THE EFFECTS OF VARIOUS ANESTHETIC AGENTS ON PROTOPLASM

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A theory of anesthesia based on the present state of our knowledge can, at best, be but a suggestion as to what may take place with loss of sensibility when an anesthetic agent is administered. The foundation of the theory will be a firmer one if it rests on direct microscopic observations. Such are the observations to be recorded here.

The theory, to which I (1) have already given support, is essentially that of Claude Bernard (2) reworded and placed on an experimental basis. Bernard, and later Bancroft (3), had the misfortune to use the words "coagulation" and "floculation." Both terms imply an irreversible clotting of the protoplasm, which means death. "Gelatinization," on the other hand, indicates reversible setting.

A further and necessary addition to the original theory of Bernard is experimental evidence that gelatinization of the protoplasm actually takes place on the addition of an anesthetic agent. This evidence is supplied by microscopic surgery. Furthermore, if the theory is to have wide applicability, it must hold for many cases of anesthesia brought on by various agents.

Some experimenters place much emphasis on the need to distinguish anesthesia, narcosis and analgesia. Anesthesia, we are told, should be restricted to those cases which involve loss of pain, or at least loss of irritability. In man, the latter would mean loss of the reflexes. The only support for such a contention is the etymologic meaning of the word. On this basis, lowly forms of life cannot be anesthetized because pain as we know it is not involved. I do not agree with so narrow a view. It is impossible to draw a line separating organisms which suffer pain from those which do not. Pain is a psychic phenomenon, anesthesia a physiologic change.

We are little better off with loss of irritability as a criterion of the anesthetic state. All general anesthetic agents do not conform to the pattern set by ether and chloroform which depress the reflexes uniformly and consistently as the depth of anesthesia increases. The use of other agents has made it evident that the concurrent loss of the reflexes and of consciousness is purely coincidental and not the expression of a fundamental rule. It is possible to induce general anesthesia in animals by means of chloralose without any demonstrable depression.
of spinal reflexes. With larger doses the reflexes may actually be exag-gerated even after paralysis of the medullary centers by toxic doses. Strychnine and morphine behave similarly. The barbiturates occupy an intermediate position between the ether-chloroform and chloralose-morphine patterns in that the behavior of the reflexes is not a useful criterion of the depth of anesthesia. Even after doses large enough to cause deep coma and profound respiratory depression, spinal reflexes may be active.

Loss of consciousness or loss of pain is thus the only acceptable and consistent criterion of anesthesia in higher organisms. For the lower organisms either the terminology or the criterion of anesthesia may be changed. In the former case it is tacitly implied that inactivation in higher and in lower organisms does not involve the same type of reaction. It cannot be assumed, without evidence, that the two mechanisms differ. The essential features of anesthesia in the higher and lower organisms may be the same. To call one anesthesia and the other inactivation evades, if it does not confuse, the issue. In both there is "suspended animation." Viewed in this light, the cessation of protoplasmic streaming in a slime mold when brought on by a recognized anesthetic agent such as cyclopropane is as much anesthesia as is the inactivation of the protoplasm in the nervous system of man produced by the same agent.

**Material and Method**

The organism on which these studies are based is the slime mold Physarum polycephalum. Slime molds are lowly forms of life with both plant and animal characteristics, for which reason they possess the dual names, Myxomycetaceae and Mycetozoa. The plasmodium is a relatively simple, noncellular, multinuclear mass of living matter, the protoplasm of which is in a constant state of rhythmic flow. The temporary cessation of this flow constitutes a state of anesthesia as here interpreted.

So primitive an organism as a slime mold has both advantages and disadvantages as research material. The disadvantage, from the medical point of view, is the obvious difference in the morphology and physiology of two organisms so far removed phylogenetically as are the slime molds and man. The distinctive and complex nervous, circulatory, excretory, and respiratory systems in man are certain to cause him to react differently to a reagent or a situation than does a relatively undifferentiated primitive mass of protoplasm. This difference becomes, however, an advantage if one seeks the direct effect of a reagent on protoplasm. Furthermore, it should be remembered that the protoplasm of man, viewed apart from those accessory mechanisms which characterize the higher organism, is not wholly unlike the protoplasm of lower forms of life. Were it possible to observe the direct effect of an anesthetic agent on human protoplasm, that effect
would probably be similar to the reaction of the protoplasm of a slime mold to the same anesthetic agent.

The microscopic study of the slime mold Physarum is carried on in small moist chambers. A bit of the plasmodium is placed on the underside of a glass slip which serves as the cover of the chamber. The subcultures thus made soon spread, all the while carrying on active streaming. The microchambers are of various types, depending on the nature of the experiment. Some are patterned so as to permit microdissection (4), and some are miniature gas chambers. The chamber has a volume of 10 cc. The gaseous anesthetic agents may be administered at any desired rate, 0.1 cc. a second being the standard rate employed in the experiments reported here. Liquid narcotics may be applied by bathing the protoplasm in the solutions. Solutions may also be administered by injecting them directly into the plasmodium with micropipets. Microelectrodes, used in experiments involving the application of an electric current, may be of platinum, or miniature swabs saturated with salt, or pipets filled with salt-saturated agar.

Electrical Shock

Electrical anesthesia met with adverse criticism during the early years of this century; it was maintained that "general anesthesia by electric current does not exist." But today, when the transmission of nerve impulses can be electrically interpreted, and the relationship between impulse and accompanying action current is measurable, electrical anesthesia does not appear improbable.

Burge (5) found it possible to anesthetize an animal by passing a current through the body, with the cathode at the head and the anode in some posterior position.

There are two general methods of producing anesthesia by electricity, by a substantial and instantaneous shock, or by allowing a low voltage current to flow constantly through the body. The first method, that of shock, is the one used in the present experiments on slime molds. Voltages of from 1 to 500, of both direct and alternating currents, were used. All shocks, unless otherwise noted, were single and instantaneous.

Anesthesia, injury and death are primarily determined by amperage, but as amperage is proportional to voltage and resistance, it is convenient to express the current applied in terms of voltage. Resistance, and therefore voltage or amperage, are greatly influenced by salt concentration, oil layers, hydrogen ion concentration, heating effects, electrolysis, protoplasmic membranes, and the nature of the electrode contact. When fine platinum electrodes are placed 1.5 mm. apart the measured resistance of slime mold protoplasm may vary from 20,000 to 80,000 ohms. Two typical examples are 34 V., 1.7 ma, 20,000 ohms, and 34 V., 0.4 ma, 80,000 ohms.
The effect of an electric shock on protoplasm may be immediate or delayed. The effect may be a reversible cessation of flow, which constitutes anesthesia as here interpreted, or mild and severe forms of injury. Mild injury reveals itself chiefly as surface disturbances; severe injury involves disintegration, coagulation and death.

Electrical anesthesia without injury occurs most perfectly and consistently between a voltage of 30 to 50 and a milliamperage of 0.2 to 1.0. A momentary shock of 10 to 20 volts causes no immediate effect but often a pronounced delayed effect. At 20 to 30 volts there usually occurs a slight immediate effect followed by a delayed effect. At 30 to 50 volts, the most successful inactivation range, cessation of protoplasmic flow is immediate. Any voltage above 60 may be lethal; it is always so at 80 to 100 volts.

Protoplasm, inactivated by an electric current without injury, is reversibly gelated. That the electrically anesthetized protoplasm is of a firm, gel consistency was proved by subjecting the protoplasm to microdissection.

COLD

The use of cold as an anesthetic agent has a long historical background which culminates in the present day practice of human refrigeration at a temperature of 40 F.

Slime molds subjected to temperatures above and below freezing, both out of doors and in refrigeration rooms, become sluggish in movement at 5 C. (40 F.). A critical temperature is reached at 3 C. below which streaming may stop at any moment and above which it never stops in a healthy specimen. Below 1 C., streaming stops within a minute. Under the freezing point no slime mold protoplasm continues to flow for more than a few seconds.

**TABLE 1**

<table>
<thead>
<tr>
<th>Temperature at anesthesia, degrees</th>
<th>6</th>
<th>4</th>
<th>3</th>
<th>2</th>
<th>1</th>
<th>0 C.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Time for cessation of movement, minutes</td>
<td>28</td>
<td>8</td>
<td>4</td>
<td>3</td>
<td>2</td>
<td>1 min.</td>
</tr>
<tr>
<td>Time for recovery, minutes</td>
<td>3</td>
<td>2</td>
<td>2</td>
<td>2</td>
<td>1.5</td>
<td>0.5 min.</td>
</tr>
<tr>
<td>Pathological damage</td>
<td>great</td>
<td>little</td>
<td>none</td>
<td>none</td>
<td>none</td>
<td>none</td>
</tr>
</tbody>
</table>

When protoplasm is removed from a cold environment immediately after the cessation of flow, recovery takes place within one-half minute to thirty minutes. When anesthesia is quickly produced at 1 to 2 C., with minimum injury, the recovery time is one to two minutes. The relationship of time of recovery to time of anesthesia is illustrated in table 1.

The foregoing data also indicate the relationship between injury and temperature; thus, one-half hour at 6 C. is far more injurious than
one minute at 0 C. Protoplasm may survive for eighteen hours at 4 C. but will suffer great injury. When, however, the time of exposure is held constant, and is of short duration, injury increases with decreasing temperature; five minutes at 6 C. is not harmful whereas five minutes at 1 or 2 C. may be very harmful, and five minutes at 0 C. invariably results in death.

Anesthesia by cold raises a time-honored question in biology, does protoplasm freeze at low temperatures? Surely the free water in a living cell freezes below 0 C. and the ice crystals thus formed disrupt the tissues, causing death. But plant protoplasm out of doors in the north survives at very low temperatures. To explain this it has been assumed that certain organisms rid themselves of all free water and retain only adsorbed or bound water through the winter. Adsorbed or bound water does not freeze, and it is sufficient to maintain life at the low metabolic rate of hibernating organisms, a tree or an insect pupa. When protoplasm cannot accomplish the change from free to bound water, as in the case of sudden freezing, it succumbs.

Considerable confusion has arisen over the relative merits of slow and fast freezing of living material. In nature, freezing and thawing are slow processes. There is better adjustment to severe cold on the part of plants when the approach to winter is gradual. Ridding themselves of free water and retaining only bound water, possibly even converting some of the free water to bound water, is probably the basis of the winter hardiness of frost-resistant species. The cells of trees which would succumb at — 5 C. can actually be made to withstand — 50 C. by hardening, that is to say, by subjecting plants and seeds to progressively lower temperatures.

Bound water is only a partial explanation of survival at low temperatures. Dissolved substances which lower the freezing temperature must also play a part, especially when the change is sudden as in the case of cold anesthesia. A slime mold subjected to a temperature of — 4 C. ceases all movement in less than a minute, and recovery without injury in about the same length of time after return to room temperature. Survival at this temperature can only mean that there is no freezing of free water, and this must be due to the salt concentration of protoplasm.

It is significant that the critical temperature for cold anesthesia in a primitive protoplasmic mass is close to the freezing point of water, a trifle above 0 C. for successful anesthesia; equally significant is it that survival is still possible at slightly below zero temperatures if the time of exposure is brief. It is also worthy of note that the temperature for the local anesthesia of man by cold (40 F. = 4.5 C.) is close to the critical temperature (3 C.) for slime mold anesthesia.

The protoplasm of slime molds which has been inactivated by cold and subjected to dissection is in all cases found to be of firm, gel consistency.
Heat

The anesthesia of slime mold protoplasm may be brought on by heat. The margin between the temperature required for the cessation of protoplasmic movement and the temperature causing severe injury or death is much less than that in the case of cold but still sufficient to permit successful anesthesia in a primitive form of life. A typical case is the following: at 35 C. streaming stops within an hour, and at 36 C. within half an hour; at 38 C. streaming may continue for several minutes but at a reduced rate; at 39 C. all flow in the smaller capillaries stops within three minutes but continues for four or five minutes in the larger capillaries; at 40, or at 41 and 42 C. in resistant specimens, flow stops in less than a minute; between 42 and 45 C. death occurs if the time of exposure exceeds two minutes. Time of recovery varies, and is usually long in all cases of heat anesthesia, up to three-quarters of an hour being required. Here again recovery is related to time of exposure.

Injury by heat is great and varied; it takes the form of shrinkage, syneresis, blistering, vacuolization, disintegration, and scattered areas which are irreversibly coagulated. The last is a startling example of the differences in resistance to injury which adjoining areas of protoplasm may exhibit. In slime molds contiguous areas of protoplasm are not separated by membranes as in the case of tissue cells. Scattered areas of dead protoplasm separated by healthy, actively flowing protoplasm often "pock mark" a plasmodium.

Heat-anesthetized protoplasm when subjected to microdissection is found to be of a firm, gel consistency.

Ethyl Chloride

Ethyl chloride was applied to protoplasm both as a refrigerant and as a toxic agent. In the former case the protoplasm was sealed in its chamber so that the chloride functioned merely in reducing temperature, which is the use to which ethyl chloride is put when employed as a local anesthetic agent on man. Used as a refrigerant only, ethyl chloride produces results on slime mold protoplasm identical to those obtained with cold, however produced.

Ethyl chloride in direct contact with protoplasm is exceedingly toxic. In gaseous form, and diluted, it may serve as an anesthetic agent with little or no injury to the protoplasm.

A dilution of one-quarter ethyl chloride and three-quarters air will stop protoplasmic flow in ten to fifteen seconds. Recovery will take place within a minute. A lower concentration of the gas is a better anesthetic agent; thus, flow is stopped in twenty seconds by an 18 per cent dilution, with recovery in fifteen to thirty seconds. Little injury results. Weaker concentrations, if above 10 per cent, produce anesthesia with less injury, but the time required for inactivation is greater. Five per cent ethyl chloride is too weak for anesthetic effects.
Slime mold protoplasm in the presence of ethyl chloride builds up a tolerance, as it does to many anesthetic agents. Thus, the time required for anesthesia with ethyl chloride on the same specimen on the second treatment is double that of the first.

When treated with ethyl chloride, slime mold protoplasm passes through a series of events which closely parallels a familiar series of reactions in the anesthesia of man. A patient goes through periods of altered consciousness, excitation, deep anesthesia and recovery. In the presence of ethyl chloride, a plasmodium will cease visible activity and remain inert for a few seconds, then recover, with active streaming, and finally quiet down to complete and permanent anesthesia until removed to a normal atmosphere.

When protoplasm which has been anesthetized with ethyl chloride, directly applied, is subjected to microdissection it is found to be a firm jelly.

To the foregoing list of anesthetic agents a number of others may be added, not only the more familiar ones, to which reference has been made in a previous article (1), but some hydrocarbons not clinically used. The research so far done on the latter is too brief to warrant more than a short reference to their effects on protoplasm, but they substantiate in full the conclusions reached in this article.

Conclusions

An hypothesis of anesthesia based on the gelatinization of protoplasm is supported by experimental evidence, that is, the physical change postulated can be verified, and it occurs no matter what the anesthetic agent may be. Such an hypothesis offers a physical basis for the interpretation of inactivation. Respiration, electrical conductivity, oxidation-reduction reactions, movement and metabolic activities in general are likely to be reduced when fluid protoplasm gelsates.

Not only does the gelatinization theory supply a satisfactory physical interpretation of anesthesia, but it, unlike other theories, is as applicable to the state of anesthesia produced by one agent as by another, even though the agents differ as much as do chloroform and electrical shock. I find it difficult to view anesthesia as a problem in adsorption, permeability or fat solubility. All of these will contribute to the reaction, but they are not the sole cause. Fat solvents will enter the cell more rapidly, but not all anesthetic agents are fat solvents. What nitrous oxide, cold, heat, and both mechanical and electrical shock can have to do with fat solubility it is difficult to imagine. Of two equally toxic anesthetic agents, that one which is more readily adsorbed will be the more effective; but anesthesia involves more than this, it involves a change in the state of protoplasm, and this change is reversible gelation.

Suppression of respiration is a possible interpretation of anesthesia which does not necessarily involve gelatinization. Chemical inactiva-
tion of respiratory enzymes may occur, for a reduction in rate of respiration during anesthesia seems likely. It is impossible to say whether the anesthetic agent causes gelation, which, in turn, inhibits respiration, or causes a reduction in respiration which is followed by gelation. The time required for anesthesia—it is often instantaneous when carbon dioxide is the agent—does not argue against respiratory inhibition, for the compounds which form between the reversible enzyme inhibitors and the enzyme itself occur at high velocities—the velocity constants are of the order of $10^{-4}$ seconds.

That reversible enzyme inhibitors form chemical compounds with enzymes is the basis on which the Warburg (6) adsorption hypothesis is rejected by some investigators. I do not question the possibility of a union between anesthetic agents and respiratory enzymes, but merely raise the question, is the reaction between agent and enzyme the essential part of anesthesia? If so, then what is our interpretation of anesthesia when the agent is not a chemical substance but shock, cold, or heat?

The precise mechanism of the reversible gelation of protoplasm in anesthesia is not known. It may be viewed as gelatinization, incipient coagulation, thixotropy (7), coacervation (8), or reversible protein denaturation. These may all be but names for one and the same phenomenon. The only statement which can be made with fair certainty is that the physical change observed is due to a chemical reorientation.

There are several generalizations having to do with anesthesia in which I have a particular interest. One of these is the hypothesis that all depressants, for example, anesthetic agents, gelate protoplasm, whereas stimulants, for example, caffeine, solute protoplasm. So far, experimental evidence obtained on myxomycete protoplasm shows this to be true (9). A second generalization is that involving a correlation between molecular structure and toxicity. I have shown that the anesthetic and the isosteric properties of both carbon dioxide and nitrous oxide are identical (10). There is also close correlation between the toxicity and molecular pattern of the xanthine derivatives, and the barbiturates; this work is now in press.

The point of view taken is that expressed by Goodman and Gilman (11) that the characteristic action of a drug is intimately related to its chemical structure. The extraordinary variety of anesthetic agents would appear to belie this generalization. There will be many exceptions, but these will fall into line as knowledge of anesthesia increases. After all, it is not the exceptions but the positive evidence which is so surprising and so gratifying.

There are, so far, no exceptions to the general rule which this paper supports, that all anesthetic agents, of whatever nature, reversibly gelate protoplasm. The gelatinization of the protoplasm of nerve tissue in higher organisms is the cause of the depression of physiologic activities and loss of consciousness.
I wish to express my appreciation of the help given me by my assistant, Mr. Hans Pollack, my collaborator, Mr. Ariel Loewy, and my colleague, Dr. Carl Schmidt whose cooperation in the form of both corroborative data and friendly opposition has been a stimulus toward a better understanding of anesthesia.

REFERENCES


NEW ENGLAND SOCIETY OF ANESTHESIOLOGISTS

The February meeting will be held in the Auditorium Building A, Boston University Medical School, Boston, on Tuesday, February 14, 1950, at 8:00 P.M.

The speaker and subject of the scientific meeting will be:

SYMPOSIUM ON INHALATION THERAPY

"The Physiological Basis for Inhalation Therapy," by Dr. Meyer Saklad, Director of Anesthesiology, Rhode Island Hospital, Providence, R. I.

"Pipeline As An Aid to Oxygen Therapy," by Dr. Ralph M. Tovell, Director of Anesthesiology, Hartford Hospital, Hartford, Conn.

"Organization of an Inhalation Therapy Department," by Dr. Bernard D. Briggs, Assistant Anesthesiologist, Massachusetts General Hospital, Boston, Mass.

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