THE ACTION OF ETHER ON PROTOPLASM.[1]

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No problem in general physiology has been investigated more frequently than the problem of anesthesia. Many workers have attempted to discover the essential nature of the action of anesthetics on living substance. Not only have animals, tissues, and cells been studied, but some physiologists have even gone so far as to study the effect of anesthetics on lifeless materials which they believed similar to protoplasm.

Many theories have been proposed to explain anesthesia. In recent years the permeability theory has had a wide following. This theory claims that anesthetics either decrease the permeability of the cell, that is to say of the plasma membrane, or that they at any rate prevent an increased permeability. The actual evidence in support of the theory is somewhat scanty. What there is has been well summarized by Winterstein (1), Höber (2), and Lillie (3), all three of whom are strong advocates of the theory.

In only a few cases has it been shown that anesthetics lower cell permeability and most of these cases are doubtful, as Winterstein admits. In some instances observers have claimed that anesthetics produce an increase rather than a decrease in permeability (see Höber). Perhaps the work most often quoted in support of the doctrine that anesthetics lower permeability is the series of conductivity measurements of Osterhout (4) on plant cells. There is however a growing realization that a decrease in the electric conductivity measurements of a group of cells does not necessarily depend on a decrease in the permeability of the plasma membranes of these cells. Many factors play a part in influencing the conductivity measurement. When an electric current is sent through a mass of cells, certainly a large part of the current goes between and around the cells. Changes in the conductivity of the spaces between cells are therefore apt

[1] Contribution from the Zoölogical Laboratory of the University of Michigan.

to be of greater moment than changes in the conductivity of the cells themselves. The factors involved include possible changes in the size and shape of the spaces between cells, as well as changes in their conductivity. One such factor is perhaps of especial importance for substances like the ordinary fat-solvent anesthetics which alter the viscosity of the medium in which they are dissolved. This is the effect of a change in the viscosity of a solution on its conductivity. Concerning this effect Walker (5) says: "The addition of a small quantity of a substance such as alcohol to water increases the viscosity of the water. Corresponding to this increase we find that the rate of diffusion is less when a substance is dissolved in water containing a little alcohol than the rate of diffusion when water alone is the solvent, no matter what the dissolved substance may be. Similarly the speed of ions in water containing alcohol is less than their speed in pure water."

Let us consider some of Osterhout's experiments more closely. He measured the conductivity of Laminaria in various solutions of anesthetics and compared it with the conductivity of the same material in sea-water. In one experiment he adds to 970 cc. sea-water 10 cc. of ether plus about 5 cc. of sea-water concentrated by evaporation until its conductivity was about double that of ordinary sea-water. In another experiment he adds to 970 cc. sea-water 30 cc. of absolute alcohol plus 15 cc. of concentrated sea-water (apparently made up as before), thus obtaining a 0.5 molecular solution of alcohol which he refers to as 0.05 molecular. This solution, according to Osterhout, has the same conductivity as sea-water. But this scarcely seems possible. Osterhout's solution has approximately the same concentration of salts as sea-water, but the viscosity of the solution is decidedly higher. Pissarjewsky and Karp (6) found that a 0.5 molecular concentration of alcohol lowers the conductivity of normal NaCl solution until it is about 8 per cent. below that of the conductivity of normal NaCl solution in pure water. On the other hand, the ether solution used by Osterhout would have, as he claims, approximately the same conductivity as sea-water, for Arrhenius (7) found that 1 per cent. ether lowers conductivity only about 2 per cent. for various types of electrolytes.

But there is also another important fact to be considered. Separating the cells of *Laminaria* is a network of cell walls. This is composed of cellulose and cellulose-like material which offers little resistance to dissolved substances and is obviously one of the main paths for an electric current. The adsorptive powers of cellulose are well known, although it apparently adsorbs electrolytes much more readily than non-polar compounds. In the finely divided condition in which the cellulose occurs in the cell walls it is not improbable that substances of low surface tension like ether, alcohol, and chloroform would be selectively adsorbed on it and more or less concentrated there. We should at least be led to expect this from Gibbs' adsorption equation. Moreover the surfaces of the cells would also tend to adsorb the anesthetic. If such adsorptive processes occur, then the conductivity of the cellulose framework as well as the conductivity of the cell surfaces would doubtless be decreased, and the decrease in conductivity would be greater than that which occurred in the mass of the solution. If this is true then it might in itself account for the variations in conductivity found by Osterhout.

It is interesting to note that the experiments of McClendon (8) on the diffusion of electrolytes from anesthetized pike eggs also fail to take cognizance of the direct effect of the anesthetic on the diffusion rate. It might be thought that the difference in diffusion rate would also explain McClendon's experiments. This is not the case. The presence of 2 per cent. alcohol would lower the diffusion rate less than 8 per cent. McClendon apparently found a lowering of as much as 50 per cent. in the total amount of chlorides diffused from the eggs. But his results are not as trustworthy as it might at first sight be thought. McClendon determined the diffused chlorides nephelometrically as silver chloride, precipitating them with silver nitrate. Now the precipitation of silver chloride varies under diverse conditions; there is a well known tendency for it to go into colloidal solution. The presence of alcohol either alone, or in conjunction with the albuminous substances also present, might act as a peptizing agent and hinder the precipitation of the silver chloride. Alcohol occasionally acts as a peptizing agent, and the precipitation of

silver chloride is known to be hindered by at least one non-electrolyte (see Bancroft (9), pp. 167, 168). Richard and Wells (10) in their first description of the nephelometer stated that "care must be taken to have both standard solution and unknown solution subjected to precisely the same conditions, for varying conditions of precipitation may lead to differences in the appearance of the precipitate far greater than the possible optical error of the apparatus. Herein lies the chief caution to be noted in its use." Later this point was emphasized again by Richards (11), who points out that "if even moderately accurate analytical results are to be had with the nephelometer, the one essential point to be heeded is this: *the unknown solutions to be estimated must be treated in exactly the same way as the known standard solutions, which serve as the basis for comparison.*" This precaution was neglected by McClendon and his results are therefore unreliable.

McClendon's work and that of Osterhout constitute a large portion of the evidence presented by Winterstein and regarded by him as trustworthy evidence in favor of the permeability theory. It would seem therefore that the support of the theory does not always rest on very solid ground.

Most of the earlier work on permeability change during anesthesia concerned itself with the attempt to demonstrate a change in permeability toward dissolved substances. More recently both Winterstein (12) and Lillie (13) have independently shown a change in permeability to water. I have repeated both sets of experiments, although in the former case I performed only a few tests and my results can scarcely be considered as constituting either a proof or a disproof of Winterstein's views. Fig. 1 shows two examples of the entrance of water into normal and anesthetized muscle as shown by the increase in weight. The abscissas represent time, the ordinates the weight of the muscle. The muscle used was the gastrocnemius muscle of the frog, and a pair of muscles from a single animal was used in each experiment.

The curves show that the entrance of water is faster in the normal muscle than in the anesthetized muscle. Winterstein obtained a somewhat more striking difference; he used the sartorius muscle rather than the gastrocnemius.

As to the interpretation of the experiments, the difference in rate of osmotic flow may be due either to a change in the properties of the fluid or to a change in the plasma membranes. Bigelow (14) and Bartell (15) found that the speed of osmotic

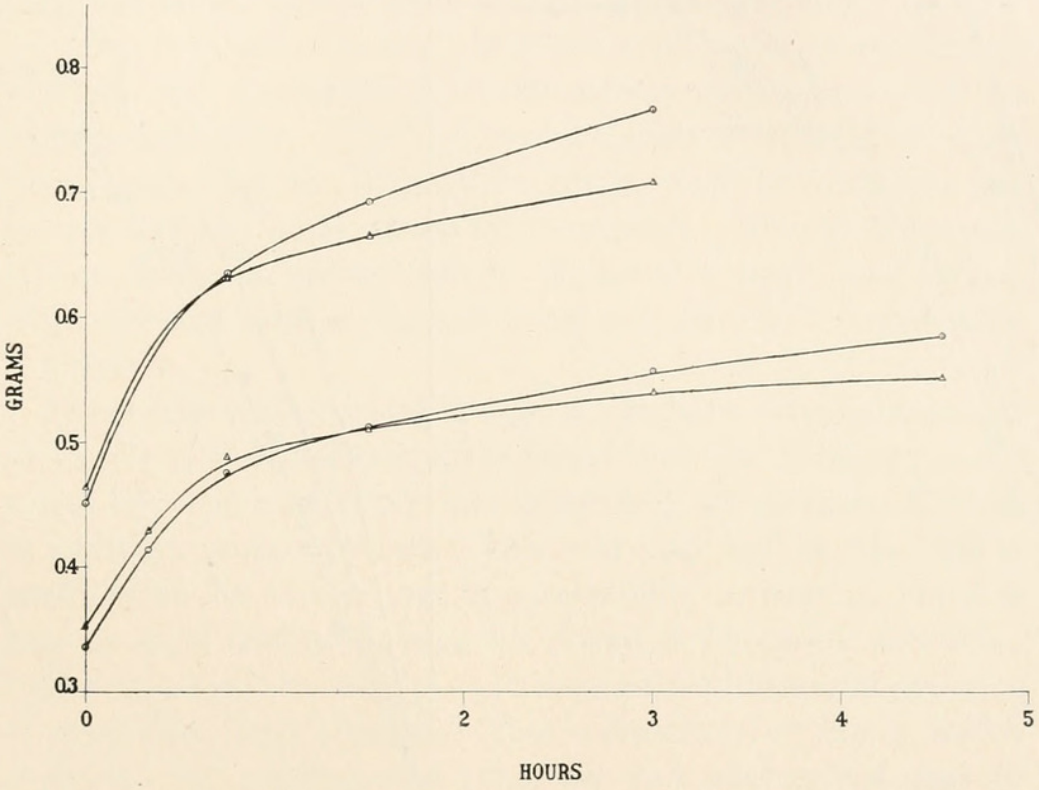

FIG. 1 shows the increase in weight of muscles in distilled water and in distilled water to which 2 per cent. by volume of ether has been added. In each pair of curves the small circles represent the increase in weight of a frog gastrocnemius muscle in distilled water, the small triangles represent the increase in weight of the corresponding muscle from the other leg immersed in 2 per cent. ether. The abscissæ indicate time in hours, the ordinates total weight of muscle.

flow of water through various osmotic membranes followed the laws of Poseuille for flow through capillary tubes. It is obvious that in general any increase in the viscosity of a fluid would tend to slow its rate of osmotic flow. The magnitude of the effect might be sufficient to account for my results, although perhaps not for those of Winterstein. There are also other factors to be considered. The anesthetics may extract materials from the muscle which tend to exert osmotic pressure in the opposite direction.

I have thus far omitted reference to Winterstein's experiments on the osmotic flow through muscle membranes obtained from

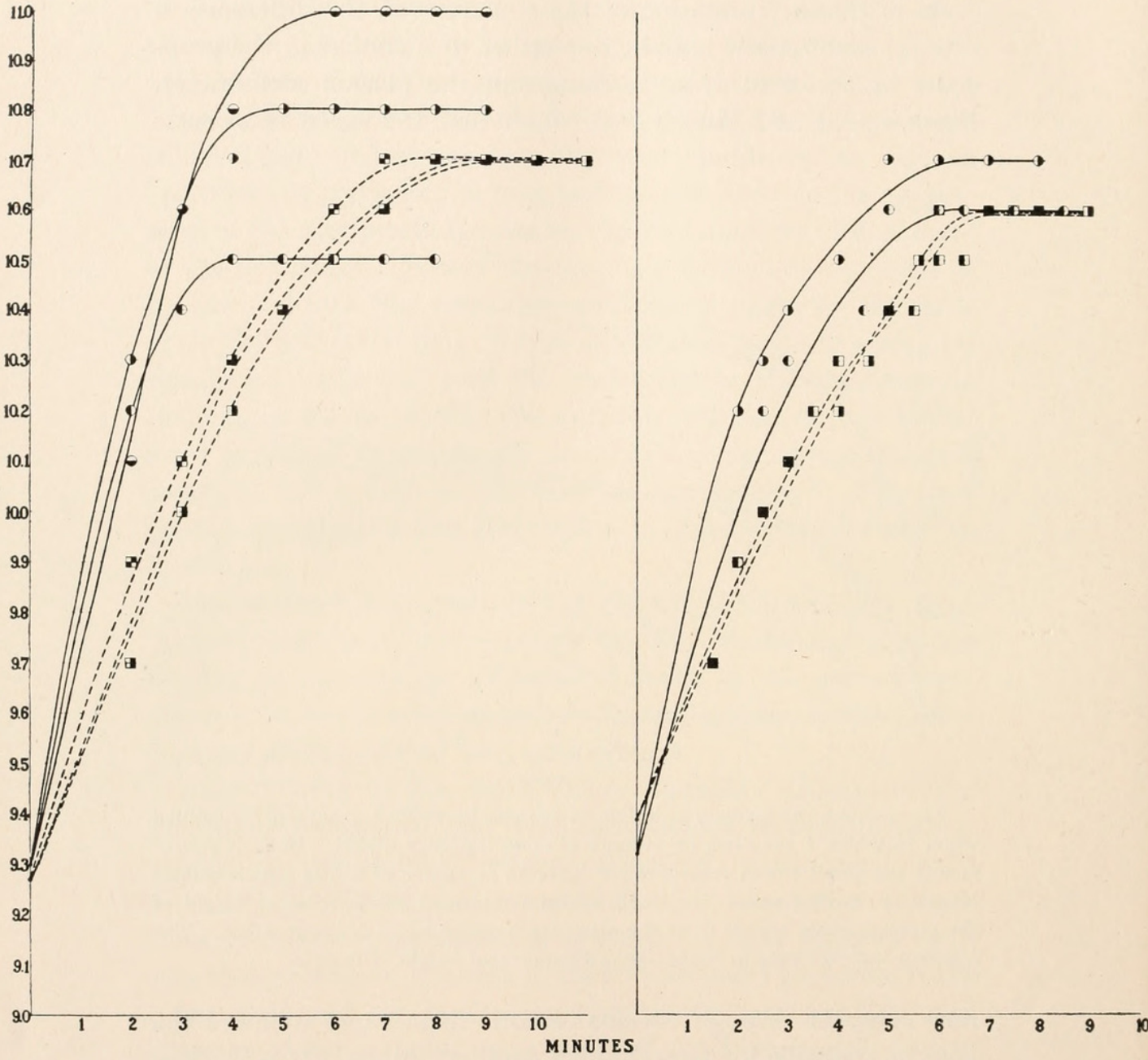

FIG. 2. In the above figures the curves represent increase of diameter of individual sea-urchin eggs in equal parts of sea-water and distilled water. (The original size is an average measurement.) The continuous lines represent etherized eggs, the broken lines control eggs, not exposed to ether. The concentration of ether used was 1 per cent. by volume, and the eggs were first treated with 1 per cent. ether in sea-water and were then transferred to 1 per cent. ether in equal parts of sea-water and distilled water. The ordinates represent the diameter of the eggs in arbitrary scale units, the abscissæ show the number of minutes elapsed after the entrance of the egg into the hypotonic solution. Similar results were also obtained with 2 per cent. ether solutions, but the measurements are harder to make as the eggs frequently rupture.

the body wall of female frogs. These experiments constitute the greater portion of Winterstein's evidence and the reader is referred to his paper for details. As I understand the experiments, Winterstein measured the osmotic flow into short glass cylinders sealed at *both* ends with muscle membranes. In such a system it would seem that the entrance of water would depend more on the distensibility of the muscle membranes than on their permeability. The high concentration of alcohol and other anesthetics used by Winterstein might tend to coagulate the membranes and make them less distensible. This would simulate a decreased permeability. However I have not worked with this sort of a set-up and I am not sure that I can judge it properly.

My results with sea-urchin eggs are perhaps more interesting. With this type of material Lillie found that the shrinkage of the cell in hypertonic solutions was delayed by the presence of ether in anesthetic concentration. This may very well be true, but it need not be due to a change in permeability to water. For it is easy to show that when eggs are placed in hypotonic solutions, they expand just as readily, or even more readily in the presence of ether than in its absence. This is illustrated in Fig. 2, which compares the expansion of etherized and unetherized eggs in hypotonic solutions. The shrinkage and expansion of a cell depends on many factors. Of these permeability is one, but not the only one. Other factors of importance are surface tension, the rigidity of the plasma membrane, and the colloidal condition of the interior. This last factor is of especial importance in Lillie's experiments. Lillie found that after fertilization the sea-urchin eggs showed a greater tendency to crenation and shrinkage when placed in hypertonic solutions, and that this increased tendency to crenation was prevented by anesthetics. As a matter of fact fertilized eggs become somewhat crenate even without being placed in hypertonic solutions (16). The crenation is concomitant with, and doubtless dependent on, the gelation of the protoplasm which follows fertilization. In view of the fact that anesthetics such as ether prevent this gelation (see below), it is easy to see that they would tend to hinder crenation.

Finally it should be pointed out that the experiments of Loeb (17) apparently indicate an *increase* in the permeability of *Fundulus* eggs to water in the presence of various anesthetics.

It is not my purpose to claim that in anesthesia there is no decrease in the permeability of the cell to salts or to water. Perhaps eventually this may be found to be a constant occurrence. But it seems at least premature to conclude a decreased permeability from the slight and uncertain evidence hitherto presented. Only by closing our eyes to the experiments that show the opposite of a decrease and only by neglecting the numerous sources of error which have scarcely been considered by previous experimenters, is it possible to consider the permeability evidence as convincing.

Even should we, some time in the future, find it true that anesthesia is always associated with decreased permeability of the plasma membrane of the cell, we would not be very much closer to an understanding of how and why the anesthetic stops cell activity. Permeability is a surface phenomenon, the activity of the cell goes on largely in the interior. Thus even if we accept the permeability doctrine, we must postulate a secondary hypothesis to explain how the permeability effect is transferred to the interior of the cell.

Many years before the permeability doctrine was thought of, various physiologists held the idea that anesthetics affected the colloidal condition of the protoplasm in one way or another. For a long time this idea remained a mere speculation, but in recent years it has been definitely shown that anesthetics do have a very real effect on the colloidal state, or at any rate upon the viscosity of the protoplasm. Both in plant and animal cells it has been shown that dilute solutions of ether cause a liquefaction of the protoplasm, and that more concentrated solutions cause a coagulation. There is a remarkable correspondence between widely different sorts of living substance. However in plants only those concentrations of ether which cause coagulation prevent the rotary movement of the protoplasm. This was therefore regarded by Heilbronn (18) as the anesthetic concentration. In animal cells on the other hand it was found that concentrations which caused liquefaction prevented cell-

division and therefore acted as anesthetic (16). As a matter of fact there is no difficulty in reconciling the two sets of observations. For it seems certain that what is anesthetic for mitosis is not necessarily anesthetic for protoplasmic streaming. An increased fluidity of the protoplasm would doubtless increase the speed of streaming, and yet such a cell with rapidly flowing protoplasm would scarcely be able to undergo a mitotic division and would therefore be anesthetized so far as cell-division was concerned. This is essentially the view taken by Weber (19) who points out that there is full accord in the investigation of both plant and animal cells.

Doubtless protoplasmic activity involves frequent changes in colloidal condition. Presumably any agent which prevents such changes in colloidal condition without causing death may act as an anesthetic. If this view is correct then either an increased liquefaction or a coagulation of the protoplasm may result in anesthesia. This idea was presented a number of years ago, and it was pointed out that there may very well be two kinds of anesthesia (20).

In earlier work it was claimed that those solutions of ether which act as an anesthetic for the process of cell-division in the sea-urchin egg cause a liquefaction of the protoplasm (16, 20). More concentrated solutions were found to cause a coagulation which was irreversible. All these facts have recently been called into question by Chambers (21). He claims that anesthesia in the sea-urchin egg is accompanied by an *increase* rather than a decrease in viscosity and that this condition of increased viscosity is reversible. Chambers' opinion is based on observations of Brownian movement as well as on a study with the microdissection apparatus.

It is evident that the findings of Chambers are in direct opposition to the earlier work mentioned above. An attempt was therefore made to repeat the older observations with the centrifuge method to determine if perhaps an error may not have been involved. In this repetition an effort was made to obtain more nearly quantitative results.

Experiments were performed both with fertilized and unfertilized eggs. We will consider the unfertilized eggs first.

When (unfertilized) sea-urchin eggs are placed in 2½ per cent. or 3 per cent ether, there is, within a few minutes, a very pronounced decrease in viscosity as shown by tests with the centrifuge. In the following table the viscosity of etherized and normal control eggs is compared. The viscosity numbers represent the number of seconds of centrifugal treatment necessary to produce a given degree of granular movement. Usually the eggs were centrifuged until the clear hyaline zone extended along one third or one half of the axis of the egg. Naturally a number of tests had to be performed to obtain any given value. The centrifugal force employed was approximately 4,968 times gravity and the centrifuge used was an ordinary hand centrifuge with hæmatocrit attachment. Details of technique are given in earlier papers (16, 22). Because of the fact that a series of tests had to be performed for each value of the viscosity and because moreover the viscosity of the etherized protoplasm is not constant, the tests had to be made hurriedly. They are not extremely accurate, and it is entirely possible that the inaccuracy amounts to 10 or 20 per cent. The decided difference between unetherized and etherized eggs makes this inaccuracy of small consequence. In the table the first column gives the percentage of ether used (volume per cent. in sea-water). The second column represents the time of exposure to ether, that is to say the number of minutes elapsed after immersion in ether solution at the time the critical test was made. The third column gives the relative viscosity of the etherized eggs as compared to that of the normal control eggs shown in the fourth column. The temperature of the experiments varied from 22 to 25 degrees. It is given in the fifth column.

Per Cent. Ether.	Exposure, Minutes.	Viscosity Etherized Eggs.	Viscosity Control Eggs.	Temperature.
2.5	11	10	25	23°
2.5	4	15	25	
2.5	8	15	25	22
2.5	10	15	28	24
3	15	15	35	22
3	8.5	20	40	23
3	3	15	30	25.3

Averaging the above values it is seen that in 2½ per cent.

ether the viscosity of the protoplasm is 53 per cent. of that of the normal control eggs in sea-water. The viscosity of the eggs in 3 per cent. ether is only 48 per cent. of that of the untreated eggs.

It must not be supposed that the viscosity of the protoplasm of etherized eggs remains constant for long periods. In the 3 per cent. solution the viscosity became lower and lower until finally a minimum was reached. Then a sudden coagulation occurred. This is shown by a series of tests on eggs in 3 per cent. ether (temp. 22.0°). When these eggs were tested after an exposure of 3 minutes it was found that centrifugal treatment for 15 seconds produced only a slight hyaline zone. The same results were obtained after 6 and 10 minutes, but after an exposure of 15 minutes, similar centrifugal treatment resulted in the formation of a hyaline zone which extended along about one third of the axis of the egg. After an exposure of 20 minutes the same treatment resulted in a hyaline zone which filled nearly half of the egg. On the other hand after 26 minutes when eggs were centrifuged for 15 seconds, although some eggs showed a hyaline zone extending through half of the egg, others showed no movement of granules at all. The protoplasm in these eggs is completely coagulated. Thus in 3 per cent. ether at 22° the liquefaction of the protoplasm is soon followed by a solidification.

The question may now be asked as to which of these two conditions represents a condition of anesthesia. In my earlier paper I had claimed that only the fluid state was anesthetic and that gelation or coagulation following ether treatment resulted only in death. But Chambers states that not the fluid but the gel state is the anesthetic condition when eggs are subjected to ether. On reading Chambers' statement I was at first led to suppose that I had been mistaken in making my claim too forcibly. I thought perhaps that although it was certain that moderately long exposure to ether after coagulation had taken place was lethal, nevertheless it might be true that Chambers had removed the eggs immediately after coagulation had begun and that in this instance the coagulative action of the ether was reversible.

The question was soon put to the test. In the experiment

cited above it was pointed out that coagulation occurred after 26 minutes exposure to ether. In this same experiment some of the eggs were removed from the ether solution after an exposure of 24 minutes and placed in normal sea-water. Others were removed from the ether after an exposure of 28 minutes. Some of the eggs exposed to ether 24 minutes were inseminated, following an interval of 18 minutes after removal from the ether, and some of the eggs exposed 28 minutes were inseminated following an interval of 14 minutes after removal from ether. None of the inseminated eggs showed any signs of development. As a matter of fact both the inseminated eggs and those not exposed to sperm went through the same series of degenerative changes. All of them disintegrated by breaking up into small globules.

Thus it is obvious that following the coagulative action of ether there is no recovery. The same sort of experiment was repeated a number of times always with the same result. If eggs are to recover from ether treatment they must be removed from the ether solution some few minutes before coagulation has begun.

The discussion so far has been concerned only with conditions in unfertilized eggs. In fertilized eggs the effects of ether are even more pronounced. Let us consider a sample experiment. In the following account many details of observation are omitted.

July 22 (Temp. about 24°). Eggs were fertilized at 3.35 P.M. Fifteen minutes later, at 3.50 P.M., they were centrifuged at the usual rate for 50 seconds. No zones appeared (control unfertilized eggs showed a hyaline zone about ¼ of the distance along the egg axis after 30 seconds treatment). At 3.52 P.M. some of the fertilized eggs were placed in 2½ per cent. ether in sea-water in a glass-stoppered weighing bottle. At 3.55 P.M. a centrifugal test for 60 seconds showed only a thin streak for a hyaline zone in the control untreated fertilized eggs. At 4.27 P.M. the etherized eggs when centrifuged for 20 seconds showed a hyaline zone extending more than a third of the distance along the egg axis. At 4.31 P.M. a test of the etherized eggs for 15 seconds showed a similar zone extending about one third of the axis, and at 4.33 P.M. a 10 second test showed a hyaline zone extending through approximately one fourth of the egg.

From this experiment we can conclude that when fertilized eggs are placed in 2½ per cent. ether at a time when the viscosity of their protoplasm is at its height, the ether reduces the viscosity to less than one sixth of its original value. Another experiment of the same sort may also be cited.

July 23 (Temp. 23°). Eggs were fertilized at 10.45 A.M. At 11.00 A.M. some of the fertilized eggs were put into 2½ per cent. ether in a glass-stoppered weighing bottle, others of the same lot were centrifuged for 60 seconds. The centrifugal treatment for 60 seconds resulted in a faint indication of a hyaline zone. At 11.05 A.M. a centrifugal test of the normal fertilized eggs for 80 seconds showed a hyaline zone extending about one fourth of the egg axis. Test samples of etherized eggs were centrifuged for 5 seconds at 11.12 A.M., for 10 seconds at 11.18 A.M., and for 15 seconds at 11.23 A.M. The 5 second test showed only faint indications of a hyaline zone, the 10 second test showed the zone not very plainly, the 15 second test showed it extending along one third of the axis of the egg. Later a 10 second test, at 11.35 A.M., showed the hyaline zone extending for ¼ to ⅓ of the egg axis. This test is not as trustworthy as the earlier tests, for the protoplasm appeared to be in chunks, and it is possible that the granules were fusing together. At 11.40 P.M., a 15 second test showed the hyaline zone ⅓, ¼, or absent. Coagulation is doubtless beginning.

This experiment showed that 2½ per cent. ether may lower the protoplasmic viscosity to one sixth or even one eighth of its original value in fertilized eggs.

As with the unfertilized eggs the protoplasm of the etherized fertilized eggs becomes more and more fluid with increase in ether concentration or length of exposure until suddenly coagulation occurs. Here too the onset of coagulation results in death. If the eggs are removed from the ether in the early stages of liquefaction they resume their development, but if they are kept in ether until coagulation has occurred or is about to occur, the eggs are permanently injured and never resume development.

The result of these experiments is a direct confirmation of my older results. These findings do not agree with the statement of Chambers previously referred to. Let us consider this statement closely. He says:

"Both A. Heilbronn and Weber agree with the coagulation theory of narcosis. On the other hand, L. Heilbrunn (1920) claims that the reversible effect of 2.5 per cent. ether on the sea-urchin egg occurs only when the viscosity is diminished. With higher concentrations of ether (3 per cent. +) the increased viscosity, according to him, is irreversible. He therefore concludes that narcosis implies a diminution in viscosity of the protoplasm. My results with the micro-dissection method and by observing Brownian movement do not agree with this. In 2 per cent. ether, Brownian movement was slowed down but

did not cease, and cleavage delayed but not stopped. In 2½ per cent. ether, cleavage was stopped and, both by means of the needle and by the cessation of Brownian movement, this was shown to be accompanied by a decided increase in viscosity (cf. p. 300)."

The first sentence of the above quotation is not quite true. As already pointed out Weber's ideas on ether narcosis agree exactly with mine. But this is a minor point. The essential fact is that Chambers has apparently obtained actual experimental results in direct contradiction with mine. How can this be interpreted?

In a previous paper I have already taken occasion to criticize the microdissection method for its over-great subjectivity (22), and this criticism has been supported by Heilbronn (23). But Chambers claims that his results were obtained both by microdissection and by the observation of Brownian movement. For some time I was puzzled as to how to explain the difference in his findings. Finally the idea suggested itself that Chambers in his studies of Brownian movement may have subjected the eggs to heat. It has already been shown that heat and ether act together, so that in low concentrations of ether, heat coagulation is hastened (24). Such coagulation is moreover reversible. On questioning Chambers it was found that in his experiments an arc lamp was used for illumination and no special precaution was taken to eliminate the heat factor. Here then is an explanation of the divergent results obtained by the Brownian movement method. As for Chambers' results with the microdissection method, these are probably not very trustworthy, for he was doubtless influenced by the results of his Brownian movement investigations. Another worker, Hyman, also using the dissection method, has roughly confirmed my views concerning the effects of ether on the protoplasm of sea-urchin eggs (25). Miss Hyman used ordinary steel needles instead of a microdissection apparatus.

Summary.

1. Water enters etherized muscle less rapidly than normal muscle, but this does not necessarily imply a change in the properties of the plasma membrane following etherization.

2. Ether does not lower the permeability of sea-urchin eggs to water.

3. Dilute ether solutions cause a very sharp decrease in the viscosity of sea-urchin egg protoplasm, both in fertilized and unfertilized eggs. Rough quantitative measurements of this decrease are given.

4. Slightly more concentrated solutions of ether produce a coagulation which is irreversible.

5. The divergent results of Chambers find a simple explanation.

REFERENCES CITED.

1. **Winterstein.**
'19 Die Narkose, Berlin.
2. **Höber.**
'22–'24 Physikalische Chemie der Zelle und der Gewebe, 5te Aufl., Leipzig.
3. **Lillie.**
'16 BIOL. BULL., XXX., 311.
4. **Osterhout.**
'16 Bot. Gaz., LXI., 148.
5. **Walker.**
'22 Introduction to Physical Chemistry, 9th Edition, London.
6. **Pissarjewsky and Karp.**
'08 Zeitsch. f. physik. Chem., LXIII., 257.
7. **Arrhenius.**
'92 Zeitsch. f. physik. Chem., IX., 487.
8. **McClendon.**
'15 Amer. Jour. Physiol., XXXVIII., 173.
9. **Bancroft.**
'21 Applied Colloid Chemistry, New York.
10. **Richards and Wells.**
'04 American Chemical Journal, XXXI., 242.
11. **Richards.**
'06 American Chemical Journal, XXXV., 510.
12. **Winterstein.**
'16 Biochem. Zeitsch., LXXV., 71.
13. **Lillie.**
'18 Amer. Jour. Physiol., XLV., 406.
14. **Bigelow.**
'07 Jour. Amer. Chem. Soc., XXIX., 1675.
15. **Bartell.**
'11 Jour. Phys. Chem., XV., 659.
16. **Heilbrunn.**
'20 Jour. Exp. Zoöl., XXX., 211.
17. **Loeb.**
'12 Biochem. Zeitsch., XLVII., 127.
18. **Heilbronn.**
'14 Jahrb. f. wiss. Bot., LIV., 357.

19. **Weber.**
'21 Biochem. Zeitsch., CXXVI., 21.
20. **Heilbrunn.**
'20 Biol. Bull., XXXIX., 307.
21. **Chambers.**
'24 The Physical Structure of Protoplasm as Determined by Micro-dissection and Injection, in General Cytology, edited by E. V. Cowdry, Chicago.
22. **Heilbrunn.**
'21 Jour. Exp. Zoöl., XXXIV., 417.
23. **Heilbronn.**
'22 Jahrb. f. wiss. Bot., LXI., 284.
24. **Heilbrunn.**
'24 Amer. Jour. Physiol., LXIX., 190.
25. **Hyman.**
'23 Biol. Bull., XLV., 254.