of sarcoma cells to propagate in 77.5 per cent nitrous oxide was tested at

several oxygen tensions from 6 to 150 mm. Hg. The cell counts per dish at the end of four days' growth are charted in figure 5, together with those of a series of controls in which nitrogen replaced nitrous oxide. In both groups the cell numbers increased in proportion to the logarithm of the oxygen tension, reaching a maximum when the P_{O_2} approached 38 mm. Hg, but it is again clear that the cells multiplied less readily in the presence of the anesthetic. With further increase in oxygen tension, the cell yields declined, so that excess oxygen did not avail against the inhibition by the anesthetic.

EFFECT OF HALOTHANE ON GLUCOSE METABOLISM

We sought an explanation for the retardation by anesthetics of cell-population growth by studying the metabolism of oxygen and glucose in the presence of halothane. Mouse heteroploid cells were incubated for four days in atmospheres containing halothane to partial pressures of 0, 0.4, 0.8 and 1.6 per cent. Fig-

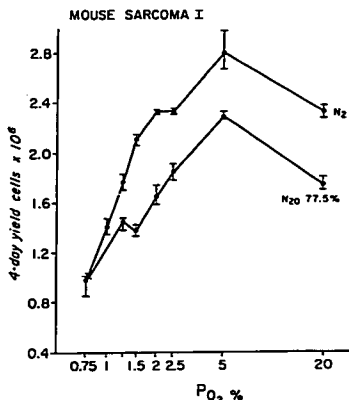


FIG. 5. Four-day yield of mouse sarcoma I cells in 77.5 per cent N_2 or 77.5 per cent N_2O ; CO_2 2.5 per cent and oxygen as shown. Nitrous oxide depresses the growth of these cells at all oxygen partial pressures above 6 mm. Hg (0.75 per cent). Optimal P_{O_2} is about 38 mm. Hg (5 per cent). Higher oxygen tensions are depressant. Medium: MEM-10HS.

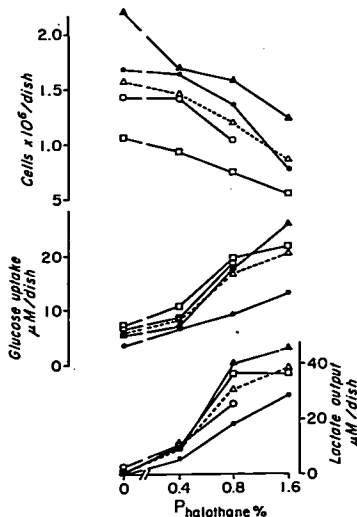


FIG. 6. Four-day effects of halothane on cell growth, glucose uptake and lactate output; note that the lactate scale is stoichiometrically equivalent to the glucose scale. Results and mean (interrupted line) of four experiments are shown. For comment see text. Medium: MEM-10HS. P_{O_2} 60 mm. Hg. P_{CO_2} ● — ● 19 mm. Hg; others, 40 mm. Hg.

ure 6 collects the results from four studies and shows the four-day cell count, glucose consumption and lactate production. In the upper set of curves there is a uniform trend: decreasing cell numbers with increasing halothane, roughly inversely to the logarithm of the anesthetic partial pressure.

The middle set of curves shows the amounts of glucose consumed by the cultures. Although the control glucose uptake was variable, within each experiment the change caused by adding halothane was qualitatively always the same: glucose uptake increased in the presence of halothane, and with anesthetic concentrations up to 1.6 per cent, the more halothane the greater the increase in glucose uptake.

The lower set of curves present the measurements of lactate concentration. Here the

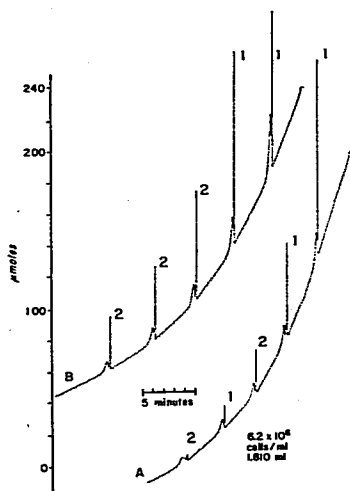


FIG. 7. From right to left: composite of two polarimetric records of oxygen uptake by a suspension of mouse heteroploid cells. Respirometer volume 1.810 ml. The numbers indicate aliquots of halothane-saturated medium injected into the medium. Ordinate zero refers to B only. Between A and B the cells were reentered in a Petri dish for five minutes to remove halothane and carbon dioxide and restore oxygen.

ordinate scale allows for the fact that two moles of lactate are the stoichiometric product of one mole of glucose. The slopes of the lactate curves approximate those of the glucose curves, indicating that lactate appeared at about the same rate, stoichiometrically, as glucose disappeared.

The inferences from figure 6 are that halothane drove the cells to increased glycolysis, that the increase was a direct function of the logarithm of the anesthetic partial pressure, and that the increase was insufficient to maintain the normal rate of cell multiplication.

OXYGEN UPTAKE

This was determined in experiments on suspensions of mouse heteroploid cells containing 6.2 to 8.3 million cells per milliliter. At the start of each of the eleven experiments a new load of cells was introduced into the respirometer and control measurements were obtained. When halothane solution was added to the respirometer there was an immediate change in the slope of the curve (fig. 7) and in about one minute the cells in the respirometer had settled down to a new, lower, steady rate of oxygen consumption. In the mouse heteroploid strain, the control rate varied between 197 and $129 \mu\text{M}/\text{hour}/\text{cell} \times 10^6$ with a mean of 153 ± 1.5 (\pm s.e.). For the mouse tumor

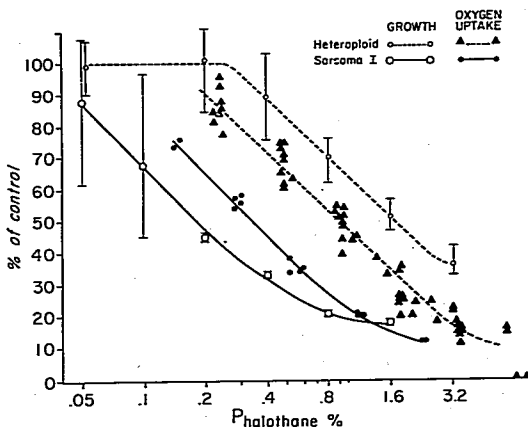


FIG. 8. Relative oxygen uptake and population growth in various halothane atmospheres. Results of short-term oxygen experiments and four-day growth experiments are shown. The best fit was judged by eye (mouse heteroploid and sarcoma I strains).

(sarcoma Ia) culture the control rate was 152 ± 11 (\pm s.e.) $\mu\text{M}/\text{hour}/\text{cell} \times 10^6$. The effect of the anesthetic on oxygen uptake rates of both strains was striking and consistent: between the limits shown the rate decreased linearly with the logarithm of the anesthetic partial pressure (fig. 8). However, the two strains were not equally sensitive; 50 per cent inhibition in sarcoma I required only 0.35 per cent halothane vapor, as against 0.9 per cent halothane vapor in the case of the heteroploid.

The other two curves in figure 8 show the relative four-day growth in cell numbers obtained with different tensions of halothane. The multiplication rate of sarcoma I was much more sensitive to halothane than that of the heteroploid line. The curves show clearly that the decline in four-day population growth occurred at the same rate as the decline in oxygen uptake, suggesting that the short-term changes in oxygen consumption and the long-term changes in cell numbers were related phenomena.

The decrease in oxygen uptake observed in the respirometer measurements occurred almost immediately on addition of the anesthetic. That there was an equally rapid change in the glucose metabolism is rendered likely by the results of an eight-hour incubation experiment with 0.8 per cent halothane. In order to facilitate the detection of small changes, the supernatants from five 5-ml. replicates (initial concentration of glucose = 1 mm.) were pooled at the end of the period of incubation, lyophilized, and the residue reconstituted to 5 ml. with distilled water, thus effecting a five-fold magnification of the change in concentration. The atmospheres contained CO_2 40 mm. Hg, oxygen 60 mm. Hg, water vapor, and nitrogen to 660 mm. Hg in the controls, and, in addition, halothane 0.8 per cent (6 mm. Hg) in the test desiccators. The results are set forth in table 1. The glucose uptake per dish after two, four, and eight hours in the presence of halothane were, respectively, 2.2, 2.4 and 1.6 times the glucose uptake in the controls. Expressed as rates per million cells, the rates of glucose uptake in the anesthetic-treated cultures were, respectively, 1.69, 2.12, and 1.65 times greater

TABLE 1. Eight-Hour Study of Carbohydrate Metabolism of Mouse Heteroploid Cells (1.37×10^6 cells/dish)

Hours Elapsed	Glucose Uptake (μM . per dish)	
	Control	Halothane 0.8%
0		0.51
2	0.38	1.40
4	0.67	2.44
8	1.54	

than the rates in the corresponding controls. We conclude that the higher rate of glucose uptake probably set in as soon as the anesthetic penetrated the cells. The figures also indicate that the time taken for the anesthetic to equilibrate between the desiccator atmosphere and the medium in the dishes was less than two hours.

EFFECTS OF NITROUS OXIDE

Attempts to measure the effect of nitrous oxide on oxygen consumption were unsuccessful, but examination of the four-day effect of 68 per cent nitrous oxide on glucose uptake and lactate output revealed a pattern resembling that of 0.4 per cent halothane. Table 2 presents the results of this experiment. The glucose consumption per million cells increased about 50 per cent in the presence of nitrous oxide. In this respect, the effect of nitrous oxide was qualitatively similar to that of halothane.

TABLE 2. Effect of Nitrous Oxide on Mouse Heteroploid Cells (Inoculum 195,000— Po_2 60 mm. Hg, Pco_2 40 mm. Hg)

Test Gas (atm.)	4-day Cell Count/Dish	Glucose Uptake		Lactate Output ($\mu\text{M}/\text{cell} \times 10^6$)
		$\mu\text{M}/\text{cell} \times 10^6$	%	
Nitrogen 0.68	2,170,000	3.85	100	0
Nitrous Oxide 0.68	1,556,000	5.90	153	6.4

Discussion

EFFECTS ON GLUCOSE-ENERGY UTILIZATION

Some interesting calculations concerning the effect of halothane on the energy obtainable by the cells from glucose can be made. For this purpose we assume that the rate of glucose and of oxygen uptake per cell was uniform throughout the four-day period of incubation, and that all the glucose consumed (or the equivalent of other substrates) was utilized for energy metabolism. We also assume that, when oxygen utilization decreased, the amount of glucose oxidized decreased in proportion, and that the remaining glucose consumption represented glycolysis. On this basis, and starting with the averages of the four experiments in figure 6, we have constructed table 3 as follows: we show in column 2 the number of cells present per dish on day 4; in column 3 the relative oxygen uptake, taken from figure 6. Column 4 lists the glucose uptake per million cells. Column 5 shows the calculated quantity of glucose oxidized per million cells. This quantity is obtained by multiplying the control glucose consumption by the fractional oxygen-uptake rate. The aerobic adenosine triphosphate (ATP) production, column 7, is derived by multiplying the figure in column 5 by 38, since 38 moles of ATP are gained in the oxidation of 1 mole of glucose. If the rest of the glucose consumed, shown in column 6, was metabolized to lactate, the glycolytic energy yield, at the rate of 2 moles of ATP for every mole of glucose reduced, would be as shown in column 8. The glycolytic energy yield per dish, calculated in this manner, may be compared with the lactate formation actually measured (column 9). In glycolysis one mole of lactate is formed for every mole of ATP gained, so that a direct comparison between the figures in column 8 and 9 can be made. The agreement between the two is good and suggests that a correct allowance for oxidative metabolism has been made in the calculations. But this does not signify that the oxidative ATP estimate is correct, because uncoupling may have been present, in which case the ATP estimate would be too high. One also can not exclude the possibility that

in the presence of halothane utilization of ATP may be impaired. Ueda³ pointed out that impaired ATP utilization may account for the inhibition of luciferin bioluminescence by halothane which he observed.

At all events, it is clear that with halothane we force the cells into increased glycolysis to a predictable and controllable degree, and that the glycolytic generation of ATP may not compensate fully for the decrease in oxidative generation of ATP.

FURTHER COMMENTS

These results define the effect of halothane on glucose energy utilization in mouse hetero-ploid cells: within the clinical range of pressures, the time rate of doing oxidative chemical work appears to be diminished in direct relation to the logarithm of the anesthetic partial pressure. We have found a similar relation (with respect to concentration) for amobarbital.⁴

Our results conform to the pattern observed by Cowger, Labbe and Sewell⁵ in L-cell suspension cultures treated with seconal and amytal. These drugs caused the production of an excessive amount of lactic acid and a stoichiometric increase in glucose utilized. On the other hand, Corssen and Sweet⁶ noted that a strain of purported liver cells, cultivated in various media containing 1.5 mM halothane (equivalent to equilibration with about 5 per cent halothane vapor) appeared unaltered morphologically. Not surprisingly, a cell's appearance under the light microscope is a less delicate indicator of cellular disturbances than cell multiplication rate, and this in turn a less sensitive criterion than the changes in carbohydrate metabolism.

The decrease in oxygen uptake and increase in glycolysis demonstrated for halothane and amobarbital in cell cultures may be described summarily as an inhibition of the Pasteur effect. "Pasteur effect" is the description applied to the decrease in glycolysis that occurs in the presence of oxygen. What we observe with these anesthetics is the reverse: increased glycolysis and decreased utilization of oxygen. Evidence suggestive of inhibition of the Pasteur effect by barbiturates has been recorded by Rosenberg and others⁷ in sliced and

TABLE 3. Mean Four-day Glucose Energy Budget of Mouse Heteroploid Cells Propagated in Halothane-containing Atmospheres (Mean Day-0 Count = 157,000)

1 P _{haloth} %	2 Cell Count per Dish (millions)	3 Relative O ₂ Uptake A	4 5 Glucose Utilized			7 Oxidative ATP μ M/cell $\times 10^4$ C \times 33	8 Glycolytic ATP μ M/cell $\times 10^4$ D \times 2	9 Lactate Produced μ M/cell $\times 10^4$
			μ M/cell B $\times 10^4$	μ M oxidized A \times B ₀ = C	μ M reduced B - C = D			
0	1.59	1.00	3.5	3.5	0	133	0	0.7
0.4	1.41	0.72	5.7	2.5	3.2	95	6.4	5.7
0.8	1.18	0.53	13.6	1.5	12.1	57	24.2	23.7
1.6	0.82	0.36	25.0	1.0	24.0	38	48	46

crushed brain, and by Webb and Elliott⁸ in excised tissue from the liver, kidney and testis. It may be contended that our results could also be explained as a stimulation of the Crabtree effect, i.e., as decreased respiration due to increased availability of glucose to the cell. This would imply that halothane accelerated the transport of glucose into the cell. However, Greene's⁹ report that halothane inhibits the rate of entry of sugars into human erythrocytes does not favor the second interpretation. Also arguing against a Crabtree effect was our observation that addition of glucose to the medium in the respirometer, to a total of 15 mM, did not decrease the rate of uptake of oxygen by the cells.

Taken in conjunction with the cited data relating to nonvolatile narcotics, our experiments indicate that alteration of the balance between oxidative and glycolytic metabolism may be a quite general response of mammalian cells to the presence of certain anesthetics, at least *in vitro*. The intensity of the effect appears to be characteristic of the type of cell. Moreover, the mechanism of the effect is not necessarily the same in every type of cell. Various points of attack are available,¹⁰ a number of which are identifiable in figure 7. The shaded area in this figure depicts glycolysis, while the lower part of the figure represents glucose oxidation via the citric acid cycle and respiratory chain, with emphasis on the tight coupling between oxidation of NADH (reduced nicotinamide adenine dinucleotide) and phosphorylation of ADP (adenosinediphosphate). The rates of glycolysis and respiration are linked reciprocally by control factors, one of the most important of which is availability of ADP. Because of the coupling, inhi-

bition of electron transport anywhere in the respiratory chain will have the same reciprocal effect on glucose metabolism as inhibition of any part of the phosphorylation mechanisms: in both cases there will be an acceleration of glycolysis and of lactate production.

A similar argument applies to the coupling between electron transport and carrier systems such as the α -glycerophosphate system studied by Estabrook and Sacktor.¹¹ Schweizer and colleagues¹² have suggested that depression of such shuttle systems or of the mitochondrial electron-transport chain may explain the high lactate levels observed in the blood during ether anesthesia. The shuttles operate, at least in some mammalian cells, to regenerate the extra-mitochondrial NAD⁺ expended in the Embden-Meyerhof-Parnas pathway. A shuttle is necessary because the mitochondrial membrane is virtually impermeable to NADH. Oxidation of extramitochondrial NADH is effected by transfer of the reducing equivalent to a carrier molecule, such as dihydroxyacetone phosphate, forming α -glycerophosphate. The latter penetrates the mitochondrion, reduces FAD to FADH₂, and in the process is itself reconverted to dihydroxyacetone phosphate, which in turn leaves the mitochondrion and is again available to repeat the cycle. Krebs¹³ recently has emphasized the importance of the malate-oxalacetate system, investigated by Sacktor and Dick,¹⁴ in the transfer of reducing equivalents from the cytoplasm to the mitochondrion in mammalian tissue. The β -hydroxybutyrate system proposed as a shuttle by Boxer and Devlin¹⁵ can no longer be accepted as such, because the activity of β -hydroxybutyrate dehydrogenase is confined to the mitochondrial space: the presence of en-

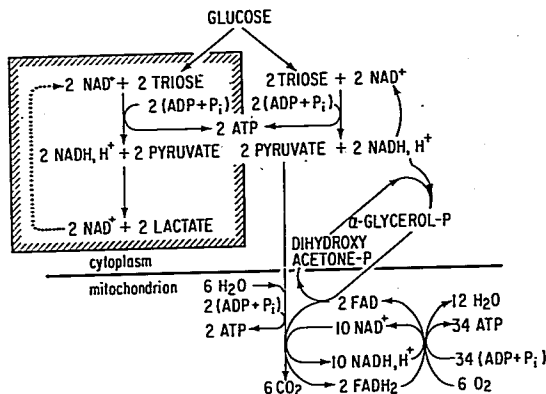


Fig. 9. Bioenergetics of glycolytic (shaded) and oxidative glucose metabolism. α -glycerophosphate shuttles reducing equivalents from extramitochondrial NADH, H⁺ into the mitochondrion. The line to 6 CO₂ represents formation of active acetate and degradation in the citric acid cycle; events in the respiratory chain are to the right of this. Inhibition of any of the intramitochondrial reactions represented in the diagram will result in decreased respiration and will force an increase in glycolysis.

zyme activity in both cytoplasmic and mitochondrial compartments is essential to the functioning of a hydrogen carrier. If an anesthetic inhibited shuttle activity, for instance, by rendering the mitochondrial membrane less permeable to α -glycerophosphate or dihydroxyacetone-phosphate, or to malate or oxalacetate, the consequences would be a failure of oxidative phosphorylation to maintain the extramitochondrial supply of NAD⁺, again forcing greater lactate production. Similarly, if anesthetics retard the penetration of pyruvate into the mitochondrion, oxidative phosphorylation and electron transport will both be slowed down, the pyruvate will be converted to lactate, and at the same time the increase in ADP + P_i from diminished oxidative phosphorylation will accelerate glycolysis by increasing the rate of phosphorylation of glucose and fructose-6-phosphate. Finally, if inhibition takes place in the citric acid cycle, the effect must again be to slow down respiration and carbon dioxide formation and to force a reciprocal increase in glycolysis. To what extent each of these inhibitions operates in narcosis with a particular anesthetic is not yet clear. The potency of our reagents as inhibitors of cell multiplication and as anesthetics parallels their solubility in lipids, so it is noteworthy that most of the mechanisms mentioned above implicate the mitochondrial mem-

branes, of which lipids compose nearly 40 per cent.

Different volatile anesthetics may well have somewhat different effects. For example, several barbiturates block electron transport from flavo-protein to coenzymes Q, but thiobarbiturates and oxybarbiturates differ with respect to their effect on oxidative phosphorylation, the former acting as uncouplers whereas the latter do not.¹⁸

Another instance of the variations that may be encountered is found in the work of Bruemmer, Brunetti and Schreiner,¹⁷ who counted the three-day yield of HeLa cell monolayers growing in the presence of inert gases and of nitrous oxide. They reported a depression of growth directly proportional to the calculated concentration of the gases in oil. This finding contrasts with the logarithmic relation observed by us with halothane, in mouse heteroploid and mouse sarcoma cells. The divergence may be accounted for partly by differences in methodology. In Bruemmer, Brunetti and Schreiner's experiments, the cells were required to attach to roller tubes in the presence of anesthetic, whereas in our studies the cells were incubated in Petri dishes for 24 hours, after which the medium was replaced and anesthetic was added to the atmosphere. Thus, in our experiments only cells already attached to the glass were exposed to the anes-

thetic. It may also be significant that the gases investigated by Brummer's group, including nitrous oxide, all have a much lower lipid solubility than halothane. Halothane is about 100 times more soluble. It should also be pointed out that cell cultures have a considerable inherent variability that renders small effects of doubtful significance.

The idea has prevailed that the molecular mechanism of anesthesia depends on interference with the movement of ions through the lipoprotein plasma membrane of excitable cells.¹⁸ The ATP depletion hypothesis formulated by McElroy¹⁹ and by Quastel^{20, 21} has had little support. It now becomes pertinent to inquire whether the movement of the relatively larger molecules of triose derivatives (fig. 9) into and out of the mitochondrial membranes of excitable cells is also impeded, and whether such an effect plays any part in the genesis of anesthesia by some of the clinical anesthetics.

The relevance of observations in dedifferentiated cell cultures to the complex conditions of specialized animal organs cannot be decided without a good deal more study. Fragments of evidence consistent with the occurrence of a Pasteur-effect inhibition during human general anesthesia do exist. Administration of thiopental and halothane is accompanied by a decrease in oxygen uptake in man.^{22, 23} Ether and cyclopropane anesthesia cause a rise in blood lactate²⁴ and a mild or moderate metabolic acidosis often can be observed in major surgical operations. Additional work will be necessary to decide whether a direct change in cellular carbohydrate metabolism contributes at all to such results, bearing in mind that homeostatic mechanisms tend to minimize the changes in mixed arterial blood. Wide regional differences may be expected, with the most active territories showing the greatest effects.

Conclusions

Halothane inhibits oxygen uptake and stimulates glycolysis in a mouse heteroploid cell culture. Population growth and, apparently, high-energy phosphate production, are diminished. The site of the inhibition is probably mitochondrial. A mammalian cell culture is a useful living system for the examination of

general cellular effects of anesthetics. The scope of these effects in animal organisms needs investigation.

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Drugs

PHARMACOLOGY There are two facets to the biochemical and physicochemical aspects of drug actions; the effects of drugs on the body and the effects of the body on drugs. In the past, pharmacology has emphasized the action of drugs on the living organism, but to be effective, a substance must have characteristics that allow it to reach its site of action in adequate concentration and to remain there for a suitable period of time. In order to reach its site of action, a drug must traverse a succession of membranes. For example, a tranquilizer given by mouth must cross the gastrointestinal epithelium, blood-brain barrier, membranes surrounding individual cells, and finally, subcellular boundaries. Even for processes of metabolism and excretion, the ability to cross cellular boundaries is important. It is obviously important that pharmacologists understand how therapeutic agents penetrate body membranes. (*Brodie, B. B.: Physicochemical and Biochemical Aspects of Pharmacology. (1967 Albert Lasker Basic Research Award Lecture), J.A.M.A. 202: 600 (Nov.) 1967.*)

CARBONATED LOCAL ANESTHETICS Local anesthetics usually are administered as water-soluble salts of hydrochloric acid. There are theoretical and experimental reasons for expecting that local anesthetic salts of carbonic acid should have a better clinical performance than the orthodox solutions. Carbonated solutions of lidocaine and prilocaine base in 1 and 2 per cent concentrations have been tested by clinical trial for conduction anesthesia in 602 patients. Quantitative assessment of analgesia was made in 566 of the patients, who received epidural blockade for surgical and obstetrical indications, and a comparison was made with the results in an additional 251 patients who received epidural analgesia with the equivalent hydrochloride solutions. The carbonated solutions were found to be greatly superior to the hydrochloride solutions for conduction blockade. Induction times were shortened by one-third and the intensity of analgesia was increased by one-third. Dose requirements were diminished, and duration of analgesia was prolonged slightly. No untoward effects were encountered in this series. It is concluded that carbonated local anesthetic solutions represent an important technical advance in conduction anesthesia. They are safe and worthy of extended clinical trial for major conduction-anesthetic techniques. (*Bromage, P.: Improved Conduction Blockade in Surgery and Obstetrics: Carbonated Local Anesthetics, Canad. Med. Ass. J. 97: 1377 (Dec.) 1967.*)