PHARMACOLOGICAL REVIEWS
Vol. 17, No. 2

Printed in U.S.A.

EFFECTS OF ANESTHESIA ON METABOLISM AND CELLULAR FUNCTIONS

A WORKSHOP HELD UNDER THE COMMITTEE ON ANESTHESIA OF THE NATIONAL ACADEMY OF SCIENCES—NATIONAL RESEARCH COUNCIL[1, 2]

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[1] The workshop was held May 1, 1964 at the National Academy of Sciences—National Research Council, Washington, D.C. It was supported by the National Institutes of Health (contract PH 43-62-167) and the Research and Development Command, Office of Surgeons-General Army, Navy and Air Force. *Committee on Anesthesia:* E. M. Papper, M.D., Chairman, Columbia University College of Physicians and Surgeons, New York, New York; John P. Bunker, M.D., Stanford Medical Center, Palo Alto, California; William K. Hamilton, M.D., University of Iowa College of Medicine, Iowa City, Iowa; Duncan A. Holaday, M.D., University of Chicago School of Medicine, Chicago, Illinois; Arthur S. Keats, M.D., Baylor University College of Medicine, Houston, Texas; Dean H. Morrow, M.D., University of Kentucky Medical School, Lexington, Kentucky; Shih-hsun Ngai, M.D., Columbia University College of Physicians and Surgeons, New York, New York; Henry L. Price, M.D., University of Pennsylvania School of Medicine, Philadelphia, Pennsylvania; J. Gordon Robson, M.D., McGill University School of Medicine, Montreal, Canada; John W. Severinghaus, M.D., University of California Medical School, San Francisco, California; Richard A. Theye, M.D., Mayo Clinic, Rochester, Minnesota; Leroy D. Vandam, M.D., Harvard Medical School, Boston, Massachusetts.

[2] The Editors of PHARMACOLOGICAL REVIEWS acknowledge the editorial assistance of Dr. Bunker and Dr. Vandam, of Dr. Milton H. Alper, the recorder of the meeting, and of Dr. Sam F. Seeley of the NAS-NRC. Mrs. Helen Gallahue skillfully collated the original manuscript.

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On May 1, 1964, under the auspices of the National Research Council and its Committee on Anesthesia, a conference on "The Effects of Anesthesia on Intermediary Metabolism" was held at the National Academy of Sciences. It was called a "workshop" to emphasize its objectives: these were to discuss current knowledge of the biochemical and biophysical effects of anesthetics and related drugs at the cellular level, and to attempt to correlate these with metabolic observations made during anesthesia in man. Another important objective was to discuss the role which metabolic events may play in the mechanism of anesthesia, and in fact this theme was evident throughout the conference. The conference was held in two sessions, the first entitled *Actions of Anesthetics on Enzymes and Cellular Systems*, the second *Action of Anesthetics on Organs and Systems*. A general discussion period, which took place between sessions, is included.

The proceedings were opened with greetings from the Chairman of the Division of Medical Sciences of the National Academy of Sciences—National Research Council and from the Chairman of the Committee on Anesthesia of the Division. Dr. John Bunker then assumed the chairmanship of the meeting.

EXPERIMENTAL MODELS AND CONDITIONS FOR FURTHER RESEARCH IN ANESTHESIA AND METABOLISM

Dr. Chenoweth: The purpose of this presentation is to establish ground rules and a common basis for further discussion of the pharmacology of those drugs which have as their common end-point production of a state which permits surgical intervention without suffering on the part of the patient. The phase of this pharmacology in which we are interested today is the effect of these drugs on intermediary metabolism.

So wide a variety of chemical structures will produce the desired pharmacological effect that it seems improbable that important gains can be made by attempting to draw as much as possible in the way of inference from the chemistry of compounds. One may divide the compounds into those which produce deep anesthesia and those which produce light anesthesia, and subseparate these into the volatile and nonvolatile compounds, although there is probably no reason to distinguish volatile from nonvolatile compounds once they are dissolved in the plasma. The pharmacologists and anesthesiologists will recognize that there are some very different types of action presented by these compounds, yet in the final analysis they all permit surgical intervention. Classical anesthesia produced by diethyl ether is different in some respects from that produced by any of the barbiturates, while both differ quite remarkably from chloralose anesthesia or from morphine anesthesia. It would seem almost axiomatic that we must be dealing with a number of mechanisms of action, both at the physiological and at the molecular-biochemical level.

To stress this point, Bennett has presented data showing that azacyclonol, or Frenquel, decidedly inhibits the narcotic action of nitrogen under pressure in the rat (8). He also has explored the effect of this drug in man under similar conditions, and reported that there is a definite effect antagonizing what is called nitrogen narcosis in divers. For comparison, we studied the effect of azacyclonol pretreatment in rats exposed to methoxyflurane vapor at barely anesthetic levels. As you can see from Figure 1, there was, if anything, a slight potentiation of the action of the anesthetic by the azacyclonol.[3] Three weeks later, the groups were reversed and essentially the same results were obtained. The use of azacyclonol shows that nitrogen and methoxyflurane may not be placed at different points on the same curve, differing only in potency, for they obviously differ qualitatively. As another example, one might mention that N-allyl *nor*-morphine will abruptly reverse morphine anesthesia in the dog, but has absolutely no effect on any other kind of anesthesia.

That the same end result can be obtained by the action of drugs which are wholly different in their mechanism is not really new or even surprising. Perhaps a good example is the dilatation of the pupil produced by atropine or by ephedrine. The mechanisms of these actions are wholly understood and completely different, yet the final result that is desired is merely the opening of the pupil

[3] We wish to thank Dr. Harold W. Werner of the Wm. S. Merrell Company for the gift of a sample of azacyclonol.

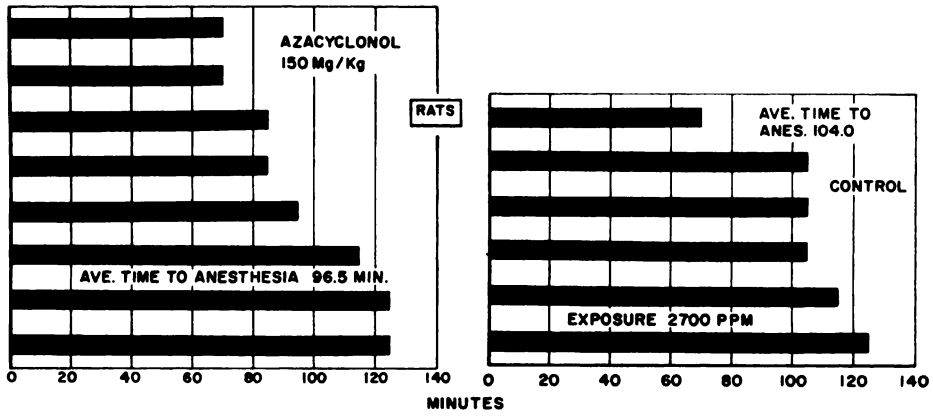

FIG. 1. Effect of 150 mg/kg azacyclonol administered in a single oral dose 48 hours prior to testing on the time to anesthesia (nonresponsiveness) of individual albino Wistar-strain rats. Methoxyflurane was administered to both control and treated rats simultaneously in a closed chamber by adding it to a continuous airflow. Concentrations were determined analytically. Each rat was code marked and its state assessed by an uninformed observer. The slight difference between groups is obviously not meaningful.

so that one can look in. There is no confusion because we know what we are doing. In our present situation, much of the confusion arises from the fact that we have no idea how we are accomplishing the end result.

Another consideration which we are only beginning to appreciate is the fact that anesthetic drugs are biotransformed to a much greater degree than we had thought previously (101). Not only are most of the anesthetic materials metabolized, in most cases to unknown or numerous, uncertainly identified end products, not all of whose pharmacological properties are known, but there is evidence now of interaction. It has been shown in our laboratory that phenobarbital will enhance the metabolism of methoxyflurane; indeed, chronic pretreatment with methoxyflurane will enhance the metabolism of methoxyflurane. The interaction of one drug upon another's metabolism is now a topic of much study in many different laboratories, and bids fair to contribute in important ways to our understanding of drug actions. It must be kept separate in our minds from our simpler understanding of pharmacodynamic interrelationships.

Now turning toward the design of the experiment, let us consider the dose-response curve which exists for anesthetics, as for all other drugs, in the typical sigmoid shape. It simply tells us that the response is related to the dose in a predictable fashion. Of more importance is the curve showing the response to an anesthetic at some constant dose with time. There is both a beginning and an end which are well defined and a constant plateau which is, in practice, laboriously maintained. This is a situation rather uncommonly met with in drug actions, even though it may be the aim. It leads to special problems: a) when and what is the response? We may define the response in this case by the clinical behavior of the patient, or, if you prefer, by a more precise examination of the electroencephalographic pattern or, under special conditions, circulatory, respira-

tory, or biochemical criteria. The anesthesiologists and pharmacologists will recognize immediately that I have passed over the hardest part of the problem, for the exact definition of how deeply anesthetized a patient is at any given time could well be the topic of an entire conference. The next question is b) once such anesthesia has been induced, how long ought the experiment to be carried on? I suggest that there are two times at which data might be obtained: the early stage, or just upon arriving at the state of anesthesia, which may be ten minutes or ten seconds, depending upon the drug chosen; and a later state, perhaps one hour after the induction of anesthesia. Carrying the experiment past this point may well introduce so many complicating factors as to invalidate it for our purposes.

Another aspect of determining the dose-response is knowing the dose that has been employed. Here again we run into particular difficulty in our special situation. With the barbiturates, we can use a gravimetric expression, that is to say, give the number of milligrams per kilogram that is given intravenously, but with volatile anesthetics, except for emulsions of methoxyflurane or roflurane given intravenously, this is not a very practical method. The alternative, the analytical expression, simply the milligrams per milliliter of arterial blood or of central nervous system tissue or other tissue under study sounds very elegant, but requires something more in the way of analytical facilities and help than is always available, although it may ultimately be the final way to get at the important information.

I will gloss over individual variation among patients as a problem, because it is common to every drug and presents no unique character here, to leap to the final and one of the most difficult problems. That is the problem of the control observations. It is necessary to differentiate "effects of anesthesia" from "effects of anesthetics." Although some data do exist on this point, I have never seen them brought together nor do I believe them to be particularly new or applicable. For control observations one might use trained, hypnotized or sleeping subjects, pharmacological or mechanical restraint, and possibly electrical anesthesia. Certainly these all should be added to the usual control, which is merely the samples taken before the anesthesia is begun. The additional problems of surgery and stress, hypotension, hypothermia and ventilatory problems seem almost hopeless until extremely careful and laborious base-line data have been obtained. A few experiments, more carefully planned and carried out than is usually the case, will be most useful.

Dr. R. A. Van Dyke: This paper is not designed to arrive at any conclusions but merely to raise some questions which are important to anyone doing work in the area of anesthetics. Generally, this paper will consider the chemically changed and unchanged anesthetic molecules and some of the effects they may have on enzyme systems both *in vivo* and *in vitro*. In short, this problem will be discussed from the point of view of the anesthetic molecule as it traverses the animal body rather than from the point of view of any specific organ system.

The first area to be considered is what one might expect to find in the way of metabolic interference if one considers only the chemically unchanged anesthetic

molecule. Several workers in recent years have discussed this point and have shown that the anesthetic molecules associate with certain body constituents. Most prevalent is the association with protein. A protein-anesthetic association has been proposed by Dr. Pauling in his recent article on a molecular theory of anesthesia (78). Another illustration of protein-anesthetic association has come from the work of Dr. Featherstone and his colleagues (93). They have shown that the highly lipid-soluble anesthetic molecules can and do associate with proteins.

Each one of these ideas is worthy of a great deal of consideration. But how do they relate to the question concerning us, namely, the effect of anesthetics on intermediary metabolism? One thing is immediately evident: that all the theories and work accomplished mentioned previously involve proteins. Since enzymes are proteins we would expect that if a protein-anesthetic association occurs with an enzyme, this may affect the enzymatic activity of that enzyme by altering either its shape or its charge or even covering up its active site. Furthermore, from what has been said, we would expect this interaction to be highly non-specific, that is, affecting any protein, either a structural one or a functional one, unless there is some consideration which limits this effect. The work which has been done in this area indicates that this effect of anesthetics on intermediary metabolism has some degree of specificity. How can we limit our thinking so that we can find some theoretical basis for specificity?

In the case of the lipid-soluble anesthetics, the anesthetics will associate with a protein at a lipophilic site on that protein. If this protein happens to be an enzyme, the enzymatic activity will not be affected unless the lipophilic area is adjacent to, or involved with, the active enzymatic site. With this in mind, we can speculate as to which enzymes might interact with anesthetics and what kind of enzymatic activity would be affected. It would be those enzymes which normally are involved with the synthesis and metabolism of lipid-soluble material. This is so because these enzymes have active sites composed of lipophilic groups to accommodate the normal substrate. Immediately, several examples of such enzymes come to mind: enzymes involved with steroid production in the adrenal, steroid metabolism and bile acid formation in the liver, oxidation of vitamin A aldehyde and alcohol in the liver, as well as the recently discovered enzymes which metabolize the anesthetics. I am certain other possibilities could be brought to light. A few years ago an observation was made in our laboratory that several volatile anesthetics accumulate in the adrenal gland (22). An explanation for this has not been forthcoming, but now let us consider the possibility that since the adrenal gland synthesizes and transports lipid-soluble, neutral materials as a normal function, then these lipid-soluble anesthetics might also be transported into, and combine freely with, the large number of lipophilic groups present in the adrenal proteins. This could be an explanation for the large amount of anesthetic accumulation in this organ, and in other organs which show an accumulation of the anesthetic.

One might expect that the enzymes responsible for the metabolism of the water-soluble substrates would not be affected by these anesthetics, mainly

because the area on the protein molecule around the active enzymatic site would be hydrophilic and therefore not allow the anesthetic molecule to interfere with or to associate with the active site. We have tested two enzymes in this category, chosen because of availability and ease of assay. With glucose-6-phosphodehydrogenase, the increase in reduced NADP was followed, and with alcohol dehydrogenase, the formation of reduced NAD was followed. In neither case did the addition of a volatile anesthetic affect co-factor reduction, even at very high levels of anesthetic. This does not mean that there was no protein-anesthetic association, but merely that the anesthetic in no way interfered with the active site.

The two anesthetics, halothane and methoxyflurane, are dechlorinated enzymatically (100). This was determined by putting a tissue slice with buffer in the center well of a Warburg flask and the isotopically labeled anesthetic in the side arm, sealing the flask, and incubating it. The anesthetic was then allowed to diffuse over to the aqueous buffer, so that in a fairly short time the buffer and tissue slice were saturated with anesthetic. Enzymatic dechlorination occurs until the buffer obtains its maximum concentration of anesthetic and then stops. Later it was found that if the rate of diffusion of the anesthetic from the side arm to the buffer is limited, as would be the case *in vivo*, dechlorination is increased, but once again the process stops when the buffer obtains its maximum concentration of anesthetic. The increased dechlorination resulted because of the longer period of submaximal anesthetic concentration. In addition, if the system is preincubated with diethyl ether in a similar manner, and then incubated with halothane or methoxyflurane, the ability of the tissue to dechlorinate or metabolize the anesthetics to CO_2 is decreased. These examples of substrate inhibition may well be the result of the anesthetics inhibiting an enzyme with several adjacent lipophilic sites surrounding the active site; if more than one substrate molecule reaches the active site at the same time, inhibition occurs.

Besides effects of anesthetics on enzymes, we can also consider the effects on certain structural proteins. Those of importance to this discussion are the lipoproteins and particularly those in the cell membranes. The anesthetics may perhaps associate with these membrane lipoproteins to interfere with normal transport. This is not a new idea and therefore it shall be mentioned only at this time.

At this time it is appropriate to enter one small plea for not jumping to conclusions as to the site of biochemical effect of these anesthetics. Certainly all investigators in this field will agree that every effort should be made to know the primary site of effect. For example, changes of blood levels of nutrients may reflect effects on transport and not necessarily changes of enzymatic production or use. An effect on carbohydrate metabolism may mean an effect on steroid metabolism.

The first point to be made, then, is that one would expect the crucial effect of the chemically unchanged anesthetic molecule to be on two places: 1) on the lipoprotein of the cell membrane, inhibiting, or at least interfering with, transport, and 2) on the enzymes which normally act on lipid-soluble materials.

The second area of this discussion is the effect of the intermediates of the biotransformation of the anesthetics on intermediary metabolism. It is well known that the injectable anesthetics are metabolized, and that their products, which are usually hydroxylated, are more water-soluble. Volatile anesthetics have also been shown to be metabolized *in vivo* (101). This biotransformation could lead to a host of theoretically possible intermediates and excretory products, almost all of whose biochemical effects are as yet unstudied. Since this is the case, we cannot fail to consider the effect of these intermediates on metabolism. Since metabolism of lipid-soluble materials usually occurs in such a way as to result in a product which is more water-soluble, these products would consequently be rapidly removed from the cell, by a route different from their entrance, and excreted from the organism. More work is needed to assess whether there is any effect of these intermediates on intermediary metabolism.

There is a possibility that some metabolic process could lead to a highly reactive intermediate which in turn could combine with an enzyme and inactivate that enzyme. Chloroform is still used to some extent as an anesthetic and therefore can reasonably be used for this example. It undergoes breakdown by two different routes. Chlorine is removed by an enzymatic process and also by a nonenzymatic process. The nonenzymatic reaction takes place in the presence of sulfhydryl groups. If the product could remain combined with the sulfur, and the sulfur happened to be on the active site of an enzyme, this would lead to inhibition of the enzyme. Yet unless the concentration of chloroform were extremely high, this inhibition would play only a small role.

The second point to be made is that most of the metabolism of anesthetics results in a more water-soluble product which is then more readily excreted. However, it is conceivable that some effects may be noted due to the formation of highly reactive intermediates.

The third area to be discussed is conveniently labeled miscellaneous considerations. Within this category there are two main points. The first is that if any studies are to be carried out, the nutritive state of the organism must be considered. What effect has the lack of nutrient supply had on the studies made to date? Certainly the normal substrate supply will be of major concern in the extent of biotransformation of the anesthetics. In this regard, one can also pose this question: would a good normal substrate supply protect those enzymes which are affected by the anesthetics by tying up the active site so that the anesthetic cannot associate with it? It has recently been reported (15) that diethyl ether is highly toxic to rats deficient in essential fatty acids. This may deserve considerable attention to see if this is specific to diethyl ether, or if all volatile anesthetics are toxic to such rats. Significant in this regard, also, is the finding that the toxicity of the volatile anesthetics is subject to circadian rhythms (61).

The second point in this area of consideration has been mentioned by Dr. Chenoweth. It has to do with knowing the concentration of the anesthetic in the various parts of the whole animal, even to knowing the concentration in the cell, before any particular enzyme system can be meaningfully studied *in vitro*. All enzymes found to be or believed to be affected by anesthetics must eventually

be tried *in vitro* under conditions which leave no question that the effect is specific for that enzyme and that anesthetic. But the concentration producing this effect must be the same *in vivo* and *in vitro*. Work has been reported in the literature in which an anesthetic was shown to have inhibitory effects *in vitro* without reference to whether the concentration of anesthetic used was similar to the concentration of the anesthetic at the site of the enzyme *in vivo*.

In conclusion the following points should be kept in mind: 1) The inhibitory effect of volatile anesthetics is likely to occur at two places: at the cell membrane where the lipoprotein is in high concentration and at those enzymes which normally utilize lipid-soluble substrates. 2) Most anesthetics are metabolized to some extent and the effect of this cannot be fully assessed at this time but must be considered in the future. 3) Such considerations as nutritional state and cellular concentrations *in vivo* should be considered more thoroughly in future studies.

STRUCTURE AND FUNCTION IN OXIDATIVE PHOSPHORYLATION

Dr. B. Chance: This paper will attempt to summarize briefly salient features and new developments in the structure and function of the oxidative phosphorylation system which may be relevant to our problem today. It is hoped that this presentation will provide a sounding board for discussions of the effects of anesthetics on oxidative phosphorylation, particularly in identifying whether their effects are on the enzymes, or on the structures to which the enzymes are bound. The mitochondrion itself may be of special interest in considering problems of anesthesia. It has some of the interesting properties of the intact cell and yet it lacks some of its drawbacks. For example, mitochondria have protein and lipid components in membranes and internal compartments as well. Yet we are able to impose many biochemical reactions upon the mitochondria that are not possible with the intact cell.

Recent developments in the study of fine structure now point to the electron micrographic identification of the enzymatic sites on the mitochondrial membranes. A few years ago, Fernández-Morán *et al.* (33) identified, with the negative-staining technique, subunits which project from the mitochondrial membrane. They believed that these projecting subunits were the elementary particles of electron transport (Fig. 2). Work in our laboratory in Philadelphia, as well as in Toronto and at Purdue, however, revealed that these projecting subunits could be removed from the mitochondrial membrane without loss of electron transport activity (20, 97, 105). These results indicated that the projecting subunits had nothing to do with electron transport and that some other function must be involved. This problem was largely solved in work between laboratories in Philadelphia, New York and Toronto (88) which showed that the mitochondrial fragments from which the projecting subunits had been removed showed a reconstitution of the subunits when the oxidative phosphorylating coupling factor (ATPase) was added under appropriate conditions (76, 88). Since it is now possible to split the projecting subunits from the mitochondrial structure and reconstitute the structure with the ATPase, we have now identified the projecting subunit as one of the coupling factors of oxidative phosphorylation.

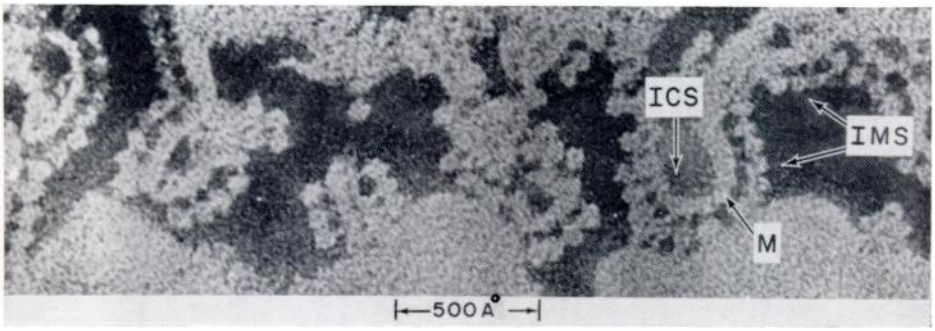

FIG. 2. Electron micrograph of extruded portions of the crista of mitochondria prepared from flight muscle of an adult bee. Staining with phosphotungstic acid. The projecting subunits (IMS), the base membrane (M), and the intercristal space (ICS) are indicated in the diagram. IMS are identified with the coupling factor of oxidative phosphorylation. The fixed components of the respiratory chain are believed to be possibly located in zone M (electron micrograph obtained in collaboration with Dr. D. F. Parsons, Medical Biophysics Laboratory, University of Toronto).

The exact location of the electron transport enzymes is not yet identified with certainty, but by process of elimination, it is most likely to be in the base membrane from which the subunits project.

At this point, we can no longer rely upon electron micrographically known structures. We have to deal with an imaginary structure of the respiratory chain, generally indicated as a linear sequence of pyridine nucleotide, flavoprotein and cytochromes (Fig. 3). Electrons flowing from either succinate or malate pass through the chain to reduce oxygen to water. Sites for conserving energy in the electron transport process are indicated, together with symbols (X, I) representing unknown intermediates of oxidative phosphorylation. The diagram indicates two general types of sites for inhibitors (*e.g.*, anesthetics) to affect the chain. The first and most obvious sites are those which prevent electrons from flowing through the chain, for example, the reaction of cyanide and carbon monoxide with the terminal enzyme (cytochrome a_3) and of Antimycin A and hydroxyquinoline N-oxide between cytochromes b and c_1. Of considerably more interest is the concept put forward in part by Hollunger (17, 45) that inhibition of the reactions which siphon energy from the respiratory chain can as well result in the inhibition of electron flow. Inhibitors of this type are guanidines, oxybarbiturates, oligomycin, and plant extracts such as atractylate.

Since seven of the components of the respiratory chain can readily be observed by spectroscopy or fluorometry, a unique opportunity is afforded for observing the pile-up of reduced enzymes on the substrate side of an inhibition site and depletion of the reduced forms of the enzyme on the oxygen side of the inhibition site. The point marked on the one hand by accumulation and on the other by depletion is called the crossover point and is based upon a number of mathematical and experimental studies (18) which lead to a general theorem for identifying sites of inhibitory action in complex enzymatic sequences.

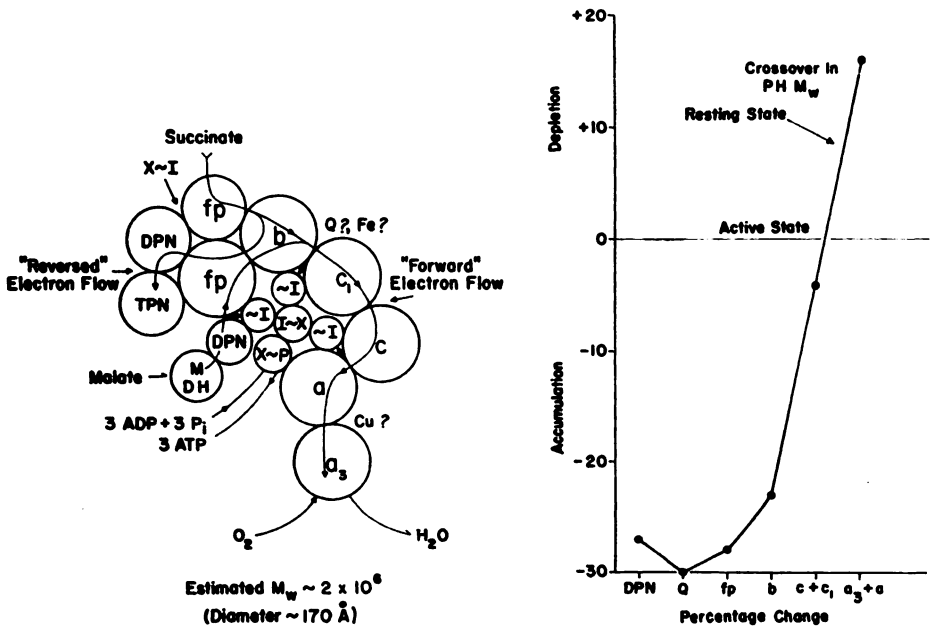

FIG. 3 (*left*). A schematic diagram of the components of the respiratory chain which comprise the functional unit or "oxysome." Cytochromes are designated a_3, a, c, c_1 and b. Flavoproteins (fp), pyridine nucleotides (DPN, TPN), components of oxidative phosphorylation ($\sim$ I, X $\sim$ I, X $\sim$ P), malic dehydrogenase (MDH), quinone (Q), nonheminiron (Fe), oxidase-bound copper (Cu).

FIG. 4 (*right*). An illustration of the crossover point of steady state change of respiratory components in a suspension of pigeon heart mitochondria caused by the transition from the active state (State 3) to the resting state (State 4). The words "accumulation" and "depletion" on the ordinates indicate an accumulation and depletion of the reduced form of the components listed along the abscissae. Reduced pyridine nucleotide (DPN), ubiquinone (UQ), flavoprotein (fp), cytochromes b, c + c_1, a + a_3. The crossover point in this transition lies between c + c_1 and a + a_3. The numbers on the ordinates are the percentage change of the oxidation-reduction state with respect to the State 3 or active state value (the designation of signs is arbitrary).

An example of physiological inhibition of electron transport in the respiratory chain is provided by the exhaustion of ADP in the presence of excess of phosphate. While ADP is present, the electron flow is rapid (State 3), but when ADP is depleted the electron flow may decrease 10- or 20-fold because of cessation of operation of the energy-transfer reactions (State 4). The accumulation of the reduced forms of DPNH, flavin, ubiquinone, and cytochrome b and the depletion of the reduced forms of cytochrome c, a and a_3 is indicated in Figure 4, which shows that one of the crossover points for ADP depletion was between cytochromes b and c.

A special spectrophotometer (16) has been developed for measuring the changes of cytochromes, flavins, pyridine nucleotides, and ubiquinone components of the respiratory chain in response to the effects of specific inhibitors. It provides a method for locating the sites of action of such inhibitors. Since oxybarbiturates

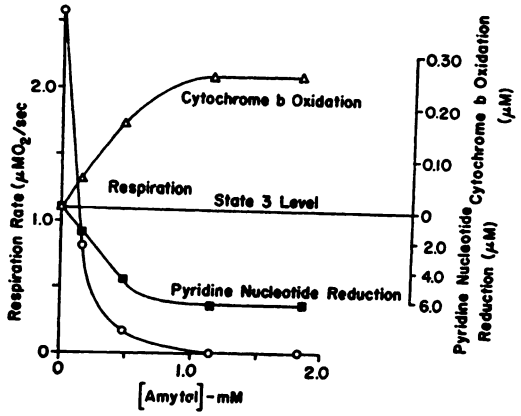

FIG. 5. Effect of amobarbital (Amytal) concentration upon the respiration rate (left ordinates) or steady state of cytochrome b and pyridine nucleotide (right ordinates) in a suspension of rat liver mitochondria. The State 3 level corresponds to the active state of respiration, due to supplementation with ADP and phosphate. The graph illustrates the inhibition of respiration and corresponding changes in the steady state of cytochrome c and reduced pyridine nucleotide.

are of particular interest to this meeting not only in the present sense, but also in view of the extensive work of Dr. Quastel's laboratory on this topic, we present our data on the crossover phenomena observed in rat liver mitochondria in inhibiting the system with amobarbital (Amytal). In this graph (Fig. 5) we plot as a function of Amytal concentration, the inhibition of the respiration rate, the oxidation of cytochrome b, and the reduction of pyridine nucleotide. It is apparent that there is a crossover point between pyridine nucleotide and cytochrome b. More detailed data, in which flavin is titrated and its oxidation is observed as well, indicate the site to be between DPNH and flavin. This corresponds actually to one of the important energy conservation sites of the respiratory chain, and for this and other reasons, our findings have indicated to us (17) that the oxybarbiturates act not upon electron transport, but upon the energy transfer reactions of intact mitochondria.

Numerous examples of the effects of inhibitors upon electron transport and energy conservation can readily be provided. Certainly the difference in response to oxybarbiturates and thiobarbiturates (21) is clearly identified, as are the sites of action of guanidines, biguanides (82), uncoupling agents, oligomycin, and atractylate. All types of inhibitors can be incisively studied and the site of their action revealed by the crossover theorem (17).

In addition to the general conclusions on the nature of inhibitors of either electron or energy transfer pathways, it is important to point out how different the responses of mitochondria may be depending upon whether their electron flow is activated by ADP or phosphate or is already inhibited by lack of ADP and phosphate. It seems elementary to state that the inhibitory response would be different in these two conditions, but in many cases, the differences have been

ignored or have not adequately been determined. The problem becomes eventually acute *in vivo* where it would not be known whether the electron transport system of a particular organ is in the ADP-activated state (State 3) or already inhibited by lack of ADP (State 4). The possibilities of determining the metabolic state of an organic tissue or even of a single cell are now gradually emerging on the basis of more sensitive optical methods developed in this laboratory. These methods can be applied to tissues in detail (19). As such methods are more fully developed, more valuable information concerning the mechanism of action of anesthetics may become available.

Dr. A. J. Trevor: This discussion will concern mainly the results of experiments with fixed anesthetic agents, such as the barbiturates, in view of the lack of information regarding the biochemical action of volatile or gaseous anesthetics. The available evidence leads to the conclusion that narcotics may act at a plurality of *loci* and in the case of the barbiturates the two most important sites of action appear to be 1) at the level of the respiratory chain, causing an inhibition of electron transfer, and 2) on some mechanism of energy utilization, causing an inhibition of functional activity with secondary effects on metabolism.

The concept that depression of neural functioning *in vivo* by narcotics might be related to their effect on nerve tissue respiration, originated largely from the studies of Quastel and Wheatley (87). It was subsequently suggested that barbiturates inhibit electron transfer at the flavoprotein level (67), but a decade ago the possibility of such a mechanism of action was obscured following suggestions that these agents in general uncouple oxidative phosphorylation (11, 12). However, Eiler and McEwen (27) and Case and McIlwain (14) had failed to demonstrate uncoupling in cerebral mitochondria using oxybarbiturates, and the results of Brody and Bain often showed only minor changes in P/O ratio until respiration was greatly inhibited. Further investigation, following adequate appraisal of the importance of correlating barbiturate concentration *in vitro* with anesthetic dose *in vivo* (2), demonstrated that oxybarbiturates neither uncouple oxidative phosphorylation nor inhibit dinitrophenol-activated ATPase of liver mitochondria. Depression of P/O ratio by thiobarbiturates was correlated with ATPase activation.

Dr. Chance and his colleagues (17) have continued and extended earlier observations (27, 28, 87) that oxybarbiturates inhibit oxidations linked to pyridine nucleotide. Their elegant spectrophotometric studies demonstrate that in phosphorylating mitochondrial preparations, amobarbital at low concentration inhibits electron transfer between NAD and flavoprotein, energy transfer (the inhibitory effects reversible by uncoupling agents) and the oxidation of succinate. In nonphosphorylating preparations the site of action of amobarbital appears to be localized between flavoprotein and cytochrome or ubiquinone.

Dr. Quastel has emphasized that such actions would both suppress the activity of the tricarboxylic acid cycle and decrease the formation of ATP. Evidence for this viewpoint includes suppression of ATP-dependent synthesis of acetylcholine and glutamine (48, 50), and decreased incorporation of radioactive orthophosphate into phospholipids (34). I don't wish to anticipate Dr. Quastel at

this point, but it is important and relevant to mention his statement that certain pyridine nucleotide oxidation systems appear to lack sensitivity to barbiturates (86).

Much of the detailed investigation leading to the conclusion that anesthetic agents, in particular barbiturates, inhibit electron transfer, has been carried out on mitochondrial preparations derived from liver or heart tissue. Justification of the use of such systems lies primarily in the fact that the overall metabolism of both liver and brain is similarly depressed and that barbiturates are considered to have a general effect, with only that on the brain producing a clinical response, rather than a specific action on cerebral metabolism (1). It may be a reflection of the cellular heterogeneity of cerebral tissue that brain mitochondria are difficult to obtain in sound biochemical condition, rarely exhibiting a high degree of respiratory control. Aldridge and Parker (2) were unable to prepare cerebral mitochondria that maintained P/O ratio in the presence of phenylarsenious acid, which does not uncouple phosphorylation from oxidation in liver mitochondrial preparations.

Considerations of the mechanism of narcotic action at the biochemical level are somewhat hampered by wide variations in structure of agents able to induce anesthesia. Conversely, certain compounds such as chlorpromazine, shown to inhibit electron transport at a site close to that sensitive to barbiturates, are not general anesthetics.

Marked differences have been shown to exist in anesthetic potency in rats between L- and D- forms of 1-methyl-5-allyl-5-(1-methyl-2-pentenyl)barbituric acid (66). At the risk of unjustified extrapolation of these findings to the situation in other species, I shall note that the two forms of this barbiturate also exhibit differences in ability to inhibit electron transfer in Tetrahymena (74).

There is no valid reason to assume that anesthetic agents act through a single mechanism. The experiments of Chance and Hollunger (17) demonstrated at least three sites of action at the mitochondrial level, and there is considerable evidence suggesting that anesthetics may interfere with chemical mechanisms associated with extra-mitochondrial membranes. The concept of action at the level of production of high energy phosphate compounds does not readily explain the findings that the major biochemical changes *in vivo* include increased levels of creatine phosphate with decreased inorganic phosphate concentration despite depression of oxygen consumption (63). This pattern of metabolic changes induced by anesthetics *in vivo* has been shown to have close correlation with events accompanying the stimulation of intact cerebral tissue *in vitro* by cations (85) or electrical stimulation (62). For example, concentrations of barbiturates (0.5 mM) having no effect on unstimulated respiratory rate or creatine phosphate levels, inhibit the respiratory response to electrical pulses and depress the rates of creatine phosphate breakdown and lactate formation (62).

In addition the presence of quite high concentrations of phenobarbital (1.7×10^{-3} M) does not disturb the resynthesis of creatine phosphate following cessation of pulses (23). Similar observations have been reported for the effects of narcotics on the metabolic response of cerebral slices to cation stimulation.

It would appear therefore that in the intact cerebral slice *in vitro* there is a parallelism with the *in vivo* situation during anesthesia, and the evidence is contrary to the idea that the primary action of narcotics is at energy-yielding processes involved in the production of ATP. Rather the viewpoint has developed that the depressant action of anesthetics is primarily at some level of energy utilization (64)—that is, an initial action on functional activity leading secondarily to metabolic changes (13). This is in some agreement with the work of Larrabee, who has suggested a dual action on respiration and utilization of energy-rich phosphates (59).

Some indication of the relationships between metabolic changes and functional activity is offered by observations that the phosphate changes during electrical stimulation of isolated cerebral tissues are accompanied by rapid progressive loss of potassium and gain of sodium (25). Appraisal of these findings, together with considerations of the effects of anesthetics on membrane permeability and the conclusions of Mullins (71) and Skou (95), has led McIlwain (64) to suggest a primary action involving diminution of ion movements during excitation, perhaps by physically inhibiting the movement or orientation of structures involved in this process. The process of active transport in cerebral systems involves considerable energy expenditure and has been suggested to act as a "pacemaker" for respiration (103). The utilization of the potential energy of ATP for ion transport at the membrane necessarily involves enzymic mechanisms and the increasing numbers of reports implicating the involvement of cation-stimulated ATPase in cation movement in a variety of systems are therefore of great interest (65). It is possible that the action of anesthetics at the neuronal membrane, in depressing ion movement and hence functional activity, would lead to inhibition of such an enzyme system. The decreased formation of orthophosphate and phosphate acceptors could lead to respiratory depression without direct action of drugs on steps in the respiratory chain (64).

As the final part of this discussion I would like to introduce the subject of phosphorylated proteins, which appear to be of some importance in a number of different pathways of phosphate exchange. For example, many highly purified enzymes concerned with phosphate transfer or proteolysis have been shown to contain phosphorylserine at their active site (3, 91).

In addition, experiments with isolated liver mitochondria implicate a protein that is capable of undergoing phosphorylation as an intermediate in the process of oxidative phosphorylation (4). Protein-bound phosphorylserine has been shown to have wide distribution through the subcellular components of cerebral cortex tissue (99) and the incorporation of radioactive orthophosphate into the phosphoprotein fraction of isolated cerebral tissue is accelerated by 1.7 mM phenobarbital (44). The application of electrical pulses to cerebral tissue slices leads to increased incorporation of labeled phosphorus from ATP into phosphoproteins (43), and experiments designed to localize the phosphoprotein sensitive to electrical pulses, indicated an occurrence in a microsomal rather than a mitochondrial fraction (98). This localization negates the possibility of involvement of the compound in oxidative phosphorylation. In this respect recent investiga-

tions concerning the metabolic relationships of bound phosphohistidine in mitochondria are of great interest (60, 83). Study of membrane phosphoprotein and the ATPase reaction in cerebral microsomes continues (90), but to date the effects of anesthetic agents on this relationship have not been reported.

In summary, it is hoped that this discussion has focused attention on the multiplicity of possible sites and mechanisms of narcotic action. Inhibition of electron transfer in the respiratory chain is well established, but considerable evidence points towards an action on energy utilization as well as on energy producing mechanisms. Further biochemical investigation of the effects of anesthetics at the level of intermediate compounds involved in both oxidative phosphorylation and membrane transport may assist in clarification regarding a primary locus of action.

EFFECTS OF DRUGS ON CARBOHYDRATE METABOLISM IN BRAIN

Dr. J. H. Quastel: I would like to discuss the effects of anesthetic agents on carbohydrate metabolism in brain *in vitro*.

Relatively recent work carried out with cation-stimulated, or electrically stimulated, isolated slices of brain cortex or with brain mitochondria preparations has shown that anesthetics, at low concentrations which cause anesthesia, bring about marked inhibition of certain respiratory processes. The concentrations of barbiturates that inhibit mitochondrial respiration are in close agreement with the anesthetic levels.

Estimates of *in vitro* respiratory rates of whole brain, examined in standard physiological media, approximate only to about half the values found *in vivo*. It is possible by stimulation of isolated brain cortex slices, by alteration of the cation concentrations (*e.g.*, increase of potassium ions in a sodium medium, or of sodium ions in a potassium medium, or decrease of calcium ions in the medium surrounding the brain tissue) or by electrical stimulation, to increase respiratory rates to approximately those found *in vivo*. The stimulation is specific to certain areas of the brain. This phenomenon does not occur in a brain homogenate or a mince; it is evident that it is linked with the integrity of the brain cell membranes. Not all substances burned by the brain permit cationic stimulation to occur but those that do, *viz.*, glucose, fructose, lactate, and pyruvate, are identical with those that allow electrical stimulation of respiration to take place. The stimulated respiration *in vitro* approximates to that *in vivo* under normal conditions of sensory stimulation. The stimulation of respiration consists of an increased turnover of the citric acid cycle, as it is highly sensitive to malonate and the malonate inhibition may be reversed by oxalacetate. Moreover, malonate (10 mM) greatly inhibits the conversion of labeled glucose to labeled amino acids, a process depending on the operation of the citric acid cycle.

Both forms of respiratory stimulation (cationic and electrical) are highly sensitive to barbiturates and other anesthetics and it is now known that amobarbitone (Amytal) (0.5 mM) not only suppresses potassium-activated brain cortex metabolism, but it has a like effect on sodium-activated brain metabolism. The stimulated respiration is also more inhibited by ethanol and higher aliphatic

alcohols, and by chlorpromazine, than the unstimulated respiration. The suppression of cation, or electrical stimulation of neuronal respiration by anesthetics, at narcotic concentrations, may be explained in one or possibly both of the following ways: 1) inhibition of electron and energy transfer in mitochondrial metabolism, 2) inhibition of the cation movements at the neuronal membrane affecting the ADP/ATP ratio in the neuron and thereby the rate of mitochondrial respiration, which is partly controlled by the adenosine diphosphate (ADP) level.

Amobarbitone as well as chlorbutanol (Chloretone) suppresses the rate of oxidation of reduced nicotinamide adenine dinucleotide ($NADH_2$) by the cytochrome system. This result is in accordance with our conclusion made in 1941 that the site of action of anesthetics (*e.g.*, chlorbutanol) in brain respiration is located with a process playing an intermediate role between cytochrome oxidase and a flavoprotein concerned with the oxidation of $NADH_2$. As it is now well known that the biological oxidation of $NADH_2$ is accompanied, and indeed in the cell is controlled, by the phosphorylation of ADP to ATP, it follows that the anesthetic is also inhibitory to oxidative phosphorylation. The suppression of $NADH_2$ oxidation by amobarbitone has the double effect of suppressing the citric acid cycle (as the rate of this is dependent on the supply of acetyl-CoA which is formed by oxidation of pyruvate by NAD) and the formation of ATP.

The barbiturates, and other anesthetics, suppress, at low concentrations, oxidative uptake of phosphate. The interference by these drugs with ATP synthesis in the brain is shown by their suppression of acetylcholine synthesis and by their inhibitory effects (*e.g.*, those of 4 mM chlorbutanol or 1 mM pentobarbitone) on ^{32}P incorporation (from phosphate) into phosphoproteins or organic phosphorus compounds in cat brain slices respiring in presence of glucose. This is also shown very well, using rat brain cortex slices, by the suppression by amobarbitone (0.5 mM) of glutamine biosynthesis, a reaction which is ATP-dependent. The suppression of glutamine biosynthesis in isolated respiring rat brain cortex slices, whose respiration has been stimulated by increased K^+, is also shown by chlorbutanol (2 mM) and other alcohols and also by salicylates (5 mM).

The effects of these drugs on oxidative synthesis of ATP and, therefore, among other reactions, on the synthesis of acetylcholine, especially that bound by subcellular particles, to be drawn upon during the functional activity of the nerve cell, may account for a diminished rate of recovery of the cell to its normal condition. This slowing of recovery is presumably one of the factors responsible for the paralysis of the nerve cells, or of the centers specifically affected by the drugs.

Thus one way by which an anesthetic may suppress the functional activity of a neuron is by suppression of the rate of biosynthesis of substances needed for neuronal function that require mitochondrial ATP (or other high energy phosphates) for their formation.

The stimulation of neuronal respiration by increased potassium ion concentration (or diminished calcium ion concentration) in the medium bathing the isolated brain tissue is held to be due to the increased quantities of neuronal ADP

(or phosphate) that are formed under these conditions as a result of cation movements at the neuronal membrane. Increase of ADP leads to increased mitochondrial respiration and presumably higher ATP levels and hence to increased rates of mitochondrial biosynthesis dependent on mitochondrial ATP. It has been shown that, when the oxidation of pyruvate by liver mitochondria is stimulated by the addition of apyrase (which releases ADP), it is the stimulated oxidation which is more inhibited by barbiturates than the unstimulated oxidation. The barbiturate acts in this manner by its suppression of the ADP-controlled $NADH_2$ oxidation. It is possible to explain the higher sensitivity to the anesthetics of stimulated neuronal respiration than that of the unstimulated respiration on the basis that the former is largely an ADP-stimulated respiration. At the same time it is to be appreciated that brain phosphocreatine may play a role as a "buffer" substance, yielding phosphate to ADP as the concentration of ADP is increased as a result of stimulation. Thus the level of phosphocreatine falls when stimulation occurs and its "buffering" action diminishes as its content falls. When, therefore, the stimulated tissue is exposed to an anesthetic that suppresses oxidative conversion of ADP to ATP, there is considerably less phosphocreatine available to convert ADP to ATP than in the unstimulated condition and the level of ATP is not restored. Hence, the anesthetic has a greater inhibitory effect on ATP-dependent reactions of the stimulated cell than on those of the unstimulated cell.

It follows, from this point of view, that drugs affecting cation transport at the nerve cell membrane in such a way as to inhibit the effects of increased potassium ions or decreased calcium ions on the levels of cytoplasmic ADP and phosphate will also block stimulated brain cell metabolism. It is to be expected, therefore, that substances affecting ionic transport at the cell membrane will have effects on cell metabolism and function which will resemble in some ways those due to drugs which suppress oxidative phosphorylation in the nerve cell mitochondria.

Ethanol and higher aliphatic alcohols have little or no suppressing effects at anesthetic concentrations on mitochondrial respiration. They act presumably largely at the cell membrane, affecting cation transport, for they are able to suppress potassium-stimulated and electrically stimulated brain cortex respiration *in vitro*. They do not uncouple oxidative phosphorylation. It should be noted, however, that ethyl ether uncouples phosphorylation from oxidation in rat brain mitochondria, the uncoupling taking place at concentrations of ether which are present in the brain during surgical anesthesia.

At present it is reasonable to conclude that anesthetic drugs bring about the suppression of mitochondrial ATP formation by suppression of cation transport at the cell membrane, by suppression of mitochondrial oxidation of $NADH_2$, or by effects on both processes. Different clinical effects will be obtained if the drugs act optimally at different sites in the nervous system.

The effects of anesthetics on metabolism of the whole brain *in vivo* may differ from those found with brain cortex *in vitro*. The reason is that, *in vivo*, the metabolic effects of the anesthetics will be confined to those sites in the brain

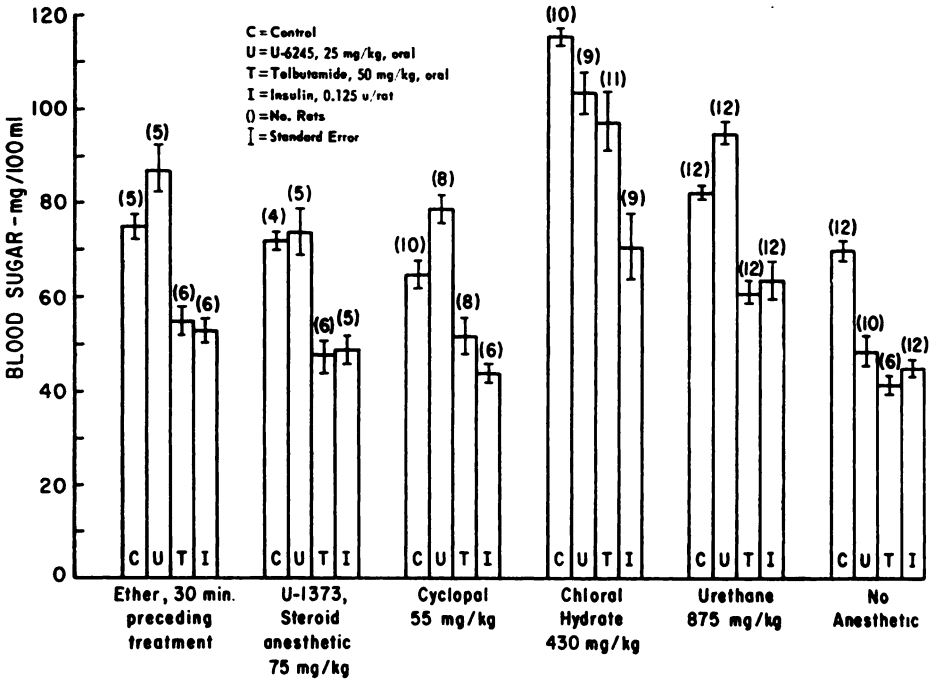

FIG. 6. Effects of various anesthetics on the hypoglycemic activity of 3,5-dimethylpyrazole (U-6245), insulin or tolbutamide in glucose-primed fasted intact rats. Anesthesia was induced prior to administration of hypoglycemic agent and blood sugars determined on blood taken two hours later.

affected in largest measure by the drug. This will result in changed cerebral activities in various parts of the brain which are no longer being stimulated by the anesthetic-sensitive sites in the brain.

It should be emphasized also that inhibitory effects on ATP synthesis in the brain cell mitochondria do not necessarily involve immediate falls in respiration. In fact the reverse may occur. But such "uncoupled" respiration can be of little value for the cell's functional activity. The manner in which the alcohols and related drugs attach themselves to sites in the cell membrane is unknown, but physical measurements, such as that of electrical impedance, may throw light on the nature of the attachment and on the physical consequences that affect ionic transport.

With liver mitochondria, the oxygen uptake may be depressed by barbiturates by more than 50% with no depression of the P/O ratio. Thiobarbiturates, however, depress the P/O ratio, a fact to be correlated with their activation of ATPase, in contrast to the lack of effect of the oxybarbiturates. Amobarbitone suppresses the activity of a ouabain-sensitive cardiac microsomal ATPase (in presence of Mg^{++}, Na^{+}, and K^{+}) but only at concentrations exceeding 2 mM, 50% inhibition occurring at 4 mM. The fact that amobarbitone, at concentrations that abolish cationic or electrically stimulated brain cortex respiration, has but

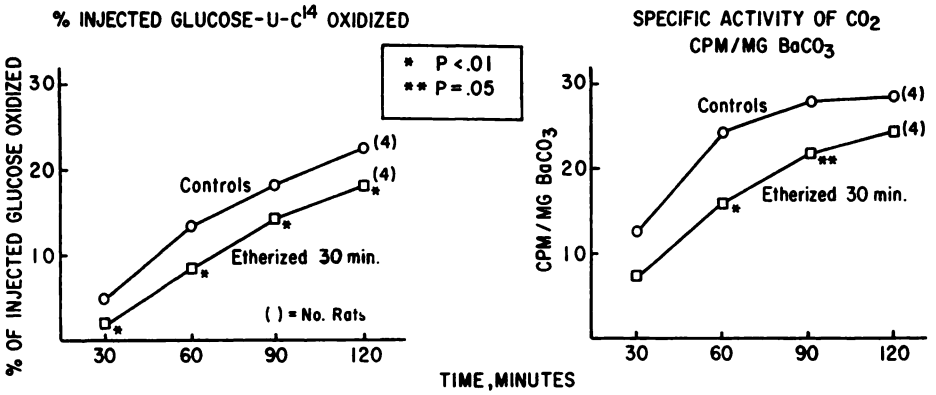

FIG. 7. Effects of ether anesthesia (30 minutes) on oxidation of glucose-U-C^{14} by fasted, intact rats. Body weight 150 g.

little inhibitory effect on the ATPase which has been implicated in the active transport of Na^+ and K^+ across membranes, throws doubt on the suggestion that the drug operates solely at low concentrations on sodium or potassium active transport at the cell membrane. Moreover, if this view were true, it would be expected that the effects of 0.5 mM amobarbitone on metabolism of brain cortex *in vitro*, examined in standard physiological media, would resemble in some respects those of ouabain that suppress the activity of the sodium pump. Yet one of the most marked features of the action of ouabain (10^{-6} M) on brain cortex metabolism is its suppression of active amino acid transport, leading to efflux of amino acids from the tissue. Amobarbitone at 0.5 mM has no such effect except under stimulated conditions where the rate of cell respiration and the ATP level have diminished in value.

Dr. W. E. Dulin: It was observed during studies on the mechanism of the hypoglycemic action of 3,5-dimethylpyrazole, nicotinic acid and pyrazinamide, that various anesthetics completely inhibited the blood sugar-depressing activity of these compounds. Figure 6 shows that all of the anesthetics examined had the same effect on one of these compounds (3,5-dimethylpyrazole) but did not block the activity of insulin or tolbutamide.

We were interested, therefore, in attempting to explain the inhibitory effect of anesthetics on the hypoglycemic activity of the pyrazoles. In order to gain some insight into this, we studied the effect of anesthetics on the ability of intact rats to oxidize glucose-U-C^{14} to $C^{14}O_2$. Ether caused a significant decrease in oxidation of glucose-U-C^{14} by intact rats (Fig. 7). A barbiturate, Cyclopal sodium, 5-(1-cyclopenten-2-yl)-5-allylbarbituric acid, also was observed to inhibit glucose oxidation.

Another possible mechanism by which anesthetics could antagonize the hypoglycemic action of these agents was to inhibit the transport of sugar into the intracellular compartment. It was found that barbiturate anesthesia had a marked inhibitory effect on the ability of galactose-1-C^{14} to gain access to the intracellular water of both muscle and brain tissue (Table 1). This observation

TABLE 1

Effect of anesthesia on intracellular distribution of galactose-1-C^{14} in muscle and brain

	Muscle[a]	Brain[a]
Unanesthetized	3.75×10^2	13×10^3
Cyclopal anesthesia (2 hr)	0[b]	9.1×10^{3b}

[a] cpm/cc tissue water.
[b] $P < .001$.

TABLE 2

Equivalent anesthetic effects of gases in isolated excitable tissues

Gas	Total Fiber Blockade	Concentration of Gas in Nonaqueous Phase
Helium	—	
Nitrogen	—	
Argon	340 atm	3.0×10^{-1}
Methane	110	2.3
Sulfur hexafluoride	None	—
Krypton	54	1.4
Difluoroethylene	15.5	1.4
Xenon	12	1.0
Nitrous oxide	13	1.4
Ethylene	12	1.5
Difluorodichloromethane	4.7	2.3
Cyclopropane	1.9	1.78
Difluoroethane	2.0	1.23

suggests that the inhibition of glucose oxidation produced by anesthetics is at least in part due to a block in transport.

EFFECTS OF ANESTHETICS ON METABOLISM OF PERIPHERAL NERVE

Dr. F. G. Carpenter: The myelinated axons that are located peripheral to the nervous system are not particularly sensitive to anesthetic agents. At concentrations some 20 to 30 times those employed for surgical anesthesia, the barbiturates, for example, will reversibly abolish the electrical responses of the entire population of myelinated fibers in a mixed nerve trunk. This property of anesthetics to block conducted action currents in nerve fibers is shared also by a large number of gases (Table 2). Some of these are well known clinically, such as cyclopropane, ethylene and nitrous oxide, while methane or krypton usually is not associated with depressed states of the nervous system under ordinary conditions. Two of the three fluorocarbons in the table have been shown to produce seizures in animals at pressures less than 1 atmosphere. In some cases the blockade can be demonstrated only at pressures considerably in excess of atmospheric. For example, over 300 atmospheres of argon are required to produce an effect equivalent to that achieved by less than 2 atmospheres of cyclopropane.

Therefore, this preparation may provide an opportunity to search for a mechanism by which a variety of chemically unreactive gases can reversibly depress axonal conduction. Blockade may arise from a generalized depression of many cellular functions, in contrast to the more specific disruption of certain brain stem functions during surgical anesthesia. Nevertheless, the actions of an anesthetic on the two systems may share something. At the characteristic blockade pressure of each gas a nearly equal number of gas molecules is distributed in a nonaqueous medium. In this case the axon-blocking potency of each substance correlates well with its solubility in benzene. Nevertheless the mechanics of the actual blockade process cannot be revealed by this correlation (Table 2).

A compound spike recorded from the surface of a mixed nerve varies in amplitude in proportion to the number of active fibers beneath the recording electrode. The population of myelinated fibers in the trunk is not uniformly depressed by a given quantity of an anesthetic like nitrous oxide. At 4 atmospheres of this gas, that portion of the spike contributed by small diameter fibers is abolished while the response of the larger fibers is not affected. At 10 to 13 atmospheres the entire myelinated fiber population is no longer excitable. In Figure 8 we see this effect on the compound spike when 10 atmospheres of nitrous oxide are rapidly admitted to the chamber. The spike height was diminished by 50% in 30 seconds and was totally extinguished in about 3 minutes, and recovery after the gas was expelled was of the same order of time. This is probably related to the diffusion of gas through the nerve trunk. If the preparation was maintained for an hour or so under these blockade conditions, recovery was not prolonged. Whatever is responsible for the loss of axonal excitability is not a cumulative process and the factors responsible for sustaining propagated responses do not "run down" during blockade. In this connection, recovery after blockade by these anesthetics is remarkably different from recovery after anoxia or substrate deprivation.

When transmission is blocked by these gases, the polarization of the nerve fibers has been found to diminish. Polarization is dependent upon energy sources supplied by the oxidation of carbohydrate. Certain metabolic inhibitors not only interfere with the polarization of nerve fibers but they substantially reduce their resting oxygen consumption. Tashiro was one of the first to show that the CO_2 production of peripheral nerve trunks at rest is diminished by anesthetic agents. In another study, conducted by Sherif, the local anesthetics, cocaine and procaine, were found to inhibit markedly the oxidative metabolism of sciatic nerves taken from rabbits. However, this property of an agent to depress the resting oxygen uptake of a nerve trunk was not unique to local anesthetic agents. Furthermore, urethane in amounts adequate for nerve block produced no significant metabolic depression. Chlorbutanol (Chloretone) and pentobarbital are known to reduce the resting utilization of oxygen by a nerve trunk at concentrations that render the myelinated fibers inexcitable. But these agents do not interfere with the polarization of the fibers under blockade conditions. For this reason it is important to know whether the anesthetic gases as well could alter the energy-yielding reactions in nerve.

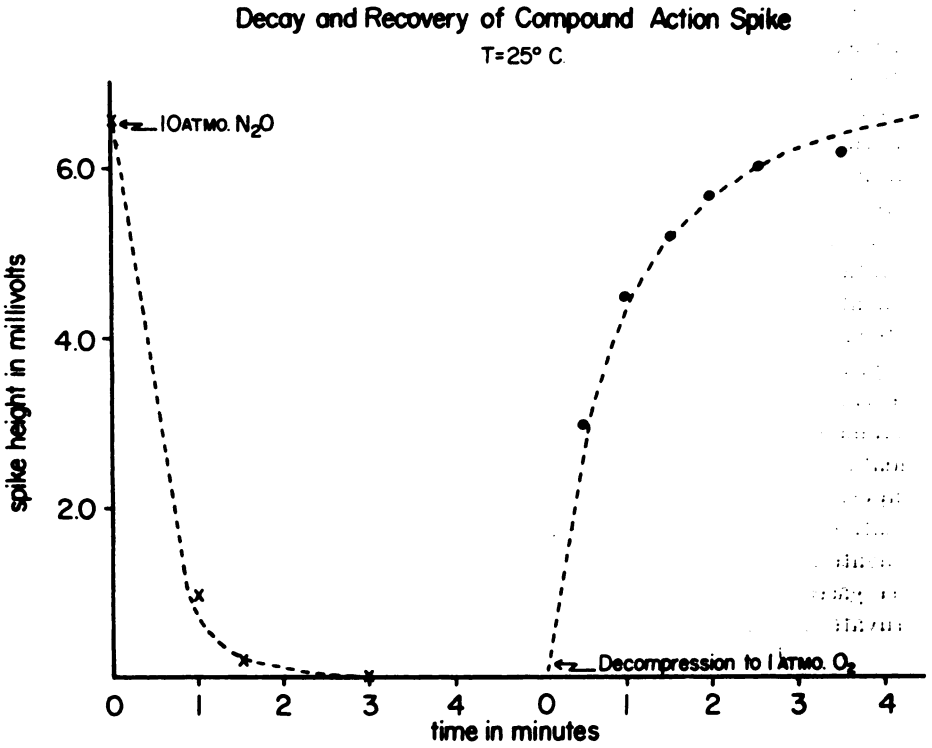

FIG. 8. Originally appeared in the American Journal of Physiology (200: 187, 1961); reprinted with permission.

The oxygen uptake of mixed nerve trunks taken from rats was measured in the presence of either 13 atmospheres of nitrous oxide, 12 atmospheres of xenon, or about 2 atmospheres of cyclopropane. These were compared with their oxygen uptake in the presence of an equal pressure of nitrogen, which displays no blocking effect on nerve trunks. In addition to the gases, phenobarbital and chlorbutanol also were employed as blockade substances to provide a measure of the effects on nerve of agents which are known to interfere with the metabolic reactions under study. The latter substances were added in specific amounts to the Ringer medium in which the nerve preparations were suspended. The resting oxygen consumption of the nerves was measured by the conventional Warburg manometric method but this method could not be employed to measure nerve respiration at pressures greater than atmospheric. For this purpose a differential respirometer was constructed, capable of operating at pressures 20 to 30 times atmospheric.

The measurement of oxygen uptake by nerve under 10 atmospheres pressure is not accomplished with quite the same precision as by the Warburg apparatus. Control values obtained with the apparatus filled to this pressure with nitrogen-oxygen mixtures were within the range of control values furnished by the Warburg respirometer. At 12 atmospheres, xenon completely abolished all conducted responses in the myelinated fibers and inhibited the oxygen utilization of nerve

by 57% (Table 3). Equivalent amounts of cyclopropane or nitrous oxide diminished the respiration of the nerves by 50% and 65%, respectively. If the latter gases were added in amounts which did not effect total blockade, the reduction in oxygen consumption was proportionately less (Table 3).

Pyruvate occupies a key step in the enzymatic degradation of carbohydrate, and its oxidation by the tricarboxylic acid cycle accounts for about 90% of the energy derived from glucose. The effect of anesthetics upon pyruvate uptake by rat nerve was measured to provide further information concerning the alteration of neuronal metabolism during blockade. The disappearance of pyruvate from a Ringer medium containing a sciatic nerve trunk proceeds quite slowly and is not related to its initial concentration. For each micromole of pyruvate removed by the nerve, 6 micromoles of oxygen were consumed. The complete oxidation of 1 micromole of pyruvate would require only 2.5 micromoles of oxygen. Therefore other endogenous substrates were undergoing oxidation more extensively. It is not at all certain that the pyruvate that did disappear was necessarily oxidized. Nevertheless, since pyruvate sustains the oxidative metabolism of nerve after glycolysis is inhibited by NaF, at least pyruvate added in this manner can be utilized along oxidative pathways. The three gases employed in this study failed to inhibit the disappearance rate of pyruvate in a manner comparable to chlorbutanol or phenobarbital (Table 3).

TABLE 3

	COMPLETE BLOCKADE OF AXONS		PYRUVATE DISAPPEARANCE		OXYGEN CONSUMPTION	
	INCIDENCE	No. OBSERV.	INHIBITION	No. OBSERV.	INHIBITION	No. OBSERV.
PHENOBARBITAL						
5 mM./L.	1	7	30%	7	27%	4
10–15 mM./L.	8	9	62%	7	63%	6
CHLORETONE						
1.5 mM./L.	0	7	—	—	37%	5
3–5 mM./L.	5	6	30%	4	85%	4
NITROUS OXIDE						
5 ATM.	0	6	—	—	35%	5
10–13 ATM.	9	10	5%	5	65%	5
XENON						
12 ATM.	2	2	—	—	57%	3
CYCLOPROPANE						
0.8 ATM.	0	4	—	—	24%	8
1.7–2.2 ATM.	6	6	5%	5	50%	6

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The metabolism of this substrate was significantly reduced by the latter anesthetics in amounts which did not abolish impulse transmission.

The depressant effects of these anesthetic agents on the electrical responses of axons cannot be attributed to an alteration of the same metabolic processes. Both the oxygen consumption and the pyruvate utilization by nerve trunks were inhibited by chlorbutanol or by phenobarbital in amounts sufficient to abolish impulse propagation in myelinated nerve fibers. No such effect was found for the gases. Perhaps their interference with the polarization of the nerve fibers is primarily responsible for blockade, while the reduction in oxygen consumption associated with this inexcitable state is of secondary importance. This conclusion must be offered with reservation. It is quite easy to detect alterations in the electrical responses of axons through the use of sensitive electronic devices. However, it is as yet impossible to discriminate between the metabolism of the components in the nerve trunk which elicit these responses and the metabolism of the other nonconducting or supporting elements.

Dr. M. G. Larrabee: For a number of years, with a number of colleagues, I have been studying the metabolism of sympathetic ganglia from several points of view. The measurements, performed on superior cervical ganglia excised from rats and rabbits, have included oxygen uptake, glucose uptake, lactate output, the conversion of labeled glucose to labeled CO_2 and lactate, the synthesis of lipids,[4] and most recently the synthesis of RNA (55–57). An excised ganglion is unique among preparations of mammalian nervous tissues, in that it includes cell bodies and synapses whose activity is not only under experimental control but can readily be induced and measured by simple electrophysiological techniques. The usefulness of sympathetic ganglia for studies on anesthetics became apparent when it was found that certain of these agents will selectively block synaptic transmission through a ganglion in concentrations similar to those occurring in the blood during general anesthesia (29, 53, 59). Moreover, effects on transmission can be measured simultaneously with the metabolic measurements, thus dispensing with the familiar problem encountered in metabolic studies on excised brain preparations, of choosing the appropriate concentration of an anesthetic for comparison with effects on mental processes in the intact organism.

Most of our results with anesthetics have previously been summarized (54, 55). Selected data are shown in Table 4. This table gives the rate of various metabolic processes in the presence of that particular concentration of an anesthetic which just suffices to reduce the action potential, elicited in the postganglionic nerve by stimulation of all the preganglionic fibers, to half the height observed before applying the anesthetic. This action potential is a measure of the proportion of cells which can still discharge a nerve impulse in response to trans-synaptic excitation. By selecting the condition of 50 % reduction in the response, a concentration is automatically chosen in which substantial depression of neuronal

[4] Previously unpublished results on lipid metabolism reported in this paper were supported by Research Grant No. NB-00702 from the National Institute of Neurological Diseases and Blindness, U. S. Public Health Service.

TABLE 4

Metabolic effects of concentrations reducing transmission by 50%

	Rate of Uptake as % of Control			
	A Oxygen rabbit	B Oxygen rat	C Glucose rat	D Lipid P rat
Chloroform	100			100
Ether	100	103		100
Pentobarbital	100	68	164	90
Chloretone	97	60	160	
Urethane	94			55
Ethanol	100			60
Hexanol		86	92	
Octanol	100			
References	(52)	(26)	(26)	(58)

function is produced, but excessive concentrations are strictly avoided. With excessive concentrations considerable but possibly irrelevant metabolic depression is always observed. It is clear from Table 4 that function could be depressed in rabbit ganglia without significant effect on oxygen uptake, by all agents tested (column A), and that agents could be found which would depress transmission through rat ganglia without depressing any particular one of the metabolic measures employed (columns B, C, and D). A possible conclusion is that those metabolic changes which are sometimes caused at these appropriately selected concentrations may result from coincidental effects not related to the cause of the interference with impulse discharge.

Since the observations on lipids given in column D of Table 4 have not previously been published, I would like to describe them in somewhat more detail. They were made with the collaboration of William S. Leicht (58). Superior cervical ganglia were excised from rats and incubated at 37° for 4 hours in a bicarbonate-buffered physiological solution, to which was added inorganic phosphate labeled with ^{32}P along with various concentrations of anesthetics. In the case of ether and chloroform a constant concentration was maintained by continually passing through the incubation vessel an appropriate mixture of two streams of 95% O_2–5% CO_2. One stream had been saturated with the anesthetic vapor at 0° by bubbling through the liquid anesthetic in an ice bath. The postganglionic response to preganglionic nerve stimulation was observed throughout the incubation. At the end of incubation the phospholipids were extracted and resolved by paper chromatography, and their ^{32}P content was measured with a Geiger counter. Further procedural details were as described elsewhere (56, 57). The particular lipids measured were phosphatidyl inositol, phosphatidyl choline, phosphatidyl ethanolamine, and phosphatidic acid. Since

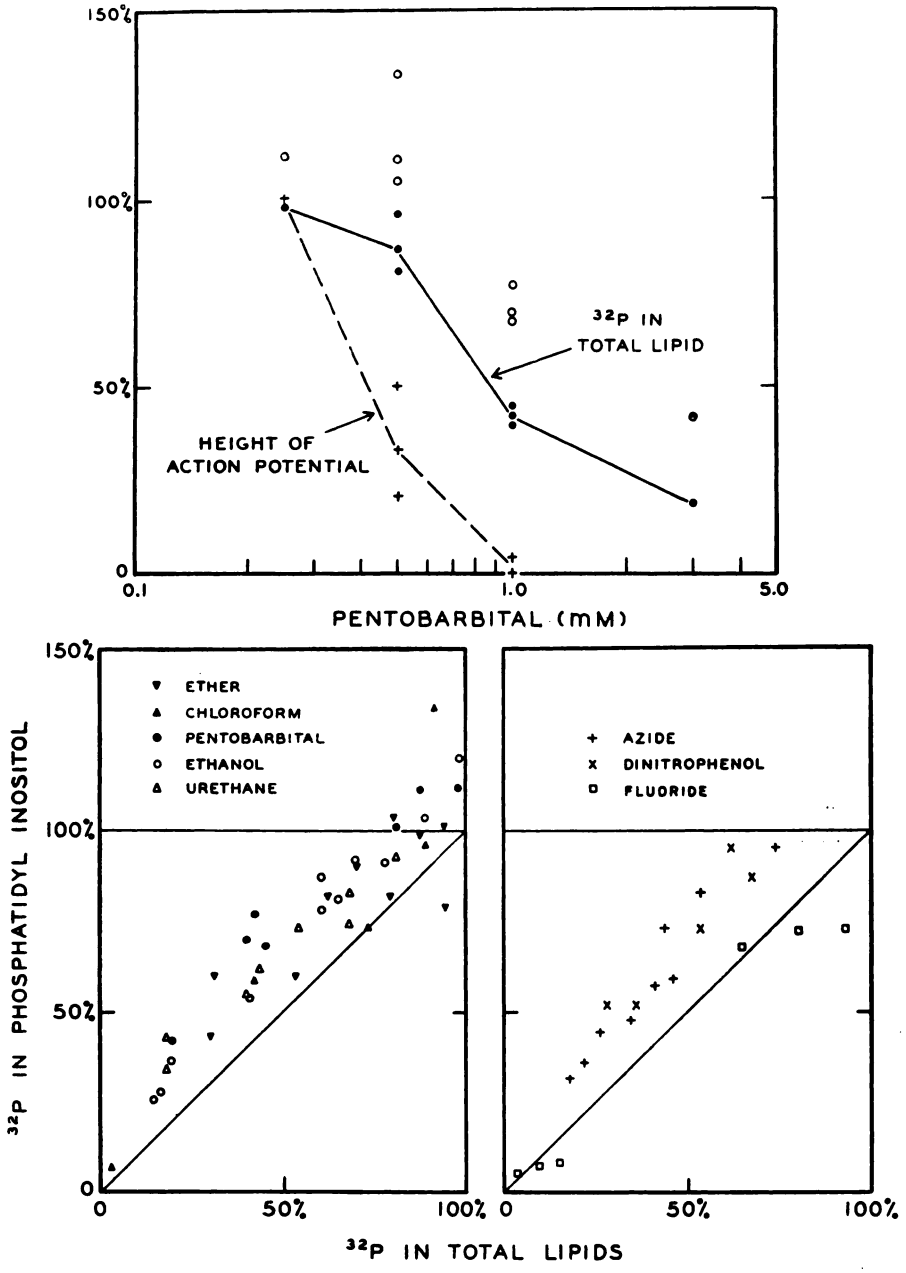


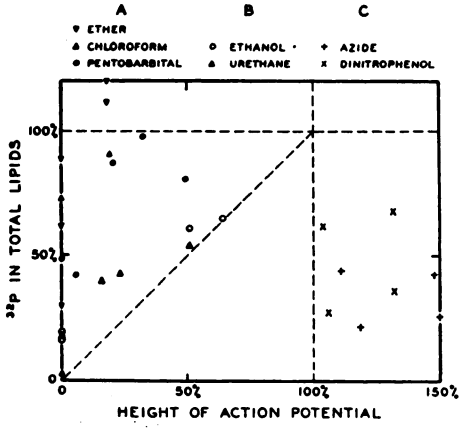
FIG. 9 (*above*). Effects of various concentrations of pentobarbital on synaptic transmission and lipid metabolism in an excised rat ganglion. Each is expressed as percent of its magnitude in the absence of the anesthetic. *Crosses:* Height of the action potential in the postganglionic nerve elicited by a supramaximal stimulus to the preganglionic nerve. *Filled circles:* ^{32}P incorporated into phospholipids (= sum of phosphatidyl inositol, phosphatidyl choline, phosphatidyl ethanolamine and phosphatidic acid) during 4 hours of incubation with inorganic phosphate-^{32}P. *Unfilled circles:* ^{32}P phosphate similarly incorporated into phosphatidyl inositol.

FIG. 10 (*below*). Effects of anesthetics (left) and other metabolic inhibitors (right) on ^{32}P labeling of phosphatidyl inositol (ordinates) compared to effects on labeling of total phospholipids (abscissae).

these were the only ones identified, their sum is herein referred to as "total phospholipids," although there may have been small amounts of ^{32}P in other phospholipids, since some radioactivity remained at or near the origins of the chromatograms. Sphingomyelin, phosphatidyl serine, and cardiolipin, if present, did not become detectably labeled under the conditions of these experiments.

Results with pentobarbital are summarized in Figure 9. At a concentration of 0.5 mM, the height of the postganglionic action potential was reduced to about 30% of the control, but there was only a slight reduction in the labeling of the total phospholipids. It is very doubtful whether the small reduction in lipid labeling could account for the large depression of response, especially since large reductions in lipid labeling can be caused by azide and dinitrophenol without impairing the response (see below). At sufficiently high concentrations, the metabolic effect was considerable. The depression was not uniform on all four of the lipids under consideration, for the labeling of phosphatidyl inositol, shown by the unfilled circles in Figure 9, was always less depressed than was that in the total lipids. In fact, there was an actual stimulation of the labeling of the inositide at the lower concentrations.

The smaller depression of the labeling of phosphatidyl inositol compared to that of the other phospholipids was a general characteristic of the anesthetics studied and of two out of three conventional metabolic inhibitors (Fig. 10). The results suggest that stimulation of phosphatidyl inositol may be caused by these various agents, being revealed by an increase in labeling when just the right low concentration is by good fortune employed, and by the smaller depression of its labeling compared to that of other lipids at higher concentrations.



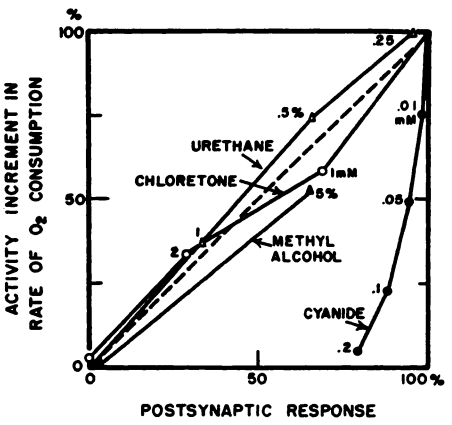


FIG. 11 (*left*). Effects of anesthetics and inhibitors on phospholipid metabolism, plotted against their effects on synaptic transmission.

FIG. 12 (*right*). Effects of anesthetics and of cyanide on the increment in the rate of oxygen uptake caused by activity (ordinates) and on the height of the postganglionic action potential (abscissae) in an excised rabbit ganglion. The preganglionic nerve was stimulated supramaximally about 10 times per second. Both variables are expressed as percent of their values in the absence of the experimental substances (37).

This inositide has also been found to be unique among the other lipids in that its labeling is increased by stimulation of the preganglionic nerve (56). This latter effect, observed in the absence of anesthetics, is apparently associated with synaptic events, since it was abolished by tubocurarine and could not be reproduced in nerve trunks (57).

Comparison of the effects of several anesthetics on ganglionic transmission with depression of total lipid labeling is shown in Figure 11. In addition to pentobarbital, it was found that ether and chloroform also depressed transmission without interfering with lipid labeling, although all of these agents depressed the labeling at high enough concentrations. Ethanol and urethane, on the other hand, depressed transmission and lipid labeling roughly in proportion to one another. In sharp contrast with the anesthetics were two metabolic inhibitors, azide and cyanide, which considerably reduced the labeling with an accompanying increase rather than a decrease in the postganglionic response.

My remarks up to this point were intended to emphasize the need for simultaneous observation of the functional and metabolic effects in experiments directed to elucidating their interrelationships. In addition it is essential either to regulate or to measure the amount of neuronal activity. This is indicated by the correlation between activity and metabolism shown in Figure 12. In this case the observations were made while the preganglionic nerve fibers were kept in a continual state of activity by repetitive electrical stimulation. Under these conditions the postganglionic response and the metabolic rate declined together as the concentration of an anesthetic was increased. A reasonable interpretation is that the anesthetics acted by interfering with synaptic transmission and that the decline in oxygen uptake was a result rather than a cause of the altered neuronal response. This is possible because the rate of oxygen uptake is known to decrease promptly in a sympathetic ganglion when the rate of neuronal activity is decreased (51, 55). Unless the nerve cells in a preparation are known to be at rest, it is thus not possible to conclude that the initial effect of an anesthetic is metabolic from the mere observation that metabolism is reduced. Special caution is required, for example, in interpreting experiments on excised brain tissue in which metabolism has been stimulated chemically, for example by increasing potassium concentration, before adding the anesthetic (37).

Finally, it should be recognized that although it has not seemed possible to establish the metabolic theory of anesthetic action, neither has this hypothesis been disproved. An obvious difficulty arises from the selective action of those anesthetics which are of practical importance in depression of central nervous functions. We found, for example, that selectivity, as measured by the ratio of the concentration required to block axonal conduction to that required to block synaptic transmission, increased with the molecular weight of the anesthetic agent. This was observed on a perfused sympathetic ganglion in the cat (59). Selective action implies localized action on some synaptic structure. Obviously a sizable but localized metabolic effect might be obscured amidst the total metabolism of a whole tissue.

It appears that more ingenious experimentation than that yet devised will be required to test the important metabolic hypothesis of anesthetic action.

EFFECTS OF ANESTHETICS ON MEMBRANE PHENOMENA

Dr. R. E. Taylor: I suppose that I should feel somewhat out of place here because it is quite clear that nothing I am going to talk about has anything to do with metabolism.

I would first like to read a sentence from a paper by Ralph Lillie published in 1916. He stated: "In anesthesia it is to be assumed that the membrane is so altered that it fails to respond to a change in its electrical potential by an increase in permeability." We may ask, "Permeability to what?" For at least one membrane and a couple of agents we can answer this question.

In the late nineteenth century Hermann suggested that the electrical activity in nerve occurred across an outer limiting membrane of high resistance. In 1902, Bernstein further suggested that in the normal resting state this membrane was selectively permeable to potassium ions. This would serve to provide a resting membrane potential because of the high internal and low external potassium ion concentration. Bernstein postulated that during activity the membrane lost its semipermeable properties and the potential would fall to a low value. As Hodgkin has noted in hit recent book. the remarkable paper by Overton on the part played by sodium ions in excitability appeared in the same volume of *Pflügers Archiv* as did Bernstein's paper.

I would like first to write down one way of making a quantitative statement of Bernstein's hypothesis. It is not unique, but useful. For the potassium ion we can write that the current (I_k) which flows from one phase to another is some function ($\hat{g}_k$) which we do not know, times the difference of the potential between the phases (E_m) and the potential at which no current flows (E_k), *i.e.*, $I_k = \hat{g}_k(E_m - E_k)$.

We have not said anything about the system, but we can use this kind of formulation, where G has the units of a conductance and is what we are trying to determine experimentally. Thermodynamics tells us that $E_k = (RT/F)\ln \cdot (C_1/C_2)$, the so-called Nernst potential, where R is the gas constant, T the absolute temperature, F the Farady and C_1 and C_2 the concentrations (actually activities) of the ions in the two phases.

We may represent the equation for the current carried by potassium ions by a diagram (center branch of Fig. 13). The diagram and the equation say the same thing in different languages.

If another ion, say sodium, is involved in carrying current and if the sodium and potassium ion currents do not interact directly but only through their effect on the total potential we merely add another parallel branch to our diagram containing $\hat{g}_{Na}$ and E_{Na}. For completeness, we add the parallel capacitance (Fig. 13) which exerts its effect as part of the equivalent electrical circuit but takes no active part in the electrical activity.

The most accurate and significant measurements of the electrical parameters of cell membranes were the result of the discovery of the half millimeter diameter single nerve fiber obtained from the squid. From 1936 to 1942, Cole, working with Curtis and with Baker, measured the conductance with considerable accuracy and showed that during activity the total conductance increased some 25-fold.

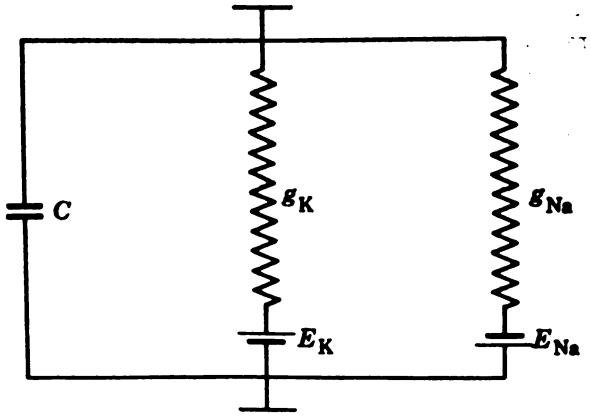


FIG. 13. An equivalent electrical circuit diagram for a membrane with capacitance and selective permeabilities to sodium and potassium ions.

Thus, one part of Bernstein's hypothesis was confirmed. The striking failure was that the potential across the membrane did not fall to a low value during activity but actually reversed in sign, as shown by Curtis and Cole, and by Hodgkin and Huxley in the same year.

In 1947, Cole and Marmont succeeded in eliminating the propagation of the action potential in squid axons during activity by the insertion of a long axial wire which essentially short-circuited the interior of the cell and eliminated the possibility of propagation as well as most of the other spatial variations in potential which made it so difficult to analyze earlier data. They further added other electrodes and connected them in such a way that they could electronically control either the voltage or the current, and measure the other. This experimental attack formed the basis of the work of Hodgkin, Huxley and Katz, who made a large number of measurements of the kind shown in Figure 14.

This shows the current through a piece of membrane with spatially uniform controlled potential as a function of time following a sudden decrease in the membrane potential. There is a rapid but transient phase of inward current followed by a rather steady outward current.

Hodgkin and Huxley analyzed data of this kind in a very remarkable way and arrived at a set of empirical equations which not only describe the behavior when the nerve is in the space and voltage clamp set up, but also predict the shape and velocity of the propagated action potential in the absence of the internal electrodes. As you may know, they won the Nobel prize for this in 1963.

Hodgkin, Huxley and Katz had identified the early transient current during a potential step as being carried by sodium ions and the late steady outward current as being carried by potassium ions. During ordinary propagation, the resting membrane ahead of an advancing action potential is depolarized by electrotonic spread. The initial effect is to decrease the resistance to the flow of sodium ions. Since external concentration of sodium ions is large compared to the internal, sodium ions flow inward. This depolarizes the membrane further,

which in turn further reduces the sodium resistance. This regenerative process results in a reversal of the membrane potential. The process is transient, however, both because the sodium permeability increase on depolarization declines spontaneously, and because the increase in potassium permeability on depolarization tends to drive the potential back to the resting value. As the potential returns to the resting value, both the sodium and the potassium permeabilities return to the resting values.

The net result of the passage of an action potential is that a little bit of sodium has gone into the fiber and a little bit of potassium has gone out. Metabolically coupled ion pumps are required in order to maintain the internal ion concentrations at the desired levels. As far as we know, this is the only point at which metabolism enters the picture.

It is now possible to very simply describe the action of procaine. In Figure 14 we see the currents flowing as a function of time following a depolarizing potential step, before, during and after the application of 0.1 % procaine. Both the initial transient, and the late steady state currents are decreased by the procaine. Analysis of data such as these shows that the permeability increase to sodium ions on depolarization is reduced by the procaine and that the effect is quite adequate to explain the blocking action of the drug. The permeability increase to potassium on depolarization is also reduced by procaine. This effect is in the direction to antagonize the blocking action, but it is not large enough to prevent it.

Figure 15 shows a sample of the effect of an alcohol. It is similar to that of procaine on the sodium currents, but affects the potassium currents very little. This figure is from a series of measurements by Binstock and Armstrong on the effects of a homologous series of alcohols. The relative effects of alcohols on the sodium and potassium current-carrying systems are concentration-dependent and are different for different alcohols.

Other agents may act in different ways. The details surely vary from one species to another, but for the giant axon of the squid and probably for most peripheral nerves, the action of the usual local anesthetic agents is just as Lillie said it was, that is the relation between the membrane potential and ion permeabilities is altered. There are no indications that metabolism enters the picture except for the important role of literally recharging the battery by active transport of ions, and no effect of these agents on this process has been reported.

Dr. L. J. Mullins: The observations just reported by Dr. Taylor concerning the effect of procaine or alcohols on the squid giant axon can be summarized by saying that they depress mainly the Na^+ current and thus prevent electric excitation. A great deal of recent electrophysiological analysis is clear in showing that excitation in peripheral nerve is independent of metabolism and of those processes that restore ionic concentration gradients. This may not be at all true in the CNS, and excitation there may be much more directly dependent upon metabolism than it is in large nerve fibers.

Calcium ions, on the other hand, have a different effect. They change the rate or voltages at which these things turn on. Therefore, they seem to be acting on

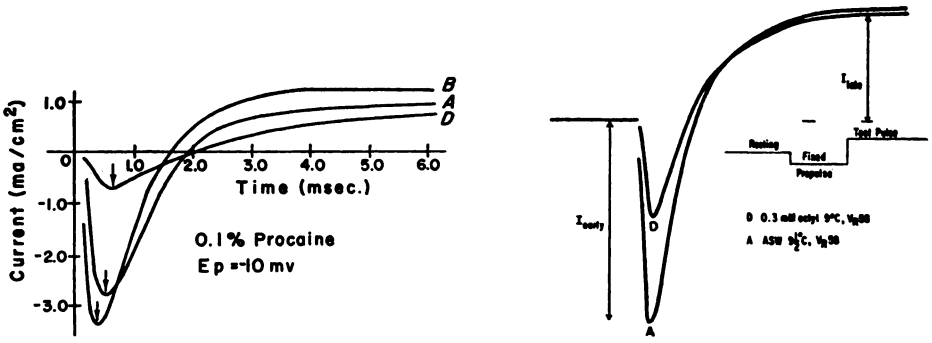


FIG. 14 (*left*). Current through the membrane of the squid giant axon following a depolarizing voltage step (outward current upwards). D: During application of 0.1% procaine. B, A: Before and after. (Reproduced by permission: Amer. J. Physiol. **199**: 1071, 1959.)

FIG. 15 (*right*). Current through the membrane of the squid giant axon following application of depolarizing test pulse. D: During application of 0.3 mM octyl alcohol. A: After recovery in artificial sea water. V_R: Resting membrane potential 58 millivolts.

the turn-on mechanism, rather than on the current itself. This suggests to me a notion that I put forth at another conference, namely, that the anesthetic gets into the channels in the excitable membrane where the currents move, and makes the ions move more slowly than they would ordinarily move; the effect is to reduce the mobility of the ions in the membrane. The reason that potassium current isn't so affected, at least initially, by low concentrations is simply that, while the sodium current is flowing, it tends to sweep out transiently some of the anesthetic molecules, and makes the oppositely directed current unimpeded for a moment, at least.

But now to metabolic effects: we have done a number of experiments measuring resting sodium influx into squid nerve by use of tracers, and these experiments show what the electrical measurements of Dr. Taylor confirmed, that this resting sodium movement is cut down by a variety of alcohols. This means, of course, that as less sodium is leaking, there is less need to pump it out, and therefore, the metabolic drain on the energy-consuming system that has to extrude sodium is less. I don't find it a great extrapolation to propose that in a very much smaller cell, such as a CNS cell, with a very much leakier state, quite possibly outward pumping of sodium is the main energy drain if the cell is to have excitability, and that therefore if one puts in an anesthetic agent, it simply cuts down on this resting inward sodium leakage, and thereby relieves the biochemical mechanisms of the necessity for performing more metabolic work.

Well, that is all I have to say about the nerve excitation, and what I would like to talk about are models which duplicate the properties of anesthetic receptors, if we could call the sites in the membrane where anesthetics appear to react anesthetic receptors.

One of the things that is in a rather tidy state is our ability to predict whether a particular compound will be anesthetic or not if we know about its physicochemical properties. If we have some measurements of vapor pressure and heat

of vaporization, and a few other easily determined physical properties, I think that we can make a rather good estimate of the dose that will be required for anesthetic action.

Prediction does not work out so well if the molecule happens to be a very large molecule, because as a molecule gets larger and larger, it gets chancier and chancier about whether it will be anesthetic, and this, I believe, is because the site into which these molecules must be introduced, in order to produce the action that they do produce, is one of limited volume, and it can accommodate only molecules below a certain size. As we don't know precisely what that size is, and only can hazard guesses, I have been most interested in trying to develop a working model that would duplicate the properties of accepting only anesthetic molecules of limited size, and achieving some kind of equilibrium between them.

This is sort of a simple-minded kind of notion and it obviously requires a very simple-minded kind of approach. I wanted some kind of porous structure where the pores were about 4 Angstroms in radius, and I didn't quite know how to get it. But one of the things that you think about is something like a crystal of sodium chloride. It has sodium and chloride atoms in it whose size we know, and then there is some space left between, because of the electron clouds surrounding sodium and chloride atoms which are spherically symmetrical, so there are little spaces in the crystal lattice where there is nothing. These spaces, unfortunately, are much too small for one to be able to get anything else into the sodium chloride lattice, but if one makes the sodium and chloride atoms 100 times bigger than they really are, then the spaces will grow correspondingly, and maybe such spaces would be the right size for the sort of thing that we want as a site model.

Hemoglobin is a protein molecule that is something like 100 times the size of sodium and in a crystal such a molecule must be expected to have spaces between its atoms of considerable size. Such spaces are normally filled up with water molecules. Our experimental arrangement attempted to fill up these spaces with something else, namely with anesthetic molecules.

Our experimental arrangement was an air-tight container with an electric balance inside. On the balance pan were a few milligrams of hemoglobin crystals. The weight of the crystals could be read on a recorder outside the chamber. When an anesthetic vapor such as ethyl ether was introduced into the chamber, the crystals gained weight, and at equilibrium with saturated ether vapor there were something like 50 ether molecules per molecule of hemoglobin. With benzene, a smaller number of molecules are taken up from the saturated vapor, and with naphthalene there was no weight gain. This latter finding suggested that indeed the model was performing as desired because it was excluding a molecule as large as naphthalene. Other experiments have shown that purely paraffinic liquids such as pentane are not taken up, while ethyl ether is taken up—since both substances have similar vapor pressures, the phenomenon is not dependent upon this parameter. This investigation has been extended to other sorts of proteins such as crystalline egg albumin, and to mercaptalbumin. It has also been concerned with the kinetics of uptake of various substances. One of the reasons we believe that the uptake of anesthetics by protein crystals is dependent

upon the transfer of the anesthetic to the intracrystalline water is that while the uptake of ether is rapid, that of chloroform is slow. Both molecules reach high concentrations in the crystal at equilibrium.

While the type of model described may not duplicate all the properties required in order to explain anesthetic action, it does appear to show some of the properties that any site-selective model should have. So I would conclude that at least these kinds of studies may offer some clue as to why large molecules are not anesthetic; and further than that, we intend to look at the alteration in X-ray diffraction structure that is produced in these proteins by the changing over of their internal structure from essentially an aqueous one to one in which the anesthetic molecules are absorbed.

CHARACTERIZATION OF RECEPTORS FOR ANESTHETIC MOLECULES

Dr. R. M. Featherstone: As I have listened to these very impressive talks this morning, I have the impression that we are all sitting around the bottom of a large mountain, and each of us has assumed the job of starting a rapid transit system of some kind, so that we are all tunneling into this mountain. Some of us are going to meet one of these days, and some of us may not meet at all, but I think Dr. Mullins and I are tunneling in a parallel fashion or maybe in the same hole.

The subject of this session is metabolism, and I assume we are to consider possible metabolic changes in anesthetic agents as well as metabolic changes in biological systems brought about as a result of the presence of anesthetic molecules. These topics are intimately related to the field of study we call "mechanisms of action of anesthetics." I believe the subject I wish to discuss is basic to all of these, but our knowledge in this area is still so primitive that we cannot easily distinguish when the term "metabolism" or "mechanism" should be used descriptively.

One can find in the literature a number of correlations of one or more sets of physical properties with anesthetic activity. These have been helpful in a predictive way. Dr. Mullins and I are members of this fraternity of correlators. We have the distinguished company of Linus Pauling, Stanley Miller and others. The predictive value of further studies of this type may be additive. However, the approach my colleagues and I at the University of California have taken is that we would like not to add further to the correlative type studies, but to move closer to a knowledge of what is happening at the molecular level.

Paul Ehrlich, at the end of the last century, gave us a most valuable guideline when he emphasized in his chemotherapy work the fact that drugs are molecules and that they must react with other molecules. It is in order, then, to consider whether we can go beyond the edge of this world of correlations and find out what actually goes on when an anesthetic molecule approaches some other types of molecules.

Our focus in recent years has been on the association of xenon, in particular, and some other anesthetic molecules, with protein molecules. I do not wish to deny that lipid molecules may be very important components in our thinking

eventually about mechanisms of anesthesia, but the protein molecules have a few more handles on which we can tie things perhaps, and they can exist in solutions, so that we can consider the role of the water that is associated with them.

We have presented elsewhere (70) data to support the contention that a varying proportion (from 40 to 53%) of anesthetic gas is associated with proteins in blood. Complete removal of lipid from protein is difficult, and the percentages may not be accurate, but there seems to be a considerable protein-gas association. The speculation that a similar gas-protein association occurs in the brain and elsewhere is not necessary at the moment, but adds to the attractiveness of these studies.

Preparation for later points in this discussion must include a brief review of the nature of proteins, water and xenon. Modern descriptions of protein structure may be found in a number of books and reviews; perhaps a reference to Perutz' Weizmann Memorial Lectures will suffice (80). The nature of the peptide chain, the variability of structures of component amino acids, the role of hydrogen bonds in forming the *alpha*-helix, and the role of weaker bonds are all important factors in determining whether some lipid-like areas may exist in the interior of a protein molecule and whether the protein exists in compact globular form, in random coils, in more regular α-helix, or in combination of these (70).

A similar review of the structure of water is also necessary. A good modern reference is Linus Pauling's latest edition of *College Chemistry* (79). Stanley Miller (68) describes liquid water as containing regions of aggregated water molecules having a hexagonal structure with spaces inside the hexagons where other molecules may enter. Pauling (77) thinks more open structure may be envisioned for aggregates of water molecules in liquid water. He has described several sizes of hydrated cages which could trap anesthetic molecules, depending upon their sizes. The general idea expressed by Pauling (78) and Miller (68) is that molecules of anesthetics would enter these clathrates, or cages, and by stabilizing the water structure, decrease the conducting properties of the biological systems. Those interested in reviewing these possibilities might also find helpful some articles from our laboratory (30, 31). The major difficulties encountered in relating these thoughts about water to reactions involving gaseous anesthetics is that these stabilizing forces are not great enough alone to account for the biological reactions observed. Many atmospheres of common anesthetic agents would have to be applied to achieve anesthesia if only water molecules and anesthetics were involved. Proteins, therefore, become likely supporting molecules.

As useful as these ideas of the structure of liquid water may be in building concepts of the effects of anesthetic molecules on biological systems, an earlier picture introduced by Bernal and Fowler (10) has been used by Vogelhut (102) in developing a method which we have used to measure the dielectric or polar properties of water as they are influenced by proteins and anesthetic gas molecules. In this more dynamic picture of water, the molecules can be thought of as being free, that is, not bound to any other water or other molecules, or as being

bound to 1, 2, 3, or 4 other molecules of water. The bond angles involved would dictate the type and degree of the open structures described by Pauling and Miller which would exist, but the picture one must keep in mind is of a more statistical nature—one in which the introduction of some charge, actual or inducible, can cause a shift from one place in the continuum of 0-, 1-, 2-, 3-, and 4-bonded water molecules toward either end. A shift toward zero-bonded water would indicate greater numbers of water molecules free to rotate in a rapidly changing electromagnetic field and a shift toward 4-bonded water would indicate the presence of more bound water molecules, called irrotationally bound water.

In the studies we have been conducting we have been using a microwave generator to measure the dielectric properties of water. Wavelengths in the region of 9 kilomegacycles, or gigacycles, were used. This is in the radar range, and until the Second World War, techniques for producing these waves were not available. They are just now beginning to be applied to biological problems. My two colleagues, who deserve most of the credit for the data I shall present, are Dr. Benno Schoenborn, a physicist working in our pharmacology department, and Dr. Paul Vogelhut, Assistant Professor of Electrical Engineering on the Berkeley campus. We have used this method, described by Vogelhut (102), to describe the nature of a possible xenon–water–protein complex. Reduced hemoglobin has been used as a model protein. The possibility exists that xenon could combine with hemoglobin by so-called hydrophobic binding, in which the nonpolar xenon would be interspersed between solvent and a nonpolar region of the protein and thereby stabilize the protein structure by minimizing the interaction of polar and nonpolar clusters. However, the structure of hemoglobin (80) does not favor penetration of xenon into nonpolar regions which are buried in the center of the protein. A more attractive thought is that the xenon may affect the degree of stabilization of the protein–water complex. Our initial studies (92) with this method indicate that there is an increase in the grams of water bound to the protein in an irrotational manner. Some of the complexities of this interpretation were discussed by Dr. Schoenborn last year in Prague (32).

An estimate of the number of water molecules associated with each hemoglobin is about 300. This number is increased to approximately 330 when xenon is present. Therefore, about 30 more molecules of water are aggregated per molecule of hemoglobin. However, the dynamic or statistical nature of this aggregated or irrotationally bound water, as described by the concept of 0-, 1-, 2-, 3-, or 4-bonded water molecules mentioned earlier, must be kept in mind. Although a complete picture of this xenon-influenced aggregate cannot be described at this time, due both to the stronger binding of water molecules nearer the charged groups of the protein and to the incomplete knowledge of the structures of liquid water and protein, these data are consistent with the clathrate structure of Pauling (78) and Miller (68) or with an enlargement of a polar surface area by an induced dipole-type bonded xenon.

We think we have a good start into the area beyond the correlations world I mentioned at the beginning of this discussion. We believe that further studies of this type with the dielectric properties of biological systems, as well as other

modern physical techniques such as modern automated X-ray diffraction equipment, will lead us to a greater understanding of the alterations in metabolism brought about by these relatively simple anesthetic molecules in the central nervous system as well as in other tissues.

GENERAL DISCUSSION

Dr. J. P. Bunker: During the presentations of section I of this conference we seem to have turned away from the effects of anesthetics on intermediary metabolism, which was our primary charge, towards the current theories of anesthesia which emphasize molecular and physical effects and tend to discount the possibility that the mechanism of anesthesia may be a metabolic one. Current theories of the mechanism of anesthesia seem to lead us toward the conclusion that the primary effects of anesthetics are on functional activity of the neuron, and that metabolic changes may be secondary.

However, Dr. Carpenter has stated in print, and I think many of you have implied, that our methods simply may be insufficiently sensitive to detect the metabolic changes that may be occurring in the central nervous system; or, to put it another way, we may simply not be looking for the correct metabolic event, and this may simply reflect our ignorance of how the energy necessary for neuronal activity is generated—our ignorance of the specific links between metabolism and function of the nervous system.

Dr. Mullins: I think if you are looking at mechanisms, you ought to try to decide what you are going to do with metabolism once you have it, whether it is affected or not. What metabolism is presumably doing, from electrophysiological knowledge, is recharging the ionic gradients which are used up in the electrical activity of the nervous system. If you use up ionic gradients quickly, metabolism has to be supplied at a high rate. If you use it up slowly, then it can be supplied at a slow rate. And when you talk about nerve fibers, which use it at a very slow rate, then there is no very obvious connection between metabolism and electrical function. But in a CNS cell, where metabolism seems to be going on at a high rate, we can only conclude that there is nothing else for metabolism to do except recharge these gradients, that it is forced to recharge them at a high rate.

A second possible role of metabolism, but I think only a minor one, is the notion that maybe structural repairs to the membrane have to be carried out all of the time, and so a certain amount of new brick and mortar has to be generated. The cell isn't growing, it isn't dividing, it isn't doing any of the other things that most cells do, so the only real channel of energy is into the ion pump.

Dr. Quastel: Then what about, let us say, the biosynthesis of acetylcholine, or doesn't that mean anything?

Dr. Mullins: Well, I should think it was a very minor thing compared with recharging.

Dr. Quastel: But locally, however small it is, it may be very important. I don't know how you equate these things. It is not simply the quantity, it is the importance physiologically that we have to grasp. Presumably acetylcholine has got to be made.

Dr. Kety: I wonder, Dr. Mullins, whether we are not moving ahead a little too rapidly? I will grant that it is a very attractive hypothesis that anesthetics act primarily by interrupting utilization, and that all the production chain of events is secondary to the lack of demand. I find this a very appealing hypothesis. I find that this would help to explain why anesthesia does not wreck the machinery as anoxia does. But nothing I have heard today has raised this above an interesting hypothesis.

One question which has been asked and answered today is what *can* happen with anesthetics, and we have seen a lot of instances of what can happen with high concentrations of anesthetics acting on various structures and producing various effects. But remember that it is quite possible to get almost any effect from almost any substance with the appropriate dosage. Another question is, what actually *does* happen with anesthetics, and here we have heard less than about the first question. But even after one has answered the question what does happen with anesthetics, there is still the next question, which is what is the *mechanism* by which it happens, or by which it produces effects, and I think we are a far cry from that. For example, on the effects of anesthetics on membranes, Dr. Taylor's paper was very interesting. I would like to see those studies done *in vivo*. I would like to see evidence that *in vivo* anesthetics in anesthetic concentrations produce changes in sodium-potassium current across a membrane.

Thus, although we have seen what can happen to membranes under doses of anesthesia, we have yet to show what does happen to membranes, and we have the still further step to show that this is in fact the sequence of events.

Dr. Mullins: It is somewhat easier to slice brains and measure oxygen consumption than it is to make the electrophysiological measurements that you mentioned. I think that is why you have the biochemical evidence that you do.

Dr. Brauer: Can we safely afford to assume, as we seem to be doing, that all anesthetics produce the same kind of effect? I am singularly unconvinced that the barbiturates, most of the ones I have looked at, should be put in the same pot as xenon or ether or chloroform.

Dr. Carpenter: Of course, Shanes has already classified and labeled these various anesthetics, hasn't he?

Dr. Brauer: I realize this, but everybody else has cheerfully kept ignoring it. What I am asking is whether this is safe. For instance, please notice that practically all of the biochemical material we have heard presented has dealt really with remote possibilities, and for concrete evidence we have been limited primarily to the barbiturates.

Dr. Carpenter: Yes, but I think Shanes' evidence was rather good that metabolic differences between anesthetic agents were unrelated to mechanism of action. If you wish to examine how an anesthetic works, I don't think there is any reason to compare enzymatic effects of ether with enzymatic effects of the barbiturates or the alcohols. Clearly if you want to study the mechanism of anesthesia, you have to find an appropriate model, such as a peripheral nerve. It is better than studying the synapse *in vitro*. It you take a brain out and make a frappé of it, are you really studying a synapse? Of course not; you are perhaps studying everything in there besides the synapse. You are studying sup-

porting cells, and I can't quite see how you can really say that you have indeed studied that part of the synapse that is contributing to excitability or inexcitability. With Shanes' approach, I think the chances are a little better that the odds are stacked in your favor. You can study a nerve. It will maintain itself viable for many hours, and perhaps it is not too good a model, but nonetheless you can say that some drugs do one thing on the membrane and some do another. This I think would be a reasonable basis for the comparison of functional effects of anesthetic agents.

Dr. Chenoweth: I would say just once again that I feel quite strongly that the mechanisms of the different anesthetics are certainly going to be found to be different. We may have classes, the hydrocarbons or the halo-alkanes or the barbiturates, but they will be very different. The end result is only the way we are bringing them together here today at all—the production of some sort of clinical anesthesia. They don't need to be the same at all.

Dr. Bunker: I think we will accept this, but since we are as ignorant as we are, I think we are tempted occasionally to talk about the mechanism of anesthesia as if it were a single fact.

Dr. Featherstone: It seems to me we are in a stage where we don't really need to worry about whether we are talking about metabolism or metabolic reactions or perhaps the more functional reactions. Anything that we can learn about any of these will be most helpful, and some day maybe we can put them together. I think some of the people on the panel this morning were moving right along in that direction. But it will be a while.

Dr. Chance: May I be bold enough to ask a scientific question on the effect of xenon? I know this is off the main topic, but I just wondered, does the stabilization of the rates by noble gases actually affect the activity of any enzyme or enzyme system, particularly hydrolases?

Dr. Featherstone: This is something that very much needs study, Dr. Chance. One of our goals when we feel that we can handle the association of xenon with any protein, is to move to some proteins where function can be measured at the same time. Perhaps someone else can move there faster than we can, and I hope they do.

Dr. Chance: But the answer then at the present time is no.

Dr. Featherstone: That is right.

Dr. Chance:—which I think is important. The second point concerns enzymatic specificity of the change of dielectric absorption at the X band frequency, as Dr. Mullins showed. He had benzene, ethanol, and so forth. Does it matter whether you use xenon?

Dr. Featherstone: We have worked primarily with xenon so far, and we are going to be working with some other substances shortly.

Dr. Quastel: Dr. Featherstone, can you see the barbiturates or the anesthetic steroids fitting with Pauling's "cagey" hypothesis?

Dr. Featherstone: I don't particularly. Dr. Harold Hodge and I have done some work with the morphine hydrates. There are very definitely morphine hydrates which one can find. For instance, we were finding them in the eye of

mice. Pictures have been taken with X-ray diffraction equipment and they are being analyzed at the moment.

I am not really sold on the idea of this "cagey" hypothesis, as you call it, but one cannot dismiss Dr. Pauling's contribution lightly. If you look back on the structure of water, his papers are among the earliest on the subject. If you look back on the structure of proteins, his papers on the *alpha*-helix are very prominent. If you consider now that xenon combines with fluoride and oxygen to form covalent bonds with strengths of 31 kilocalories per mole—in comparison to the kilocalorie per mole we are dealing with here—again you find in 1935 that Pauling predicted these things.

Dr. Quastel: So you think it is possible for the steroids or barbiturates to be enclosed, as it were, in a sphere of water molecules?

Dr. Featherstone: At the moment I have to think of the possibility of a water envelope around any molecule. There are lots of water molecules available, and the degree to which they are free or bound might be expected to shift as you move away from any molecule which may affect their binding. This is a little easier for me to picture at the moment than Dr. Pauling's cage.

Dr. Chance: Dr. Kety brought up a topic which I think is basic, and perhaps has even a common interest to the physiologists and the biochemists, namely that the state of anesthesia is reversible, and therefore distinct from anoxia. I think it is an interesting thing that at least in isolated mitochondria and cells the state of anesthesia is a high energy state, in which some aspects of the membrane transport are not highly inhibited, but can go along slowly. The average of activity is depressed, not completely as it is in anoxia. I think that perhaps anesthesia is a fairly delicately balanced state in which many processes of energy metabolism are possible, but a few crucial ones, which may be highly localized and selective in an electrical sense, are depressed. I think there are real differences, subtle, important ones. I think Dr. Quastel's work showing the many subtle influences on carbohydrate metabolism is difficult to ignore in this respect. In other words, we are not dealing with a gross phenomenon. It is a delicately balanced one. Now, whether that is a point of departure of biochemistry, I don't know. Perhaps you would like to comment further.

Dr. Kety: I would like to hear Dr. Quastel's comment.

Dr. Quastel: If ATP is responsible for the ionic transport movement in the membrane, and an anesthetic in some way blocks this, there must be an interference with the utilization of ATP for this particular job. I believe that this is valid for certain groups of anesthetics, maybe for all. But this is the only aspect of ATP utilization for which we have evidence of interference. Other forms of ATP utilization, such as in various acetylation phenomena *in vitro*, and other reactions involving ATP are not blocked by anesthetics at the concentrations we are talking about. With very high concentrations of compounds like urethane, you do certainly get effects, but most ATP-utilizing processes are relatively inert, and this includes sodium-potassium-activated ATPase which we have already mentioned.

The effects of anesthetics on ionic movement and the role of changes in ATP

are not well understood, but they may be at the bottom of some of the results Dr. Mullins has been discussing. Certainly this is a process which requires a great deal more investigation.

Then I think we have to consider the direct effect of the anesthetic on mitochondrial processes. So far as I can see, these two processes are being affected, probably in different parts of the nervous system.

Dr. Taylor: I consider it more than a reasonable hypothesis that in the squid axon membrane, the electrical activity is not connected with any metabolic system. It has not been shown to be, and I think there is plenty of evidence that it is not, and that the anesthetic agents act not through any metabolic system. But this is merely a peripheral nerve membrane. I would suspect the same is true of the frog sciatic. But you have other parts of the nervous system to think about, and you have got to synthesize transmitter substances. You have got to get the excitation across the neuromuscular junction. I do think that the electrical aspects of a small piece of membrane are pretty clearcut, but I also suspect that the physico-chemical phenomena of mitochondria are probably very much the same as for the membrane, just because they look alike.

Dr. Mullins: I think that one should not rely too much on the evidence from squid axon in judging whether there are metabolic influences. The biochemists are preoccupied with making these cycles go round and round, and they don't worry too much about what you do with the energy after you have got it. But the physiologists, who are somewhat more practical-minded in this regard, are concerned with what happens to the energy. I think it far more than likely that it is not the ATP concentration in the nerve cell that is important, but rather the ATP–ADP ratio, which is the thing that drives the pumping.

It used to be thought that these pumps were electroneutral, that is, they did not contribute anything to the electrical situation, but evidence seems to be accumulating more and more that they are capable of generating electric potentials themselves. So it is quite conceivable that a nerve cell has a natural membrane potential in the absence of metabolic drive that is too low to fire it, and that you need a continuous pumping to raise the electric potential sufficiently to keep the membrane working.

Dr. Chance: We seem to be in agreement that the mitochondria are no farther from the realities of anesthesia than is the squid axon.

Dr. Taylor:—unless you talk about local anesthesia in a squid axon.

Dr. Larrabee: Dr. Featherstone, do your concepts give us any clue as to the significance of the constant thermodynamic activity rule among anesthetics? Certainly in some systems narcotic action correlates more closely with thermodynamic activity than with molar concentration.

Dr. Featherstone: I think Dr. Mullins has answered this in part by saying that the thermodynamic changes that one can note tell about energy changes, but they don't really tell us how these come about.

Dr. Chance: It does not matter how it comes about, so we can't have that one.

Dr. Featherstone: This is probably how it does come about on a molecular basis.

Dr. Taylor: But isn't it true that Pasternak, or Brink and Pasternak, found that this broke down for the ganglion?

Dr. Larrabee: There was a progressive change in axonal block, but with respect to block of the synapse by different molecules, the thermodynamic activity was almost invariant.

Dr. Trevor: I would like to make two small points. There has been a lot of discussion about correlation between anesthetic dose levels *in vitro* and *in vivo*, but time is a fundamental difficulty in making this correlation since there may be binding of anesthetic agents with nonspecific functional groups, and thus when you measure anesthetic levels in blood or in brain, this may give you quite an untrue estimate of the concentration of the anesthetic required to inhibit a specific mechanism.

The second point was a bit more specifically biochemical. You have seen that there is inhibition of the electron transport in mitochondria. I wonder if either Dr. Quastel or Dr. Chance would comment on the finding that in certain more purified NADH dehydrogenase systems there is insensitivity to amobarbital?

Dr. Chance: The experimental data seem to support the hypothesis that amobarbital acts on different sites in the intact mitochondrion than it does in purified dehydrogenases. The work of Ernster, which was very good at the time, largely beclouded the true site of amobarbital action, because he was using artificial electron receptors, and came to the conclusion that amobarbital was working between flavin and the respiratory chain. This is actually not correct in the mitochondrion, where amobarbital works on one of the compounds that transfers energy from the respiratory chain to ATP. This only underlines the fact that you can get as many effects of anesthetics as there are systems for study.

ACTION OF ANESTHETICS ON THE BRAIN

Dr. Cohen: It is important to stress that the complete understanding of the events with which we are dealing during anesthesia will be reached only when we have a complete understanding of consciousness. In order to understand anesthesia, the lack of consciousness, we must first understand the totality of events occurring during consciousness.

We have observed that during nitrous oxide or halothane anesthesia there was either no change due to the anesthetic agent or a slight diminution in the rate of cerebral oxygen consumption; changes in cerebral glucose consumption paralleled this. These studies were all conducted at a normal P_{CO_2}.

Additional measurements also have been made. These involve the analysis of arterial and jugular venous blood for lactate and pyruvate. We have attempted to determine, in each study, whether cerebral excess lactate (Huckabee) is present. In addition we have sought to partition the fate of glucose into aerobic and anaerobic pathways of metabolism. In order to do the latter, we have made use of the above-mentioned measurements in order to derive an aerobic and an anaerobic metabolic index (24). The calculations involved will be briefly described. If there is complete anaerobic metabolism during the degradation of one molecule of glucose, we would obtain the formation of two molecules of lactic acid; we would term this 100% anaerobiosis or an anaerobic metabolic index of 100%. Since we can measure the actual amount of lactate produced,

this may be compared with the lactate equivalent of the actual amount of glucose consumed. Thus:

$$\text{Anaerobic metabolic index} = \frac{\text{A-V difference in lactate}}{2 \times \text{A-V difference in glucose}} \times 100\%.$$

Similarly, we may calculate an aerobic fraction of glucose consumption by comparing the actual amount of oxygen consumed with that which would be necessary theoretically to oxidize the glucose consumed. Thus:

$$\text{Aerobic metabolic index} = \frac{\text{A-V difference in oxygen}}{6 \times \text{A-V difference in glucose}} \times 100\%.$$

Measurements of the anaerobic metabolic index, the presence or absence of excess lactate of significant degree, and the aerobic metabolic index showed essentially no differences between cerebral carbohydrate metabolism in the conscious state and that occurring when the subjects were anesthetized with either thiopental-nitrous oxide or with halothane and oxygen; these measurements were obtained while P_{CO_2} was maintained normal (Table 5). We can thus say that during normocarbia, regardless of any alterations in the overall *rate* of glucose and oxygen utilization produced by nitrous oxide or by halothane, the *pathways* of carbohydrate metabolism remain essentially unchanged.

One of the events which frequently occurs during anesthesia is an alteration in pulmonary ventilation. The resulting changes in P_{CO_2} will produce marked effects upon cerebral blood flow. They may also produce changes in cerebral metabolism, either directly through an alteration of tissue P_{CO_2} or pH, or indirectly by affecting the cerebral circulation. It was one of the goals of our investigations to determine whether the diminished cerebral blood flow resulting from hypocarbia would produce cerebral hypoxia.

We have studied the biochemical effects produced by changes in P_{CO_2} during anesthesia using two different agents. During thiopental-nitrous oxide anesthesia, the study involved the inhalation of about 20 to 25 % oxygen in the presence of drugs which may slightly increase the resistance within the cerebral vasculature. In contrast, during halothane anesthesia nearly 100 % oxygen was inhaled in the presence of a drug which by itself produced cerebral vasodilatation.

In some individuals, when anesthesia was maintained with nitrous oxide, it was possible to demonstrate increased cerebral production of excess lactate and an increased anaerobic metabolic index when P_{CO_2} was markedly lowered (to approximately 15 mm Hg). In these individuals, examination of an eight-channel bipolar electroencephalogram showed a pattern compatible with cerebral hypoxia. In other individuals there was neither biochemical nor electroencephalographic evidence of hypoxia during hypocarbia. It would thus appear from these initial investigations that if P_{CO_2} is greatly lowered during nitrous oxide anesthesia, mild and apparently completely reversible cerebral hypoxia may result. It should be stressed that these levels of P_{CO_2} were obtained in young healthy

TABLE 5

Effect of anesthetics on cerebral glucose utilization

Anesthetic	Cerebral Excess Lactate	Anaerobic Metabolic Index (%)	Aerobic Metabolic Index (%)
None	0	5	92
Thiopental + 70% N_2O	0	7	98
Halothane, 1.2%	0	8	92

TABLE 6

Effect of anesthetics on cerebral oxygen and glucose consumption

Anesthetic	% Depression		Temp.	Expected Depression with ↓ Temp.
	$CMRO_2$	$CMR_{glucose}$		
Thiopental (conscious)	0	—	—	—
Thiopental (surgical anesthesia)	52	—	35.6	21
Thiopental + 70% N_2O	23	27	36.5	8
Halothane, 1.2%	10	7	36.4	10

subjects not subjected to the stress of operation; in addition, this degree of hypocarbia is far greater than that normally obtained during clinical anesthesia even when intentional hyperventilation is used. Biochemical and electroencephalographic evidence of cerebral hypoxia was not observed when P_{CO_2} was above 25 mm Hg.

When hypocarbia was produced during halothane anesthesia, we observed no biochemical events that would be associated with cerebral hypoxia. In this instance, 100% oxygen was administered, P_{CO_2} had a mean value of 25 mm Hg, and a drug (*i.e.*, halothane) producing cerebral vasodilatation was being inhaled.

An interesting finding was obtained during the studies performed with halothane. The ratio of oxygen consumed to glucose consumed by the brain appears to be related to P_{CO_2}. When P_{CO_2} was 40 mm Hg, this ratio was 5.5:1. The theoretical ratio would be 6:1; however, the small amount of lactate normally produced results in a slightly lower value. A possible explanation of the change in the ratio occurring with changes in P_{CO_2} is as follows. During hypocarbia, there is increased incorporation of glucose into other metabolic pathways, for example into protein synthesis and fatty acid formation. This would result in a lower ratio of oxygen consumed to glucose consumed. On the other hand, during hypercarbia, the observed oxygen consumption contributes not only to the oxidation of glucose but to other substances as well; this would produce a higher value of this ratio. In both these situations, a maximum of only 10% of the metabolized glucose appears to be involved.

In conclusion, we have attempted to examine some of the aspects of cerebral carbohydrate metabolism during anesthesia. Different agents will produce greater, lesser, or no depression of cerebral glucose consumption. Halothane appears to produce little or no depression of glucose and oxygen consumption if

temperature is considered. Changes in P_{CO_2} appear to be able to alter slightly the pathways of cerebral carbohydrate metabolism. Extreme hypocarbia produced during nitrous oxide-oxygen anesthesia may produce cerebral hypoxia by diminishing cerebral blood flow.

Dr. Alexander: I would like to discuss with you the effects of anesthetics on whole brain oxygen and glucose consumption in man.

Table 6 summarizes some data from several laboratories on the effects of anesthetics on cerebral oxygen and glucose metabolism. All these data were obtained at a normal level of arterial P_{CO_2}. The first condition I would call to your attention is during thiopental seminarcosis. Dr. Kety *et al.* administered intravenous thiopental to psychotic patients in a dose sufficient to produce personality changes without loss of consciousness (49). Although there were definite changes in the personalities of these individuals, there was no depression in the rate of oxygen consumption by the whole brain.

We pass to the next state, where thiopental was administered by Pierce *et al.* in a dosage sufficient to produce surgical anesthesia (81). When the measurements were made, jugular venous thiopental concentration was approximately 20 milligrams per liter. This thiopental level produced a 52% depression in cerebral oxygen consumption.

Next we may examine the data obtained in our own laboratory, on the effects of thiopental-nitrous oxide-oxygen and of halothane. The methods employed have all been described elsewhere (24). These studies were performed on normal young adult male volunteers; no surgery was performed. Inspired anesthetic concentrations were maintained at a constant level. Since the cerebral circulation, and perhaps cerebral metabolism, is altered by changes in arterial P_{CO_2},

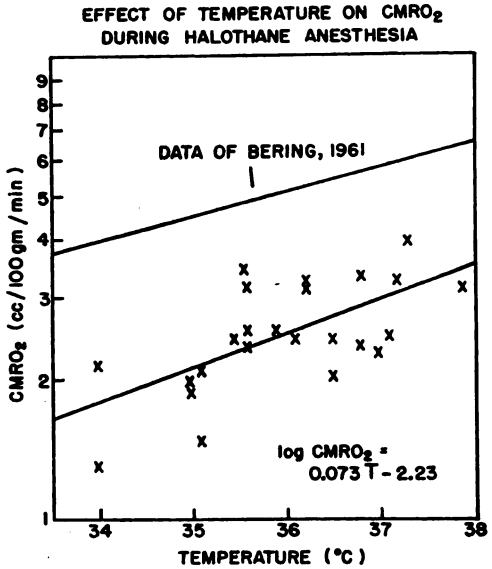


FIG. 16.

these subjects were artificially ventilated with a Bird respirator and arterial P_{CO_2} was controlled by adding CO_2 to the inspired mixture. Finally, it was necessary to control the body temperature of these subjects, because body temperature has a rather marked effect on the rate of cerebral glucose and oxygen metabolism.

In Figure 16 a semilogarithmic plot of the rate of cerebral oxygen consumption as a function of body temperature during halothane anesthesia is shown. The regression line depicted in the figure has approximately the same slope as that obtained by Bering during pentobarbital anesthesia in monkeys (9). These data indicate that cerebral metabolism falls about 15% for each °C fall in body temperature.

Let us now look at the effects of thiopental-nitrous oxide-oxygen anesthesia (Table 6). Five mg/kg of thiopental were administered to healthy normal male volunteers as a single intravenous dose; 70% nitrous oxide in oxygen was then administered in a nonrebreathing circuit. At a time when jugular venous thiopental concentration was less than 3 mg/l, cerebral oxygen consumption was depressed 23%, and brain glucose consumption was depressed an approximately equal amount. This thiopental concentration does not ordinarily produce unconsciousness in normal adults and, therefore, we can conclude that most of this depression was due to the presence of nitrous oxide.

Finally, we come to the effects of halothane anesthesia. Halothane 1.2% in oxygen was inhaled in a nonrebreathing system and end-tidal halothane concentration was 1% at the time these measurements were made. Notice that there was only a 10% depression of cerebral oxygen consumption and a 7% depression of cerebral glucose consumption.

As noted earlier, cerebral metabolism is very sensitive to temperature. Data are available on the body temperature existing in the last three studies cited in Table 6. In all three of these studies there was some fall in temperature. Using the relationship shown in Figure 16, it is possible to calculate the expected depression in cerebral oxygen consumption of these subjects due to the fall in body temperature alone, and thus to determine how much of the depression of oxygen consumption was due to the direct action of the anesthetic on brain metabolism. In Table 6 it can be seen that approximately 40% of the depression noted by Pierce during thiopental anesthesia could be accounted for on the basis of a fall in body temperature. Likewise, over one-third of the depression noted during thiopental–nitrous oxide–oxygen anesthesia can be accounted for on the basis of a slight depression of body temperature. Finally, virtually all of the depression in overall brain oxygen consumption during halothane anesthesia can be ascribed to a small decrease in body temperature. This is somewhat surprising because the subjects anesthetized with halothane appeared, by clinical criteria, to be more deeply anesthetized than those given thiopental–nitrous oxide–oxygen. Therefore, these data indicate that the mechanism of action of general anesthetics may not be due to a depression in overall brain metabolism.

However, Dr. Kety and others have shown the brain to be a very dishomogeneous organ with the rates of blood flow and metabolism varying widely in different intracerebral structures. Although cerebral metabolism in the whole brain

of man was only slightly depressed by halothane, it is possible that certain selected areas were markedly depressed; such a localized depression could account for the production of anesthesia.

DISCUSSION

Dr. S. S. Kety: The studies we have just heard reported are contributing a great deal to an understanding of what goes on, at least in some areas, under anesthesia. But I think you may be as perplexed as I am in using this information to explain the mechanism of anesthesia.

Perhaps it may be worthwhile to review our own thinking with regard to the importance of metabolism in cerebral function. We started these studies some 20 years ago at a time when metabolism was very important. Biochemistry was essentially intermediary metabolism, in those days. DNA and RNA had not yet been discovered to have any important functions and great emphasis was placed upon the Krebs cycle. Coming from a background of physiology, we expected to find in general rather good correlation between the oxygen consumed by the brain, energy produced by the brain, and cerebral function.

Interestingly enough, we have revised our concepts of the relationships between cerebral oxygen consumption and cerebral and mental function somewhat since that time. We very soon learned that there were a large number of mental states which were unassociated with any change in oxygen consumption: schizophrenia, sleep, seminarcosis with thiopental, and mental arithmetic, all of which produced or were associated with marked changes in thinking or in consciousness but were associated with no detectable change, by our methods at least, in oxygen consumption.

This led us to the great insight which retrospectively seems quite self-evident: that the brain is not a pump or a motor, and there is no reason to expect a good correlation between its oxygen consumption and its functions. In fact, by that time, computers were coming into vogue, and it was quite obvious that the brain was a computer, and not a pump. Of course, if it were a computer, there would not be any reason at all to expect that the output, the accuracy, or the productivity of a computer would necessarily have anything to do with the power supply except where the power supply was seriously altered.

But we thought that anesthesia was certainly the situation in which there was a primary defect, enzymatic probably, in the availability of energy and that this was why anesthesia was associated with changes in function.

We were impressed with Dr. Quastel's earlier work *in vitro*, but of course we raised the question which I raised earlier this afternoon, that these were simply findings of what might happen or what could happen with anesthetics. It did not necessarily imply that this did happen with anesthetics in the intact living brain.

So when Himwich and we independently made some of the early measurements of cerebral oxygen consumption in thiopental anesthesia, we were gratified to find that there was a marked reduction in cerebral oxygen consumption,

which paralleled or appeared to parallel the depression in functional activity. And everybody seemed happy.

Then Martin Larrabee came along with his little brain in a perfusion chamber in which he showed, as he did this morning, that one could get marked effects by anesthetics on transmission without any effect on oxygen consumption. But the depression in mood which this might have generated was short-lived because Martin did the very gratifying experiment of testing the effects of anesthetics on stimulated sympathetic ganglia, and showing that when the ganglion was under a stimulated condition even small doses of anesthetics which were associated with blockade of transmission also produced a profound and gratifying fall in oxygen consumption. So again everyone was happy, because obviously the brain was much more comparable to the stimulated cervical ganglion than to the unstimulated ganglion. The brain was after all a complex of 10 billion neurons, each interacting with every other one, and if one simply depressed the interaction between neurons, this would certainly account for the fall in cerebral oxygen consumption, and therefore, the results obtained in man were quite comparable to those obtained in the more carefully controlled molecular situation, which he was studying.

However, this did mean that metabolism took a seat farther back in the arena, because now metabolism was the handmaiden of activity, so to speak, instead of being its precursor. But even so, metabolism could still have been the important primary site. It takes metabolic processes to make acetylcholine which is so essential to conduction, and the biochemical effects of anesthetics on enzymes could still be expressed as a primary action on the synthesis of acetylcholine. This possibility still exists, and has not necessarily been dismissed, although I must confess that the possibility that anesthetics have nothing primary to do with metabolism, but act in a physical-chemical, or physical, or electrical way on membranes, has a certain attractiveness.

Now, on the basis of the findings of Dr. Alexander and Dr. Cohen, we have to revise even our impressions of what is going on in the intact human brain under anesthesia, or at least under certain forms of anesthesia. They have quite cogently shown that body temperature is a factor which has to be controlled, although here I wonder whether there is not something a little circular in the problem. If a drug depresses metabolism, it is of course bound to depress body temperature as well, and therefore, the mere fact that there is a component in the depression in oxygen consumption, which is accountable by depression of temperature, does not in itself depreciate the possible importance of a metabolic effect from the anesthetics.

But then with halothane, which certainly produces anesthesia satisfactorily enough to account for its widespread use, there is an inappreciable reduction in cerebral oxygen consumption. We have now an illustration of a phenomenon in anesthesia which may be quite comparable to the concept which we had already accepted in sleep, that here again it is not necessarily a primary effect upon metabolism which accounts for the changed function, but a primary effect some-

where else in the system—on the circuits, on the relationships between the circuits, on what components are activated, what components are inhibited—so that even though the total energy consumed may be the same, the content of the message and the information processing efficiency of the computer are seriously altered.

It is not impossible that when more facts are available it may be that in anesthesia, as in some of these other conditions, we may be witnessing a situation in which metabolism plays a secondary role, a role in which it is called upon by activity, rather than being the immediate and important determinant of activity.

METABOLIC EFFECTS OF ANESTHETIC AGENTS ON THE LIVER

Dr. R. W. Brauer: Effects of anesthetic agents on the liver should, I suggest, be thought of under two headings: one of these comprises those effects which are exerted by anesthetics on the liver as such, while the second category should cover indirect effects upon the liver resulting from the action of anesthetic agents upon other parts of the organism. I shall attempt to deal largely with the former, while Dr. Price may present some data pertaining to the latter subject.

In my opinion, studies of this topic are conducted best upon intact livers, supplied with a blood circulation as nearly normal as possible, but isolated from the remainder of the organism. I shall deal, therefore, essentially with the preparation with which we have had most experience, *i.e.*, the isolated rat liver perfused with whole blood. Apart from the fact that in such a preparation the circulation is at the command of the experimenter, and can therefore be maintained constant or subjected to small variations as seems indicated, such preparations also permit one to maintain the organ temperature constant at whatever level is desired. In view of recent evidence suggesting that with respect to carbon tetrachloride at least, the effects of some of the procedures considered to be "protective" may be largely if not wholly dependent upon reduction in liver temperature, this would appear to be a real advantage.

The anesthetic agent with which we have had most experience has been chloroform. We chose this originally because of its close relation to carbon tetrachloride, a well-known liver poison which at that time had just begun to return to prominence as an interesting test substance for the study of liver injury. Chloroform in anesthetic concentrations (we use in general levels below 2%) produced prompt and rather marked effects on liver function: bile secretion begins to fall almost as soon as chloroform is introduced. Blood flow rate, after initial dissipation of the hepatic vasoconstrictor substance from whole rat blood tends to remain constant in an undisturbed preparation. Chloroform then produced marked further vasodilatation. Finally, blood glucose, again following some initial rise, attains fairly constant levels in normal isolated preparations. The introduction of chloroform, after a brief period of indecision, results in a progressive rise in blood glucose levels which apparently continues for a considerable period of time if chloroform exposure is continued. A summary of data from a number of experiments relating the intensity of several effects to chloroform dosage shows that, on the whole, while there is some dose-dependence at the lowest levels of

chloroform, the effects, once they have been established, show relatively little further dose-dependence. In addition to other parameters, the sodium space also responds to the exhibition of chloroform by a marked increase.

These prompt and striking effects of chloroform upon some functional parameters describing the biochemical and physiological status of the liver parallel to some extent the morphological events as revealed by electron microscopy. After about 20 minutes of exposure to chloroform at a level of about 0.9 vol %, one begins to see a loosening up of the relation between ribosomes and endoplasmic reticulum, probably the earliest morphological effect seen. So far as we can tell, the mitochondria are left intact at this point. After 90 to 120 minutes of chloroform exposure at the same level the breakdown of the endoplasmic reticulum has progressed considerably further. There is vacuole formation, the organization of the endoplasmic reticulum is substantially lost, and ribosomes appear to float free in the cell sap without any particular organization. One may well wonder whether this stage does not already represent substantially irreversible cell injury.

In passing, one might mention that at about this point, when bile secretion in general has substantially subsided, the microvilli in the bile canaliculi still maintain a remarkably satisfying appearance, suggesting that the presence of intact microvilli by no means suffices to guarantee continued bile secretion.

I should next like to say a few words about some of the gross biochemical parameters we have examined so far. A marked decrease in oxygen consumption was observed. Here again, it would appear that dose-response relations are none too pronounced. Under these conditions, the decrease in oxygen consumption by these livers cannot be attributed, as has been suggested in the past in connection with carbon tetrachloride, to impaired delivery of oxygen: these livers are being supplied with fully oxygenated blood, and the blood flow, as has already been pointed out, is in fact somewhat greater than that in livers not exposed to chloroform. The effect therefore must be exerted upon the metabolic apparatus itself.

It is nonetheless profitable to make some comparisons between the effects of chloroform and the effects of imposed hypoxia, because qualitatively these two situations present a good many similarities, though quantitatively there are important differences. Livers not subjected to any special treatment but merely transferred from the animal to the perfusion apparatus and perfused as best we know how, show an initial loss of glycogen associated with the surgery, but subsequently the fall in liver glycogen levels becomes quite slow. Chloroform markedly accelerates this fall in liver glycogen levels. Hypoxia also decreases hepatic glycogen levels, but we feel that for levels of hypoxia producing a comparable decrease in oxygen consumption, the glycogen decrease is much less marked with hypoxia than with chloroform. In addition to glycogen levels, our experiments have given us data on some other metabolites. As one might expect, the fall in pH is reflected in an increase in lactic acid levels that just about accounts for the decrease of pH. Pyruvic acid levels fluctuate a bit in a manner which, in a series of experiments, is none too reproducible; lactic/pyruvic ratios

invariably show a transient increase. In passing, please note that I have studiously avoided calculating any excess lactate production figures, since we have some data which lead me to feel that in the case of the liver, the metabolite data from blood are not necessarily representative of those in the tissue, so that in order to make such a calculation meaningful, one would have to resort to repeated biopsy analyses.

So far then what has been said amounts to this: 1) one can produce very early changes by exposing isolated livers to chloroform at anesthetic levels; 2) these changes are not dependent upon impairment of oxygen supply to the tissue; 3) there is some qualitative resemblance between the effects of chloroform and of hypoxia upon the livers, but quantitatively the chloroform effects appear to be more severe than can be accounted for by levels of hypoxia which reduce oxygen consumption to an extent comparable to that produced by chloroform.

To pursue the subject further, one must look at three separate sets of relations: one of these involves the question of the extent to which the reactions we have looked at so far are peculiar to relatively hepatotoxic anesthetic agents like chloroform, or to what extent they are shared in general by volatile anesthetic agents. The second one, closely related, concerns the extent to which the changes we have seen so far are reversible. Could it be, for instance, that the effects of a relatively hepatotoxic agent like chloroform differ from those of agents which affect the liver to a much smaller extent merely in that the effects of the former are less rapidly and less promptly reversed? And finally, there is the important question of the interplay between oxygen supply and liver injury by volatile anesthetic agents, a question which particularly in the case of chloroform has a long and honorable history. In view of the limited space, I shall go into this last point merely to the extent of calling attention to two sets of observations. If one exposes rats to carbon tetrachloride in a pressure chamber under oxygen pressures of two or three atmospheres, one obtains a distinct protective effect even if this exposure extends only over the first 3 hours after administration of the agent. The effect is more marked if the oxygen exposure is continued, but this can be understood in the light of evidence that carbon tetrachloride stays in the system of the rat for a good while. These observations suggest that the oxygen effect is exerted on the production of the primary liver lesion by halogenated hydrocarbons rather than on the reversal or perpetuation of the primary lesion. The second set of observations concerns some preliminary studies on the significance of the centrilobular location of the chloroform lesion. This has been attributed in the past to differential oxygen tensions, the portions of the tissue closest to the venous end of the blood supply getting the poorest oxygen supply and hence being more sensitive to chloroform. We have carried out a series of perfusions in which the blood stream has been reversed, a complicated and not altogether satisfactory procedure, and exposed these preparations to chloroform. Under these conditions the distribution of the lesion is not altered; even when the blood stream flows from the hepatic vein toward the portal vein, it is still the centrilobular portion, the portion that is now receiving the most highly oxygenated blood, in which the early lesions, most particularly

the glycogen loss, are observed. Tentatively, we would like to interpret this as indicating that the zonal distribution of lesions in the liver reflects not the oxygen gradient, at the time of exposure, but rather more subtle biochemical changes brought about in the tissue as a result of the biochemical gradients which must prevail along the blood stream for a good period of time before chloroform exposure; in other words, it is a matter, so to speak, of adaptive enzyme formation and induced chloroform susceptibility of the cells composing different tissue zones rather than of oxygen tensions or other blood composition gradients prevailing at the time of chloroform exposure.

Let us turn now to the matter of reversibility. During a typical experiment with a relatively short exposure to chloroform, some of the changes after the removal of chloroform are reversed while some persist. Reversible, in particular, are the decrease in oxygen uptake and the vasodilatation. By contrast, bile secretion does not recover. Glucose levels merely stop increasing. Glycogen stores remain depleted. Unfortunately we do not have data to tell us to what extent the morphological changes persist over the periods of time that we have observed.

One might wonder whether the failure of such organs to recover completely might in part be a response to the continued exposure of these livers to blood which had been in contact with the organ during the exposure to the injurious agent. One might conceive that metabolic changes would be reflected in such blood, and that whatever metabolic breakdown products might be present could influence the recovery of the organ. We have tried to obviate this effect by transferring chloroform exposed livers to fresh blood, treated only to the extent of passing it through a preliminary liver so as to remove the vasoactive substance of fresh whole rat blood. While, with intact liver, transfer from one blood supply to another is invariably successful, in the limited series of experiments with chloroform treated livers this procedure has regularly produced sizable vascular changes. It would be interesting to ascertain whether such enhanced susceptibility to vasoactive substances is observed *in vivo*. This might have important bearing on the ability of an injured organ to recover from the effect of an agent like chloroform. Recovery of bile flow is poor, but we do not know to what extent this is merely a consequence of the vascular change. Oxygen uptake similarly shows an unsatisfactory recovery; and again this probably reflects the fact that now we are no longer measuring the oxygen uptake of the entire tissue. The original reason for doing these experiments was to look into the recovery of blood pH, and the removal of lactic acid. In fact, however, such reversal is not observed: on transferring the chloroformed liver to fresh blood, the initially normal pH of the perfusion blood is rather swiftly lowered by the release of what we assume to be lactic acid, the final value being approximately the same as prevailed in the blood from which the liver had just been removed. Thus, what we thought would be a clean-cut experiment has turned out to raise more questions than it has answered. These questions, however, appear to me to be quite germane to the problem of recovery in this tissue, and we plan to devote a good deal further study to them in the near future.

Finally, I should like to say a few words about the comparison of chloroform with another volatile anesthetic, diethyl ether. Diethyl ether in anesthetic concentration—4.8% was used—also produced marked effects on the isolated liver. Here again there is a fall in bile secretion, more readily reversible perhaps than with chloroform. There is also a decrease in perfusate pH associated with a rise in lactic acid, as well as a transient increase in lactate-to-pyruvate ratios. Most interesting, however, is the fact that in none of the preparations have we seen with ether the decrease of oxygen consumption which is so characteristic of chloroform. We feel that this observation may hold the key to the whole subject of relative hepatic toxicity of these anesthetic agents. It is appropriate to close with this observation since it is from this point that our next investigations in this field will take off.

I have deliberately presented something of a grab-bag of phenomena. It seems to me that this collection is beginning to circumscribe some of the questions one can meaningfully ask by means of the type of tool which we have here chosen to describe. It appears clear now that effects of at least two anesthetic agents on the liver can be observed at a very early period of time. A number of changes persist for some time after withdrawal of chloroform, and it would seem reasonable that some of these may play a role in that fixation of injury which must occur if cell necrosis is to occur eventually. It has been possible to begin to define the relations between oxygen supply, oxygen uptake, and the deleterious effects of chloroform. And finally, there are beginning to emerge the outlines of some phenomena which hold some promise of distinguishing anesthetic agents of varying degrees of hepatotoxicity.

Dr. H. L. Price: The study that I would like to report to you was performed in normal human volunteers, and was conducted in somewhat the same manner as that detailed by Dr. Alexander.[5] These individuals were studied first under resting conditions, then under the influence of an anesthetic which had been kept at a constant inspired tension for about one hour. They also were studied during an additional period while they remained under anesthesia. The body temperature was maintained constant by means of thermal blankets. The alveolar P_{CO_2} was maintained constant when necessary by means of assisted ventilation when the patients were under anesthesia. The oxygen tension in the inspired air also was maintained constant. Splanchnic blood flow was measured with indocyanine green dye.

Two anesthetics were studied. Cyclopropane was chosen because it apparently causes sympathetic nervous system stimulation, accompanied by a considerable vasoconstriction in areas of the body which are under sympathetic innervation. The question whether or not cyclopropane produces enough constriction to cause splanchnic ischemia has been raised previously. The second anesthetic studied was halothane. This was chosen because it is so different from cyclopropane in its hemodynamic actions. Its administration is not accompanied by any con-

[5] This study was performed in collaboration with Dr. R. M. Epstein, Dr. S. Deutsch, Dr. L. H. Cooperman, and Dr. A. J. Clement. It is still incomplete and the data are preliminary.

spicuous liberation of catecholamines, and it produces arterial hypotension in consequence of both central and peripheral actions.

In man it is not possible to study the circulation to the liver uniquely, because of the intestinal contribution by way of the portal vein. Since this vein is inaccessible unless one performs a laparotomy, we were obliged to include the entire splanchnic circulation, that is, the circulation between the celiac and mesenteric arteries and the hepatic vein. Hepatic venous samples were withdrawn through a radiopaque catheter which was introduced through an antecubital vein. The arterial samples were obtained from the femoral artery.

Concentrations of both anesthetics were chosen which would just permit normal ventilation without any reaction to an endotracheal tube, inserted in order to provide a patent airway once the subject had been anesthetized. These concentrations were 18 % cyclopropane and 1.2 % halothane, both measured in end-expired air.

In brief, the effects of *cyclopropane* were to increase the perfusion pressure (arterial minus venous), to reduce splanchnic blood flow by increasing the splanchnic vascular resistance, to reduce the rate of clearance of indocyanine dye by the liver, and to increase the "excess" lactate (Huckabee) produced by the splanchnic viscera. Hepatic venous oxygen tension and splanchnic oxygen consumption were inconsistently affected.

In order to analyze the mechanism responsible for these changes, 10 mg of hexamethonium were slowly administered intravenously during the administration of cyclopropane but after the foregoing measurements were made. The effect of this ganglion-blocking agent was to return perfusion pressure toward normal, to increase hepatic blood flow until it was not significantly different from normal, and to reduce splanchnic vascular resistance. Oxygen consumption increased after the administration of the blocking drug. The clearance of the dye was essentially unchanged. The venous oxygen tension was also unchanged. But "excess" lactate, in the four measurements which comprised this group, was reduced in those cases where it had previously been elevated.

We can infer from these facts that certain metabolic changes occurring during cyclopropane administration, including the appearance of excess lactate, may be consequences of increased sympathetic nervous activity. However, the fact that the dye clearance was unaltered seems to indicate that besides the sympathetic effect, there is a direct action of cyclopropane on the functional ability of the liver parenchyma.

The administration of *halothane* was accompanied by diminished perfusion pressure and a reduced hepatic blood flow. The reason for the reduction in flow was not vasoconstriction but hypotension, *i.e.*, diminished perfusion pressure. The oxygen consumption was not significantly affected. Again the clearance of the dye was reduced. The venous oxygen tension was significantly diminished. In contrast to cyclopropane, a significant increase in "excess" lactate did not occur with halothane.

One might ask how it is that two drugs which both reduce splanchnic blood flow, and both of which usually reduce the venous oxygen tension, have such

different effects on production of lactate. It seems to me there are two possibilities. The first is that the change in lactate that we are seeing is not an evidence of hypoxia, but some kind of a metabolic effect of sympathetic nervous activity. We cannot rule this out. We hope that we can go on to explore the effects of *beta*-sympathetic blocking agents which might get rid of any metabolic action of catecholamines without changing splanchnic vascular resistance. The other possibility is that under cyclopropane anesthesia the circulation in the splanchnic area is functionally different from that during the administration of halothane, and if in fact these measurements do represent hypoxia occurring in the splanchnic bed during cyclopropane, then they suggest that cyclopropane is causing some kind of a restriction in the circulation, so that as the flow diminishes, the diffusion distances become greater and some of the tissues suffer hypoxia.

EFFECTS OF ANESTHETIC AGENTS ON MYOCARDIAL METABOLISM

Dr. S. J. Galla[6]*:* Depression of myocardial function during anesthesia is observed frequently in the operating room and laboratory. This depression may be due primarily to the effects of anesthetic agents upon the myocardium, or secondarily to ischemia resulting from decreased coronary blood flow or arterial hypoxemia. The effects of hypoxia on myocardial metabolism have been studied (42) but little attention has been given to the direct effects of anesthetic agents. Since efficient operation of energy mechanisms is essential for myocardial function, cardiac depression and failure must be explained ultimately on a biochemical or biophysical basis. Impairment of these mechanisms by anesthetic agents could conceivably explain their myocardial depressing properties.

It is useful to divide cardiac energy mechanisms into liberation, conservation and utilization. Anesthetic agents might act upon one or all of these processes. The heart is a versatile organ and metabolizes a number of substrates in proportion not only to its requirements, but also to the arterial concentration of the substrate. Anesthetics could alter myocardial activity by impairing enzymatic function in glycolysis, in the tricarboxylic acid cycle, or in the electron transport chain. Chemical energy released through oxidation must be conserved by formation of high energy phosphate bonds (ATP). Evidence has been presented that ether anesthesia can uncouple oxidative phosphorylation (47). Finally, the energy conserved as ATP must be utilized in the performance of cardiac work. Virtually no information is available relative to the effects of anesthetics on energy utilization.

Our work has been concerned with the manner in which anesthesia influences the myocardial uptake of substrates utilized in energy production. Since many workers have reported that ether anesthesia increases blood glucose, lactate, and pyruvate (39), the effects of these changes on myocardial metabolism in dogs were investigated (35). During ether anesthesia arterial glucose, lactate, and pyruvate concentrations rose, but nonesterified fatty acids (NEFA) decreased. The myocardium readily extracted pyruvate and lactate, but the extraction of NEFA was

[6] Supported by Grant No. HE 06967-04, National Heart Institute, Public Health Service.

decreased. Furthermore, in every case except glucose the myocardial extraction was a linear function of the arterial concentration. Associated with these changes, the myocardial respiratory quotient rose, suggesting that the extracted carbohydrate substances were oxidized. The pattern of lactate and pyruvate extraction did not differ significantly from that in unanesthetized animals receiving lactate or pyruvate infusions. The decreased extraction of NEFA was probably related to the decreased arterial NEFA concentration seen during ether anesthesia.

With glucose the situation was slightly different. In the unanesthetized animal receiving a glucose infusion, the myocardial extraction exceeded 20 mg% if the arterial concentration was above 120 mg% (38). With hyperglycemia during ether anesthesia, however, the extraction was usually less than 20 mg% at arterial concentrations greater than 120 mg%. Ether anesthesia appeared to alter the ability of the myocardium to extract glucose.

Because myocardial glucose extraction was reduced below normal during ether anesthesia, further studies were performed to elucidate the mechanism (36). Mongrel dogs were prepared by inserting polyvinyl catheters into the carotid artery and the jugular vein, enabling us to study the animals repeatedly over long periods of time in both the conscious and anesthetized states. A series of experiments was designed to test the response of the dogs anesthetized with ether or halothane to an insulin tolerance test, a glucose tolerance test with and without insulin, and a fructose tolerance test. To eliminate bias and residual effects a Latin square experimental design was used in which treatments were systematically randomized at 48-hour intervals and each dog was used as its own conscious and anesthetized control. This design contributed 96 degrees of freedom to an analysis of variance. Glucose was measured enzymatically and fructose by the Somogyi-Nelson method on blood samples obtained from the carotid artery. The depth of anesthesia was controlled by anesthetizing the animals until the lid reflex had just disappeared. A nonrebreathing system was used with oxygen as the diluent gas. An inspired concentration of 1 to 1.5% of halothane (continuously monitored with an ultraviolet analyzer) was required to keep the animals anesthetized. Similar conditions were attained with 3 to 5% ether.

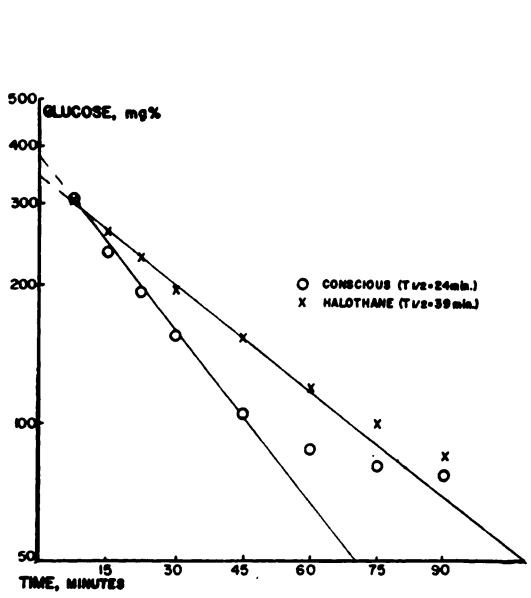
Arterial glucose concentrations were determined at 15-minute intervals for 2 hours in conscious animals and those anesthetized with halothane. Glucose decreased to lower levels in the anesthetized animals and these animals were less able to reverse the hypoglycemia and stabilize the blood glucose than were the conscious animals. When the same two groups were given a glucose tolerance test (1 g/kg), the mean half-times of return of blood glucose to normal were significantly prolonged in the anesthetized compared to the conscious animals (Fig. 17). This suggested that plasma removal of glucose was impaired during halothane anesthesia. If insulin (0.1 unit/kg) was administered simultaneously with the glucose tolerance test, not only were the mean half-times reduced in both groups, but they became identical (Fig. 18). Thus, insulin was effective during halothane anesthesia.

The same pattern of experiments was repeated with fructose, which is known

to pass freely across liver cell membranes and is not taken up to any appreciable extent by extrahepatic tissues. The mean half-time for fructose removal was extremely short, and identical in conscious and anesthetized groups. The results agree with those of Wick *et al.*, who showed that removal of fructose from plasma is independent of membrane transport or the action of insulin (104). A rise in the arterial glucose level was seen shortly after the fructose was given, and this was greater in the anesthetized group. Apparently there was no impairment in the conversion of fructose to glucose in the liver, and the rate of conversion to glucose exceeded the rate of removal of glucose from the plasma. Finally, if an endogenous glucose load was given by injecting glucagon (150 μg/kg), the arterial glucose rose in both groups and was higher in the anesthetized group. Therefore, the liver of the anesthetized animal was responsive to glucagon at this dose.

Similar results were obtained in studies during ether anesthesia in dogs. Contrary to the situation seen during halothane anesthesia, ether was accompanied by the characteristic hyperglycemia (Fig. 19). The administration of insulin (0.1 unit/kg) decreased arterial glucose concentration. Administration of insulin simultaneously with the glucose tolerance test resulted in a significant decrease in the half-time; this finding provides further evidence that insulin was effective during ether anesthesia. The rate of removal of fructose was rapid and the findings were much like those during halothane anesthesia. Results similar to those seen with ether and halothane have been observed with one animal during trichlorethylene anesthesia.

Prolongation of the glucose tolerance test during ether or halothane anesthesia



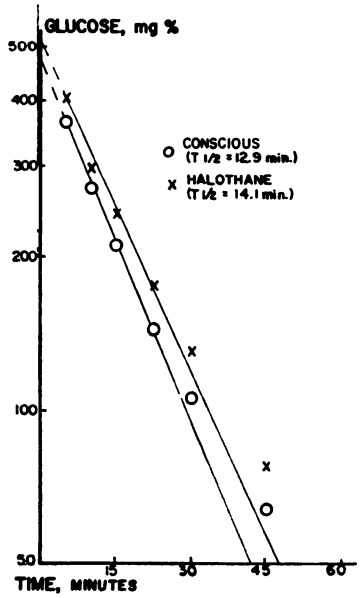


FIG. 17 (*left*). Prolongation of glucose tolerance test during halothane anesthesia. (Eight dogs; $P < .0005$.)

FIG. 18 (*right*). Effect of insulin, given simultaneously with a glucose tolerance test, in reversing effect of halothane anesthesia (statistically identical).

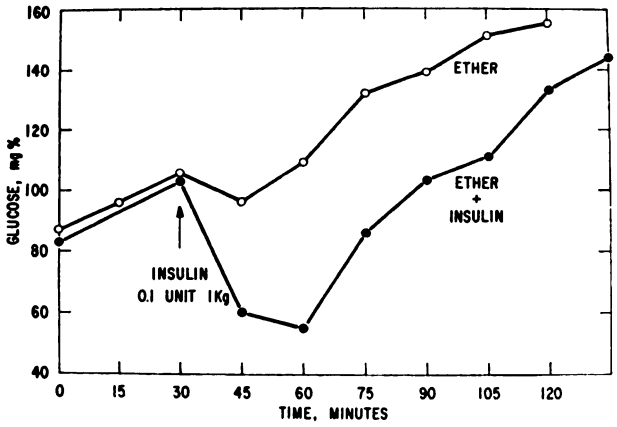


FIG. 19. Effect of insulin in lowering arterial glucose levels during ether anesthesia.

and the reversal of this effect by insulin suggest that these agents alter cell permeability in muscle or adipose tissue. The lack of effect of these agents on the fructose tolerance test supports this view. This observation of hypoglycemia, rather than hyperglycemia, during halothane anesthesia probably reflects the diminished or absent catecholamine response with halothane (84). Why the anesthetized animals were unable to supply their own insulin and glucagon in response to hyperglycemia or hypoglycemia is not clear. Possibly, ether and halothane interfere with the release of insulin and glucagon from the pancreas, transport in the plasma, or action at the cell membrane. Recent workers have shown that insulin can exist in plasma as a biologically inactive complex (5). Stadie *et al.* have indicated that fixation of insulin to tissues is necessary for its physiological action and that inhibitors may act by alteration of the bound form or prevention of binding (96).

Ether and halothane may possibly compete with insulin at the cell membrane; and if so, increasing the insulin concentration might overcome this inhibition. Park *et al.* have postulated a mechanism for the transport of glucose across cell membranes and have demonstrated that it follows Michaelis-Menten kinetics (75). Hackel demonstrated that insulin lowered the threshold for myocardial glucose extraction (41). Failure in the dog heart-lung preparation has been associated with decreased myocardial glucose utilization (40). Administration of insulin increased myocardial glucose utilization and restored the competence of the preparation (6).

To illustrate our hypothesis in another way, insulin opens the gate for membrane transport of glucose, whereas ether and halothane, as counterbalances, tend to close the gate. The actual rate of transfer of glucose then results from the balance between the two conditions. Our results could explain why myocardial glucose extraction is reduced during ether anesthesia.[7]

[7] The author wishes to thank Grant T. Phipps, Ph.D., for assistance with the experimental design and statistical analysis.

Dr. T. J. Regan: The effects of a number of anesthetic agents on the heart involve interaction with the sympathetic nervous system. The studies reported here are concerned with the response of the left ventricle to *l*-epinephrine infused into the left coronary artery in regard to the extraction and utilization of substrates and the myocardial contractile response. The animals were anesthetized with morphine and pentobarbital, and artificially ventilated. Catheters were placed in the coronary sinus and the aorta for blood sampling, in the left ventricle for pressure measurements, and in the left coronary artery to minimize systemic effects during the arterial infusion of epinephrine, 5 μg/minute for 90 minutes.

The well-established effect of catecholamines in stimulating glycogenolysis was reflected in a diminished glucose extraction by the left ventricle, presumably mediated by the high tissue levels of hexose phosphates. There was an associated increase in contractility for 50 minutes, using the maximum rate of left ventricular pressure rise as an index of contractility. After 50 minutes contractility progressively diminished, and before the end of the epinephrine infusion contractility was generally below control.

Coronary blood flow by the Kr^{85} method increased an average of 15 % during infusion and declined after the infusion to control values. After an early uptake of potassium and phosphorus, increased concentration of these ions was found in coronary venous blood beginning at 60 minutes; the later appearance of glutamic oxalacetic transaminase (SGOT) is consistent with a necrotic process in the myocardium. Since coronary flow was not diminished, this was presumably a direct tissue effect of the hormone. From the low R.Q. of the fasted state when free fatty acids largely provide for oxidative needs, a rise in the R.Q. occurred during epinephrine infusion, coincident with glycogen breakdown and a transient period of excessive production of lactate. These changes reverted to control before the end of epinephrine infusion. However, substrate uptake was substantially altered, as evidenced by the reduction in extraction of free fatty acids and glucose. The substrate requirements were apparently met by plasma triglyceride, since the extraction of triglyceride was substantially enhanced in the presence of a relatively constant arterial concentration. This switch in substrate dependence was not seen in control animals infused with saline. On analysis of left ventricular tissue after 1½ to 2 hours of epinephrine infusion, the triglyceride content was found to be two to three times the normal concentration.

Accumulation of triglyceride in the liver has been repeatedly demonstrated after ethanol administration and there is evidence that this acute effect is dependent upon the sympathetic nervous system. Since plasma catecholamines are increased in this circumstance, the reaction of the myocardium was studied in light of the above epinephrine studies. Fifteen percent ethanol was infused systemically at a rate of 0.1 ml/kg/minute for 2 hours in 20-kg animals; this produced peak plasma ethanol levels of between 150 and 250 milligrams per 100 ml. Liver triglyceride levels were increased 2- to 3-fold at the end of 6 hours.

The hemodynamic effects of the infusion of ethanol consisted of a transient increase in heart rate during the 2-hour infusion. There was no effect on systemic arterial pressure, but an early and persistent rise of left ventricular end-diastolic

pressure was present, associated with decreases in the maximum rate of pressure rise and in the cardiac output. During the 5-hour period of hemodynamic observations there was a persistent depression of left ventricular function.

After the first hour of infusion, the potassium and phosphate ion concentrations in coronary venous blood began to increase, followed later by leakage of the enzyme SGOT, a finding analogous to that associated with necrosis produced by coronary artery ligation. Although coronary blood flow was reduced for the first 90 minutes after ethanol, this response appeared to be secondary to a decrease in contractility, rather than a primary vasoconstrictive action. Further, whereas ligation of a coronary artery is attended by enhanced oxygen extraction and lactate production in the myocardium these changes were not present after ethanol; this suggests that the coronary blood flow was probably adequate for the reduced cardiac activity.

The myocardial metabolic response to alcohol was biphasic. There was first a rise in respiratory quotient by 90 minutes, reflecting increased carbohydrate oxidation, with a concomitant rise in glucose and lactate extraction. The R.Q. subsequently reverted to lower levels; this change was presumably related to a predominance of lipid oxidation. Yet free fatty acid, the major substrate in the fasting state, was extracted in diminished quantities after the infusion of ethanol had been stopped. The predominant substrate was plasma triglyceride, with an enhanced extraction persisting through 5 hours of observation (Fig. 20). Infusion of an isocaloric amount of glucose instead of alcohol did not affect contractility, and had no significant effect on the extraction of triglyceride by the heart. By 6 hours left ventricular triglyceride concentration was significantly increased after ethanol, compared to those of fasted or glucose-fed animals (Fig. 21).

Thus infused ethanol produced a substrate response in the heart that is similar to that after epinephrine, and may well be mediated by this hormone. The diminished free fatty acid extraction would limit the role assigned to this substrate in the tissue accumulation of triglyceride, while plasma triglyceride appears to be the major source of energy. If the pathways for lipid oxidation remain open, this metabolic process may be taxed by an excess uptake of triglyceride or inhibited at some point by catecholamines or ethanol. Although the altered myocardial lipid levels may contribute to the pathophysiology described, a response secondary to other tissue injury has not been excluded.

COMMENTS ON ROLE OF ENDOCRINES IN METABOLIC EFFECTS OBSERVED DURING ANESTHESIA

Dr. G. F. Cahill: Rather than review the role of the endocrine system in anesthesia, a subject which really has not progressed much in the past 2 or 3 years, I thought I would make a few general comments related to endocrines, and present my philosophy of the utilization of fuel.

We have heard several papers discussing glucose utilization, the importance of it, and the possible diabetogenic effects of certain anesthetic agents, which may be related to stimulation of the adrenal cortex and medulla, or to the sympathetic nervous system. I would like to point out that exclusion of glucose from tissues

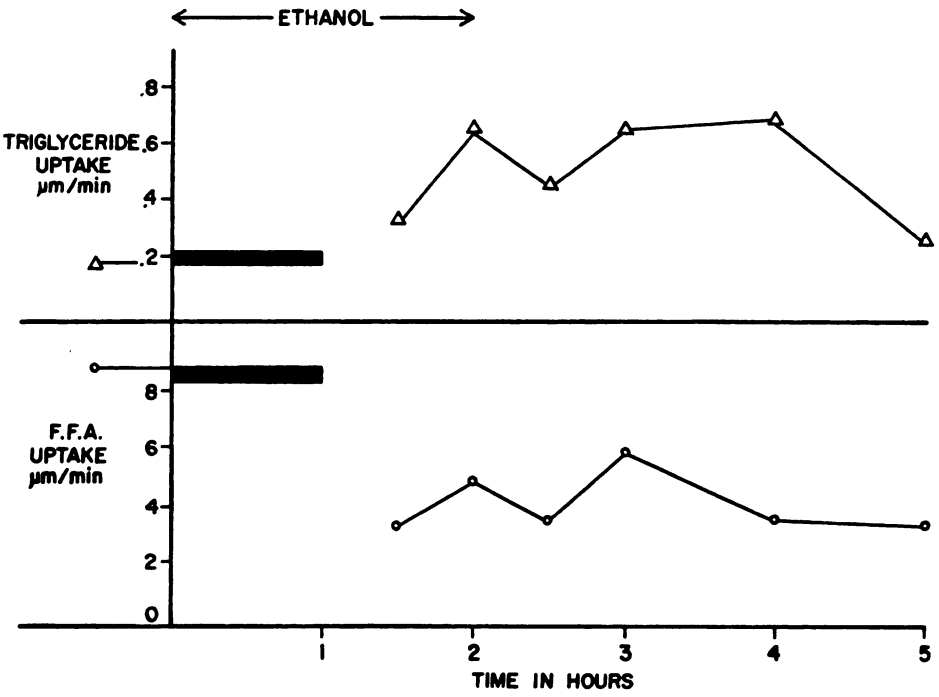


FIG. 20 (*above*). Substrate uptake was calculated from the product of coronary plasma flow and the arteriovenous difference. This was not done during the first hour because of a dilutional decline in arterial concentration of substrates.

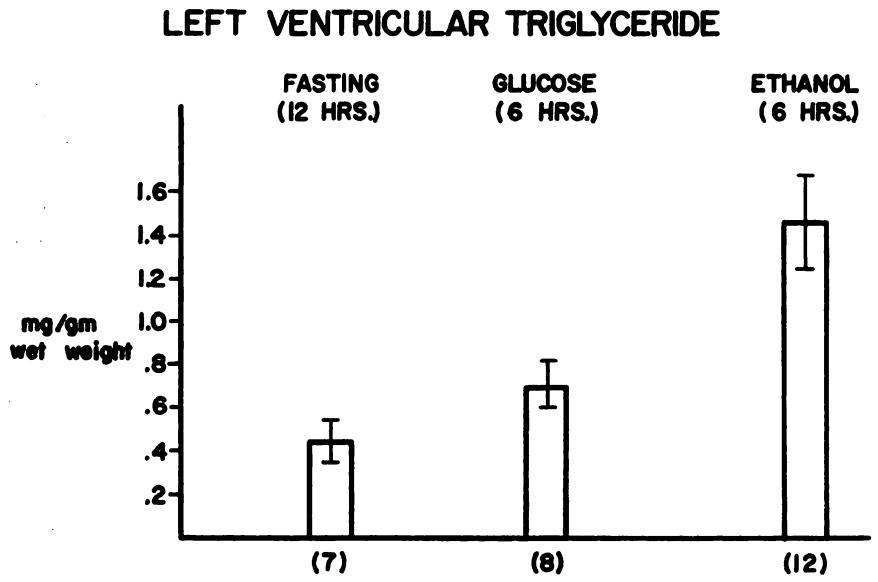


FIG. 21 (*below*). The relative increase in triglyceride depicted here represents an absolute substrate increment, since tissue water content was unchanged.

in general is probably more important to the survival of the animal than the capacity to get glucose into the tissue, with one single exception.

Dr. Galla has presented the so-called Park hypothesis of the mobile carrier system for getting glucose into tissues. Consider the membrane of a typical muscle or fat cell, the two primary tissues into which glucose disappears under the influence of insulin. Glucose is able to get into these tissues in the presence of insulin, and once inside the tissue, in a very small quantity, usually not measurable, it reacts with hexokinase, which has a very high affinity for glucose, and is phosphorylated. If this is fat tissue, the glucose is converted into fat. If it is muscle, it is either utilized by glycolysis and the tricarboxylic acid cycle for energy, or else a small portion of it is stored as glycogen. As far as we can tell, the major proportion of glucose in the body which disappears under the influence of insulin probably goes into fat tissue. Now, this is the so-called "fed" state.

During fasting, as we know from many experiments, glucose is almost totally excluded from all tissues with the single exception of the nervous system. Obviously the importance of this in evolution is paramount, because the nervous system requires glucose for energy. If an animal is going to fast for a long period of time, it wants to spare every calorie that it can. The central nervous system for all practical purposes can use only glucose. Therefore, it benefits the animal to spare glucose from all tissues except the central nervous system. In fact, the current theory now is that the mutation for diabetes, which must have occurred centuries ago, may have been a selective factor favorable to survival. Now, in current well-fed society, diabetes is a serious detriment.

In other words, the capacity to exclude glucose from tissues when an animal ate every 2 or 3 months, rather than three or four times a day, and twice at night as we Americans do, may have been an extreme evolutionary advantage. I think this really bears more than a casual thought, because the diabetic gene is pandemic to all civilizations and races throughout the world. So the original derivation of the gene occurred far before *Homo sapiens* appeared on the scene.

So it is the role of tissues during fasting or stress to exclude glucose. In other words, this is Jay Tepperman's "keeper-outer" theory. More important, whenever an animal is exposed to any stressful situation, the endocrine and nervous systems attempt to keep glucose out of tissues, again excepting the brain.

One of the most important of these is the catecholamine system. The exposure of an animal to catecholamines excludes glucose from many tissues. The cell fills up with glucose-6-phosphate from glycogenolysis and, according to the classical theory, glucokinase is inhibited and free glucose accumulates in the cell. This is one way of sparing glucose from being metabolized.

Perhaps more important, if one exposes muscle to any of many other substrates, "ketone bodies" being perhaps the best example, there is some signal that other fuel is coming into the muscle, and somehow glucose entry is inhibited. If one gives diethyl ether to an animal, the sympathetic nervous system is stimulated, perhaps there may also be a direct action of the anesthetic agent on the adipose tissue itself, and free fatty acids in the serum increase; the muscle cell gets the signal somehow, can direct the cell membrane to exclude glucose, and can me-

tabolize the fatty acids or the ketone bodies (even when insulin is present) instead of glucose.

Other energy systems can also feed into this area. Glucagon exerts a definite effect on muscle metabolism, by mobilizing amino acids, and it is clear that glucagon also provides increased ketone production in certain species. In fact, in lower forms, glucagon may serve primarily as a gluconeogenic agent.

Now let us get on to the brain cell. One fascinating point is that no matter what the blood glucose is, as long as there is enough of it (over 30 mg%) the brain will take out a given mass of glucose per unit time. What is the signal for the brain cell to extract this quantum of glucose? We know that 95% or more of the energy of the brain is probably derived from glucose. What tells the cell membrane or the so-called barrier which we all read about, to let in more glucose? Apparently there is a control. The reason I am pointing this out is because I heard a most fascinating point by Dr. Dulin in his presentation, that he feels the brain can concentrate galactose. People have looked for free glucose inside the brain; we have looked for it, and we have been unable to find any. Of course it is difficult to do it, because the brain is always utilizing glucose, and even in the extracellular fluid you have a lesser glucose concentration than you will have in circulating plasma. So to get adequate glucose spaces in the brain is extremely difficult. But, if Dr. Dulin is correct, the brain can concentrate carbohydrate. This makes the brain unique compared to all other tissues, with two exceptions, namely, the renal medulla and the gastrointestinal mucosa. These are the only two places in the body where carbohydrate can be concentrated against a gradient. So I am just pointing this out, because I think this may be one of the most exciting new areas for research.

Why should brain be taking up all of this glucose? As a general physiologist, I look at the brain as one large "ATPase" system, to use the loosest sense of the word. It is doing absolutely nothing but making heat, taking up glucose and oxygen and putting out CO_2 and calories. Dr. Quastel pointed out one thing fascinating to me, namely, that the brain has to make a lot of acetylcholine, and this of course takes energy. The other thing it may be doing is pumping out ions through leaky membranes, which again is another "ATPase" system. I tried to look where this brain energy is going. Could it be calculated just by the sodium pump? Thermodynamically one can correlate the sodium ion pump in the kidney with total energy exchange. Whether anyone has looked at this in the central nervous system, I don't know.

Now let us end with 2 or 3 minutes on the liver. The liver is unique in that it has no membrane barrier to the simple entry and egress of glucose or any other monosaccharide. There are probably good reasons for this, because the liver is producing glucose about 20 hours a day, and only about 4 hours a day is there a net inflow. In addition, other monosaccharides, such as galactose or fructose, must enter in order to be converted to glucose for assimilation by other tissues.

Many people recently, including Dr. Vester, who is here today, have shown that the liver is unique in possessing two enzymes which phosphorylate glucose. One of these has a low affinity for glucose, but is present in large quantities, and

is insulin-dependent. The other enzyme is independent of insulin and is there unchanged in activity day in and day out. In addition there is recent evidence that there are probably two pools of glucose-6-phosphate, one pool destined to go to glycogen, namely that glucose-6-phosphate coming *via* the insulin-sensitive enzyme, and the other pool feeding glucose to glucuronic acid, glucosamine, and many other compounds. The insulin-sensitive enzyme is extremely labile. I point this out because, as you remember, the liver also has another important enzyme, glucose-6-phosphatase, the enzyme which is lacking in von Gierke's disease. Whether or not liver takes up sugar or produces sugar is the net result of the summation of all of these enzymes. If one enzyme should be inhibited in any way, then the net effect is for the liver to produce sugar, or to take up sugar, and this is certainly what happens. In Dr. Brauer's interesting experiments on the perfused liver exposed to carbon tetrachloride, glycogen was breaking down. The liver originally was in a steady state with a blood sugar about 110 mg% because these enzymes were in balance. If you inhibit one enzyme, perhaps one of the two glucokinases, the balance is disturbed and the liver begins to produce glucose to try to reach a new steady state with a higher blood glucose level. This may be the cause of the rise in blood glucose in certain types of anesthesia.

EXCESS LACTATE AND ANAEROBIOSIS

Dr. W. E. Huckabee: One point I think worth emphasizing, especially in intact creatures, whatever the stress being studied, is this: whether or not tissue hypoxia is present is a difficult decision to make. It cannot be made on the basis of arterial or venous blood oxygen or rates of blood flow, or rates of oxygen consumption. All you can ever get from such measurements is rate of delivery of oxygen. If the notion of tissue "hypoxia" has any meaning significant to the function of the tissues, it seems certain that it must mean, as Barcroft said many years ago, an inadequacy of oxygen delivery relative to the needs of the tissues. Measurement of the amount of oxygen delivered will never lead us to the answer to the important question, namely: what are the needs of tissues? What is the relationship between the delivery and the need? It seems likely that the answer to the question of whether there is inadequacy of oxygen delivery (or inadequacy of oxidation due to interference by anesthetic or other agents with electron transport, for that matter) will have to be decided by having a look at the metabolism of the tissues, rather than the delivery or the concentrations of molecular oxygen.

As you recall, proposals were made many years ago that the production of lactic acid by cells was the metabolic indication of inadequacy of oxygen delivery that everyone wants. This proposal did not hold up very well. It was grasped eagerly by everyone working with intact animals because an approach was badly needed. Techniques usable *in vitro*, and in various kinds of highly artificial animal preparations, were extremely difficult to apply in real life situations, whereas measurements of metabolite exchange are within the range of possibility in intact animals. The difficulty about lactic acid production by tissues in relation to hypoxia was not that there was any doubt that lactic acid production did (at least usually) occur under circumstances in which there were other good reasons to believe that

oxygen delivery had been interfered with. The big problem was that lactic acid production occurred under a lot of other circumstances in which it was just not possible to believe that oxygen delivery had been interfered with.

A similar situation was being seen *in vitro* by biochemists over these years, and they coined terms like "aerobic glycolysis" and "anaerobic glycolysis"; that is to say, lactate production from glucose sometimes did occur if you gassed the Warburg chamber with nitrogen, but it also sometimes occurred when you gassed the tissue with oxygen. If you got lactate production because there was not any oxygen, that clearly was "anaerobic" glycolysis, and was not due to hypoxia. In the intact animal, I think you will see in a great variety of experiences that there are similarly two kinds of lactate production. There are those which occur, as has been known for many years, in situations which one can reasonably believe are associated with interference with oxygen delivery, relative to work performance or other characteristics which reflect need of the tissues. Then there are a lot of increases in body lactate which occur in a variety of physiological circumstances that do not seem likely to be hypoxic.

Perhaps one could revive the terms "aerobic and anaerobic glycolysis," but instead of applying "aerobic" or "anaerobic" in the sense of there being oxygen or no oxygen (in the tissues of the intact animal, that is something we don't actually know), one might decide to say that there is an "aerobic glycolysis," *i.e.*, lactate production associated with no increase in the DPNH/DPN ratio within the cells, and there is lactate production which is associated with, and caused by, an increase in the DPNH/DPN ratio.

Now, if these separate kinds of glycolysis would just occur by themselves, we could make some kind of judgment in each case about whether the DPNH/DPN ratio is changed or not. If it had changed, then we would assign all the lactate production in that situation to anaerobic glycolysis. This would be simple. The chances are, I am afraid, that what one might picturesquely call aerobic glycolysis and anaerobic glycolysis sometimes occur simultaneously; of the lactate produced by an organ, part may be produced because the oxygen delivery is not adequate to keep the DPNH/DPN ratio normal, and part is produced for other reasons.

The equation of the lactic dehydrogenase system would itself make one think of there being two factors which would lead to a change in the amount of lactate. That is, if pyruvate levels change and there is no change in the adequacy of oxygen delivery, lactate nevertheless would change. If pyruvate concentration rose, lactate would rise, and in an organ being perfused by blood, lactate would come out into the blood. More than this one should not attempt to say in intact animals because the difficulties on that particular point are multiple. The question of whether this theoretical system can be applied at all in the intact animal is based upon two facts: this metabolic system is cytoplasmic, and its components, with the exception of DPN, are highly diffusible. The hope is, as it so often is among physiologists of intact tissues, that analysis of the extracellular fluid, that is, of the blood, will reflect events in the interior. Certainly the concentrations in blood are not the same as those inside the cells.

In addition to this possible cause of lactate change, namely a primary change in pyruvate, there is the other one named above, change in DPNH/DPN. But

if this equation entirely represents the lactic dehydrogenase system, then there are no others. It is possible that the hydrogen ion ought to be entered into this, but the molar changes in hydrogen ion concentration are extremely small in the intact animal not subjected to cataclysmic experiments. If there are then two, but only two, causes of lactate change we are in a fairly nice position to try to figure out which of these is accounting for the lactate production in any experiment.

A variety of calculations might be used to separate the two moieties of lactate. A suggestion for a way of doing this is simply: a) the amount of lactate which appears in the process of changing the lactate-pyruvate ratio, and b) the remainder. The lactate-pyruvate ratio has a relationship to what one wants to know when one is looking for hypoxia, that is, it bears a relationship to the DPNH/DPN ratio in pure systems at equilibrium. Now, what relationship the blood L/P ratio will have to the cellular DPNH/DPN ratio is quite another question, but I want to reserve the discussion of that for a little later. If lactate appears when there is no change in DPNH/DPN, this is what one might refer to (by analogy with the older term) as aerobic glycolysis. But if lactate is produced because DPNH/DPN has increased, we might refer to it as anaerobic glycolysis, and look upon it as a reflection of inadequate oxygen delivery inside the cells. This quantity of lactate is what has been referred to as "excess lactate."

The question of whether blood concentration ratio of lactate and pyruvate has any realistic relationship to cytoplasmic DPNH/DPN ratio in intact animals is one that cannot really be answered definitively. The kind of information one wants to have first of all is just how diffusible these substances are. There has been quite a number of studies on the actual concentrations in tissues and blood at a variety of production rates and steady state levels and these indicate, of course, that the concentrations are not the same. But they do indicate that the levels change by the same amount during physiologic experiments, like, for instance, hypoxia and muscular exercise. This is the kind of thing physiologists of the intact animal regularly use in applying principles of biochemistry to the real living animal. We will not try to judge what is going on in the cells at one moment, but the change occurring from one steady-state period to another steady-state period will be free of many objections, especially those having to do with diffusion gradients occurring between tissues and blood, and within tissues and cells. I want to stress that these kinds of data are really difficult enough to interpret if you do have two steady states compared with each other. But if you don't, the experiment really is hardly worth doing. The thought that measurements of blood lactate and pyruvate will, without any trouble at all, give you what you want to know, I am afraid is wishful thinking. It is very important to work with differences between steady states; although these are sometimes difficult to obtain in the experiments we want to do, I am afraid it is unavoidable.

Several problems of application of these principles to intact animals were evaluated in experiments that were previously published (46) on the effect of infusing pyruvate into animals. First of all, blood pyruvate was made to increase 5- to 10-fold to a new steady-state level in 5 to 10 minutes. The blood lactate also rose to a new steady-state level very quickly. Of course, this is what the equation of

the LDH system predicted would happen in a pure system. It turned out that it happened also in the intact body, which therefore behaved sufficiently like an *in vitro* system to permit us to go on. This rise in lactate occurred without any change in any parameter of oxygen supply, of course, and without oxygen debt. It serves to illustrate what I have called "aerobic glycolysis" insofar as lactate is concerned. When we calculate the so-called excess lactate in this experiment, we find there is none in the steady state. In the 5 minutes of unsteady state during rising and falling concentrations, there was a little excess lactate, but not an amount we regard as significant ordinarily. Thus the lactate from tissues adjusted itself very rapidly to the pyruvate from blood and related itself to pyruvate in the same way as it had been related in the control state.

It is interesting, I think, that there is a double transit of these substances. Pyruvate was infused into the blood, had to cross the capillaries into the tissues, there reach a new equilibrium with lactate, a larger amount of lactate being produced in the tissues; and the lactate had to diffuse back out again into the blood. And yet the delay in matching of these two levels to each other in such a way as to keep excess lactate zero was surprisingly slight.

Several physiologic circumstances seem to cause primary changes in pyruvate and the associated "aerobic glycolysis." The most troublesome of these circumstances, because it is the most common and because it causes the most marked change in pyruvate, is a change in hydrogen ion concentration. Hyperventilation of the intact animal, for instance, will cause marked increases in blood lactate; a 10-fold change in the body lactate of an animal, in the course of 15 or 20 minutes, is something which cannot be regarded as a mere detail. It is much larger than the lactate changes observed in many severely and even fatally hypoxic experiments. During hyperventilation, from which I need not remind you the animals recover uneventfully (and so do the medical students who do this same experiment, and so did I), there is no evidence, from any other means we have of measuring it, that there is any hypoxia. No oxygen debt is formed, there is no decrease in arterial blood oxygen, there is no decrease in cardiac output, and there is no alteration in oxygen consumption except during the unsteady state. Well, there is a lot of lactate production, but there isn't any excess lactate production. That is, there is no change in the lactate-pyruvate ratio. It becomes very difficult to interpret glycolysis of this sort without having the notion available to you that there is such a thing as aerobic glycolysis, and that it has a different physiologic meaning from anaerobic glycolysis, the latter suggesting inadequacy of oxygen delivery to the tissues—a very important difference.

It is difficult to test the idea that anaerobic glycolysis, that is the amount of lactate produced in the process of changing the lactate-pyruvate ratio, has a meaning in terms of the amount of oxygen needed by tissues but not supplied. I don't think a critical experiment has been done, but there is one that was done to test the possibility that there is such a physiologic significance, and that is the comparison of excess lactate with the oxygen debt. The standard technique is to produce an oxygen debt with exercise. The correlation between the cumulative lactate and oxygen debt, whether in exercise or in respiratory hypoxemia was

once thought to be good, but this cannot be substantiated today. The blood lactate may vary widely during the time an oxygen debt is being paid off. Oxygen debts fall along a very standard kind of curve, a pattern very reproducible in animals and man; as this occurs, the quantity of excess lactate falls off in a similar pattern with a similar time period. Most of these measurements are subject to a lot of technical error, but an approximation having in several hundred comparisons a mean variation of 15% seems to me to suggest that the two estimates had a similar meaning in the particular experiments in which these were done.

It is possible to make the lactate curve gyrate around in a very strange way by simply trying to keep it from looking like the oxygen debt curve. Exercise in a well-trained athlete may be associated with a fall in pyruvate. In this case the lactate is lower than the oxygen debt, as would have been predicted from the equation. In other words, it is possible to have more excess lactate than there is total lactate by this calculation.

In the original comparisons of excess lactate with oxygen debt, a great point was made of carrying out this comparison both in muscular exercise and in respiratory hypoxemia, although oxygen debts are a great problem to measure in respiratory hypoxemia. The reason for this was as follows. Although oxygen debt was classically described in muscular exercise, this is a hypermetabolic state. When the total rate of turnover of the electron transport system is increased, there is the possibility that a change in the state of oxidation of DPN (*i.e.*, of that portion of cellular DPN affecting our results) would be caused by inherent slowness of one of the steps, which would then become rate limiting. On the other hand, hypoxemia is not associated with hypermetabolism but is accompanied by a slightly reduced oxygen consumption. Similar correlations between excess lactate and oxygen debt occurred with respiratory hypoxemia in a large number of animals. The "oxygen debt equivalent" of a mole of excess lactate was the same in both kinds of hypoxia, as nearly as it could be determined. There was no evidence that excess lactate was produced without hypoxia in hypermetabolic states. More recently we have produced markedly hypermetabolic states in animals with dibromo- and dinitrophenol. No excess lactate is found in these experiments. In fact, blood L/P ratio goes down just as one would have predicted from the mitochondrial DPNH/DPN expected in this situation. These are the attempts that have been made to assign some quantitative value, like oxygen equivalent, to the excess lactate. Actually they all come out to be about the same as Meyerhoff's old figure for the equivalence of lactate and oxygen debt. One could reason that actually a half mole of oxygen is needed per mole of lactate disappearing, and that also comes out to be about the same as Meyerhoff's figure. A lot of other different values could be given to it. Its ATP equivalent, for instance, is different from its oxygen equivalent, and so on. But these are perhaps issues that will some day be resolved by more definitive experiments.

We have done a number of experiments attempting to elevate the blood lactate and pyruvate by means other than hypoxia. One of the ways is to use oxythiamine. Hyperventilation also, of course, adds a great deal.

In animals which have an initial very high lactate with no excess lactate in

normal ratio, if various kinds of hypoxic experiments are done, there is suddenly an accumulation of excess lactate. These accumulations of excess lactate tended to remain standard, even though they started at very high levels of lactate and pyruvate. But what happened in these experiments was that there was a very small change in lactate/pyruvate ratio, relative to that of the same experiment done from normal starting values. If one applies a whole series of hypoxic experiences designed to be increasingly severe, one gets increasingly large accumulations of excess lactate. At first the lactate/pyruvate ratio changes considerably; but the changes grow smaller and smaller until they become almost negligible, so that there is a curvilinear relationship of lactate/pyruvate ratio with other estimates of intensity of hypoxia. This is the only objection I can see to the use of the lactate/pyruvate ratio as a qualitative indication of hypoxia. I think there are some objections in principle in the sense that this is a dimensionless number. It is very difficult to use with other physiologic values; excess lactate may be erroneously dimensioned, but it at least can be assigned dimensions.

This has been a somewhat heterogeneous presentation of examples of problems that have had to be faced, that a lot of people have faced, in working with the question of whether oxygen supply is adequate to metabolic needs in intact animals. These have been some of the situations in which a slightly more complicated view of lactate metabolism seemed to produce a workable and usable idea. Now, the extension of this to experiments in which these tests have not been applied of course is done by each man at his own risk. Whether there would be this same kind of relationship of excess lactate and oxygen debt in somebody else's experiment is a guess. There is that risk.

"EXCESS LACTATE" AND ANAEROBIOSIS

Dr. W. G. Vester: For most animal species, molecular oxygen is essential for life. Cells deprived of oxygen experience severe metabolic changes and, in the main, cannot survive for long periods of time in the oxygen-free state. Accordingly, studies of the biochemical indices and consequences of oxygen lack at the cellular level have been, and continue to be, of great interest. Huckabee (46) has reported that "excess lactate" in the blood reflects changes in the ratio of DPN^+ to DPNH in the cell. We would like to present two series of studies of the relationship between oxygen deprivation and circulating levels of lactate and pyruvate in the blood. The first series is abstracted from a recently published paper (89) describing observations of the biochemical consequences of various forms of oxygen deprivation in the freshwater turtle. The second series contains data derived from as yet unpublished studies in humans.

Some animals can tolerate anoxia for surprising periods of time. Knowledge of the adaptations permitting survival undoubtedly will help provide an understanding of the biochemical consequences of oxygen deprivation in all species. The ability of the turtle to withstand prolonged periods of diving is well known. Others have suggested that this facility is dependent upon extraction of oxygen from water (72). Belkin has recently shown that the administration of iodoacetate to a turtle blocks its ability to tolerate anoxia so that it rapidly dies when placed

under water (7). These data, of course, suggest that the ability of the turtle to withstand prolonged diving is related to its ability to depend on anaerobic metabolic processes for long periods of time. Accordingly, the effects of oxygen deprivation on freshwater turtles of the genus Pseudemys have been studied. The turtles used weighed between 1 and 3 kilograms. Blood sampling on a repetitive basis was made possible by intubating a peripheral vein in the lower extremity with a small polyethylene catheter that was tied in place and filled with a heparin solution between samples. Blood pH was measured by the use of a glass electrode and plasma CO_2 tension was determined with the Severinghaus CO_2 electrode. Plasma oxygen tension was determined by a polarographic technique using a modified Clark platinum oxygen electrode. Blood lactate and pyruvate were determined enzymatically, with modifications of the techniques of Scholz and Bücher (94). With these modifications, duplicates agree within 2% and recovery averages 100% ± 3%. Excess lactate was calculated in the manner described by Huckabee (46). The first group of turtles was immersed in water equilibrated with 100% oxygen. The head was sealed in a gas-tight mask and the cloaca was tightly sewed. This prevented any internal access to water and the oxygen dissolved therein. In this group the blood O_2 tension declined to values approaching zero within 1 to 3 hours and remained there for the remainder of the dive—as long as 8 days. This observation indicated that no significant oxygen extraction from water takes place. Comparative studies indicated that diving animals without face masks and cloacal occlusions behaved in a fashion identical to these animals. In the next group, the interrelationship of dissolved gases and blood lactate and pyruvate levels during diving was measured. Figure 22A presents pH and P_{CO_2} changes for the first 24 hours of a representative turtle dive. In all of these animals, the plasma pO_2 dropped to levels approaching zero in 1 to 3 hours and remained there throughout the entire period of the dive. CO_2 tensions rose steeply during the dive and reached a plateau of approximately 100 to 150 millimeters of mercury within 24 to 48 hours after the dive commenced. Plasma pH fell sharply and remained low. Not illustrated is the plasma bicarbonate concentration, which likewise fell; this finding indicated that the development of acidosis is not merely related to CO_2 retention but is contributed to by increased concentrations of fixed acids. Figure 22B presents the changes observed in blood lactate and pyruvate in the same turtle during a representative dive. The lactate concentration in all of the animals rose sharply, reaching very high levels. Pyruvate concentrations did not change in a consistent fashion in that they rose in some animals and fell in the others. With prolonged diving, pyruvate concentration tended to fall. In all animals studied in this fashion, the increase in lactate greatly exceeds the increase in pyruvate. As a result, excess lactate as calculated by Huckabee's method increased rapidly in a fashion similar to that shown for the animal in Figure 22B.

The next group of animals studied was subjected to inhalation of 100% nitrogen for the period of study. In this series of animals also, the plasma oxygen concentration fell to values approaching zero within the first 1 to 3 hours and remained there. Figure 23A shows the data obtained by measurement of plasma

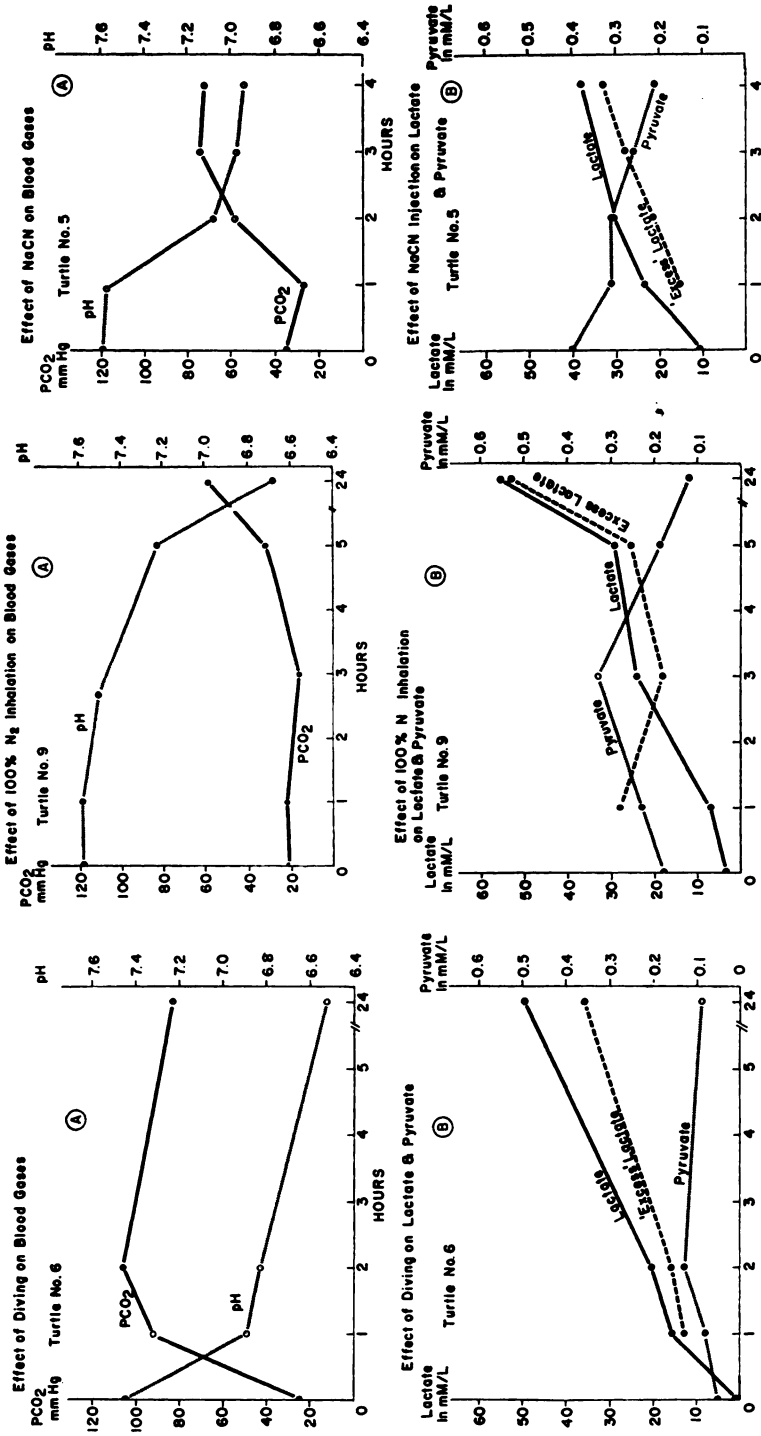


FIG. 22 (*left*). Effect of prolonged diving on the freshwater turtle. A. Changes in pH and P_{CO_2}. B. Changes in blood lactate and pyruvate.

FIG. 23 (*center*). Effect of 100% nitrogen inhalation on the freshwater turtle. A. Changes in pH and P_{CO_2}. B. Changes in blood lactate and pyruvate.

FIG. 24 (*right*). Effect of sodium cyanide injection on the freshwater turtle. A. Changes in pH and P_{CO_2}. B. Changes in blood lactate and pyruvate.

CO_2 and pH in a representative animal inhaling 100% N_2. Plasma CO_2 in this animal remained constant, but in some animals the level dropped. Whereas in the animals shown here, the plasma pH slowly fell, some of the animals showed an initial slight rise in pH because of the hyperventilation producing a loss of CO_2. In all animals, however, with continued exposure to nitrogen, the generation of fixed acids during anaerobic metabolism exceeded the loss of hydrogen ion resulting from CO_2 excretion by hyperventilation and the pH fell. Plasma bicarbonate concentrations, not shown here, fell progressively, reflecting the buffering of anaerobically generated organic acids. The changes in lactate and pyruvate levels seen in 100% nitrogen inhalation were similar to those seen in diving (Fig. 23B).

The final group of animals was given 15 to 25 milligrams of sodium cyanide intravenously. This dose represents approximately 5 to 15 times the lethal dose, on a weight basis, for mammals. In the first several hours after cyanide injection, plasma oxygen tensions rose steeply to values approximating atmospheric oxygen tension. As tissue oxygen utilization ceases because of the effect of cyanide, the body fluids come into equilibrium with atmospheric oxygen tension. In some animals, there was a late drop in plasma oxygen tension that may have been due to a continued slight utilization of oxygen coupled with an impairment of ventilation. In other animals, the effects of sodium cyanide disappeared and plasma oxygen tensions returned to normal levels. Figure 24A presents, in a graphic fashion, the effect of sodium cyanide injection on plasma pH and P_{CO_2} in a representative experiment. As a rule, CO_2 tensions declined in these animals and then gradually rose to normal or above normal levels. Plasma pH immediately following cyanide injection tended to be equal to or higher than in the control period. This probably represented a greater loss of hydrogen ion equivalents *via* ventilation than generation of hydrogen ion equivalents *via* anaerobic metabolism. With time, plasma pH generally dropped as is shown here as the organic acidemia associated with sodium cyanide administration became more intense. Plasma bicarbonate concentration, not shown here, decreased following sodium cyanide injection; this reflects generation of lactic and pyruvic acid. Figure 24B shows the response of lactate and pyruvate to cyanide injection. It can be seen that the effects were similar to those seen in diving and 100% nitrogen inhalation.

These studies demonstrated that the turtle is capable of relying on anaerobic metabolism for long periods of time. The very large production of lactate supports this concept. Deprivation of oxygen either at the total organism level or at the molecular level had similar effects on blood pH, P_{CO_2}, lactate and pyruvate. The production of excess lactate in these turtles was due primarily to the constant high level of blood lactate produced in the anoxic turtle. In the large group of animals studied, the pyruvate levels varied so widely that no consistent change could be recorded as occurring.

A chance encounter with observations by Miller *et al.* (69) provided a means to study the relationship between circulating levels of lactate and pyruvate and oxygen saturation in man. These investigators described the marked rise in

human blood lactic acid levels following intravenous infusion of fructose. Accordingly, normal subjects were selected and given 1 gram of fructose per kilogram body weight by intravenous injection of a 10% solution over a 30-minute period. Data similar to those obtained for the turtles were collected. There was no significant change in arterial pO_2, P_{CO_2} or pH during this period. Figure 25 demonstrates that there was a great increase in both blood lactate and blood pyruvate in all subjects studied. Nine subjects were studied in this fashion and excess lactate was calculated as before. The value for this figure ranged from −1.0 millimole per liter to +1.3 millimoles per liter. The next step was an attempt to determine whether hypoxia would have any effect on lactate and pyruvate levels in patients studied in this fashion. Also in Figure 25 can be seen the effect of hypoxia plus fructose on blood lactate and pyruvate levels. The subjects breathed 10% oxygen during the infusion. Arterial oxygen saturation at the end of the infusion ranged between 65% and 87%. Plasma pH and P_{CO_2} both dropped slightly. This illustration shows that the changes in lactate and pyruvate were very similar to those observed without hypoxia. Excess lactate values were calculated as before and ranged between −0.8 millimole per liter and +1.5 millimoles per liter. There was one value of 3 millimoles per liter. Inspection of Figure 25 reveals that hypoxia had no real effect on the changes in blood lactate and pyruvate resulting from fructose infusion. Figure 26 shows excess lactate plotted as a function of arterial oxygen saturation. It is quite apparent that these data do not demonstrate any correlation between excess lactate and arterial oxygen saturation.

These studies demonstrating that, in the turtle, hypoxia increases blood lactate strongly support the concept that this animal's prolonged survival in the absence of oxygen results from an ability to depend on anaerobic metabolism. The human data demonstrate that it is possible to produce marked increases in blood lactate

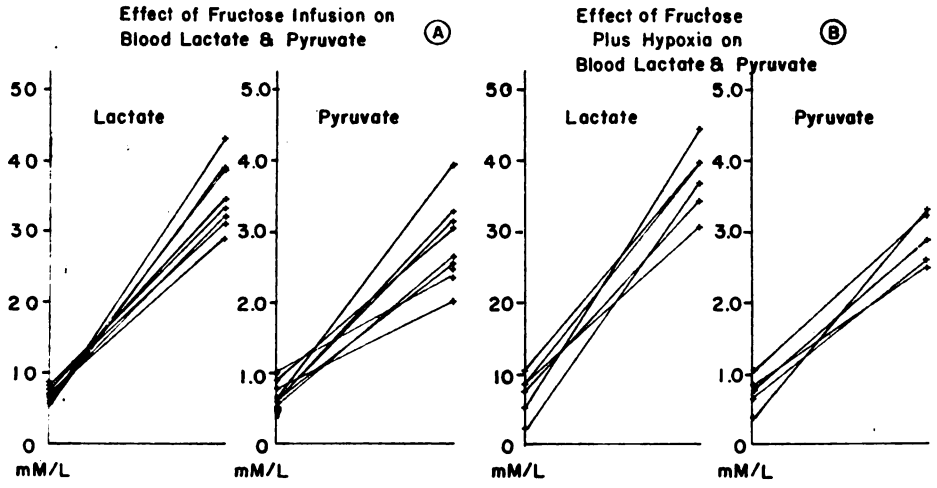


FIG. 25.

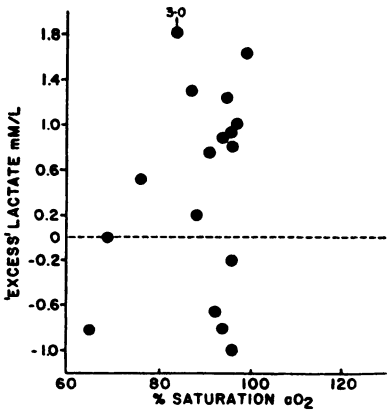


FIG. 26.

and pyruvate as well as excess lactate by the infusion of fructose. This is accomplished without any change in plasma oxygen saturation or suggestion of cellular hypoxia. Exposure of the subjects to 10% oxygen, resulting in clear-cut, measurable, and significant decreases in arterial oxygen saturation, failed to affect these changes in any direction. Finally, these data provide no correlation between arterial oxygen saturation and excess lactate.

When the glycolytic generation of pyruvate exceeds its utilization by oxidative degradation, increased blood levels of pyruvate or lactate, or both, may occur. The ratio of lactate to pyruvate is more than likely a resultant of a number of simultaneous processes. The cytoplasmic DPN/DPNH ratio that has been mentioned by others is itself a quantity difficult to measure. The processes whereby cytoplasmic DPNH may be reoxidized are only dimly understood and poorly characterized. When one reflects that the plasma turnover times of both lactate and pyruvate in the human are exceedingly rapid—in the neighborhood of 4 to 6 minutes—one realizes that levels obtained at any given time represent isolated samples of very busy systems (73). In view of these considerations and the data presented, it is difficult for us to agree that excess lactate has been established as a valid indicator of cellular hypoxia as presently understood.

Dr. M. B. McIlroy: I have been asked to tell you something about McArdle's syndrome, a very interesting but rare congenital condition in which, as a result of myo-phosphorylase "B" deficiency, the patient is unable to produce lactic acid from glycogen in his exercising muscles. This defect produces a condition during exercise which is best thought of as a deficiency of substrate. In view of the lateness of the hour I will not present evidence for this view but just give you our present thoughts about the condition.

If the patient starts to exercise too rapidly his muscles stiffen and become painful and swollen, and if he goes on too long he develops myoglobinuria. This stiffening of the muscles occurred in the patient whom we studied at a mild load of 250 kg/minute. His muscles would usually stiffen in about 4 or 5 minutes at this load. The interesting thing is that you can prevent the muscles from stif-

fening by various maneuvers which increase the amount of available substrate, notably the infusion of glucose, fructose or norepinephrine. The other interesting thing about it is that if the subject starts to exercise very slowly and gradually builds up the level of exercise bit by bit, he can go on for a very long period of time at a level at which he would have had to stop if he had started suddenly.

Patients with McArdle's syndrome are unable to produce lactic acid in their exercising muscles. There are one or two interesting things about this which might possibly bear some relationship to what we have been talking about. We believe that there is a deficiency of substrate in McArdle's syndrome. It looks as if there is not enough ATP to keep cellular metabolism going in the muscles during exercise. One possible reason why the myoglobin leaks out of the cell may be that there is not enough ATP to keep the cellular membrane intact or to keep the sodium pump working adequately. The second thing about McArdle's syndrome is the lack of any relationship between excess lactate and oxygen debt. There seems to be no doubt that these patients are capable of accumulating abnormally large oxygen debts during exercise in spite of the production of no lactic acid whatsoever in the exercising muscles.

The thing that fascinates me about this condition is that the respiratory rate, pulse rate, and ventilation increase remarkably during exercise in which there is substrate deficiency in the muscles. I would like to know what the mechanism is which lets the central nervous system (or whatever controls the heart and lungs) know that times are bad in the exercising muscles. The results in the patient with McArdle's syndrome indicate that the mechanism is independent of lactic acid and also probably of anoxia or increase in carbon dioxide tension. In closing I would like to comment on the work from Dr. Robin's group in Pittsburgh and say that turtles with McArdle's syndrome would do very badly.

Dr. P. J. Cohen: I would like to say a few words about what our philosophy has been in evaluating the meaning of excess lactate. After beginning these studies, we felt that it would be wise to see if we could correlate excess lactate with hypoxia in the organ we were studying, namely, the brain. When studying any organ or system, if one is going to say that excess lactate is or is not a good index of hypoxia, one ought to produce hypoxia in this organ and see whether or not excess lactate accumulates.

We therefore had subjects breathe a mixture of from 7 to 7.5% oxygen in nitrogen. We added carbon dioxide to maintain a normal P_{CO_2} because the subjects hyperventilated in response to a hypoxic stimulus and we wished to examine the effects of hypoxia independent of any effects produced by hypocarbia. In all of the subjects examined, there was an increase in cerebral excess lactate as well as appropriate changes in the aerobic and anaerobic metabolic indices during hypoxia. Thus, for this system, the brain, we defined hypoxia by producing it and reasonably well convinced ourselves that excess lactate was a valid measurement.

There are, however, various criticisms that have been raised with respect to excess lactate. Among them is the fact that we are obtaining measurements in blood in order to examine events which presumably take place in the mitochondria. We are examining blood entering and leaving an organ and using the results

obtained to ascertain intracellular events, and, in particular, events which may be taking place within a localized compartment of the cell. In addition, the derivation of the formula used in computing the value of excess lactate makes the concept strictly applicable only to an equilibrium situation. There is some evidence that the lactic dehydrogenase system is not necessarily in equilibrium during hypoxia. Factors other than hypoxia have been shown to change the ratio of DPN/DPNH. The metabolic state, as was pointed out earlier, may have a profound influence on intermediary metabolism and may change this ratio. This, in turn, may or may not change the value of excess lactate. These objections are valid and should always be considered.

Nevertheless, we convinced ourselves empirically that in this particular situation, excess lactate was a valid measurement. I think that I can conclude by saying that there are theoretical objections to excess lactate as a measurement; nevertheless, in a great many instances, this approach works. Hypoxia will produce excess lactate.

The converse, namely, whether an increase in excess lactate invariably means that there is hypoxia, is another case. Some of the studies that Dr. Price has presented have shown an increase of excess lactate across the hepatic circulation. I think it still remains to be seen whether this is an effect of hypoxia, or whether this is an actual metabolic alteration induced by cyclopropane or epinephrine in the absence of hypoxia.

DISCUSSION

Dr. W. R. Drucker: Since the hour is extremely late, I shall try to be brief. I want to say a word in defense of the concept of excess lactate. An article written by Weil and Broder, published in *Science* last month (102a), summarizes, I believe, the thoughts of people interested in this field. There are cogent objections to the concept of excess lactate, if it is interpreted as an absolute biochemical indication of tissue anoxia. On the other hand, from an empirical point of view, this is an exceedingly useful guide, both from the standpoint of the management of clinical patients and in the experimental laboratory. I think we have heard data today that would confirm this point of view.

Dr. Fritz Hinz, Dr. Joe Larner and I used biopsies to study the biochemical alterations in patients with myoglobulinuria (44a). We concluded they did not have McArdle's disease, because there was no deficiency of phosphorylase A. Also, they didn't have a glycogen storage problem. In fact, none of the tissue enzymes that we measured were found to be abnormal. Quite interestingly, however, the subjects had an exceedingly high rise in blood lactate with exercise, with little or no accumulation of excess lactate. Infusion of lactate into these subjects led to a decreased rate of disappearance of the lactate from the blood. In line with the studies which John Vester talked about, when glucose was infused into these subjects there was a much higher rise of lactate than in control subjects. This is a circumstance, therefore, in which there were evidences of altered lactate metabolism not associated with either anoxia or demonstrable defects in muscle enzymes.

I mention these studies primarily to illustrate the use of tissue biopsies in

clinical biochemical studies and to caution that even this technique may not give a definitive answer to the problem under consideration.

Now, just a brief word about epinephrine, because it is so clearly involved with the metabolic effects of anesthesia. Because of the work of Dr. Price and his associates at Pennsylvania, all of us are aware that halothane has a direct inhibitory effect on the sympathetic nervous system. The proposed central mechanism of action is fascinating. In view of this work, I think we might be able to attribute the fact that the concentration of blood glucose does not rise under halothane anesthesia to suppression of epinephrine release. But even more interestingly, the stresses such as surgery or hypovolemia which ordinarily cause a rise in blood glucose, fail to do so in the animal anesthetized with halothane (25a). It might be worth noting that we have recently demonstrated that an infusion of epinephrine into a normovolemic dog can cause all of the metabolic changes of a severe hypovolemia with an accumulation of excess lactate and fall in blood pH, changes which we have interpreted as indicative of tissue anoxia (25b). Currently, when we perform surgery upon an acutely traumatized patient, we choose cyclopropane as the anesthetic because of its support of blood pressure. Since this pressor effect is achieved through the release of catecholamines, it is possible that in time, cyclopropane might exert a detrimental effect on metabolism similar to that produced by a continuous infusion of catecholamines.

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