tion. Five years later Sumner published his observations that the action of copper salt can be deferred through the addition of cane-sugar, which is of course similar to the observation by Gies and myself. In the case of the antagonization of $ZnSO_4$ by another electrolyte we are, however, dealing with the action of both electrolytes on the same colloid.

2. Sumner states also that distilled and fresh water are toxic for *Fundulus* and that there exists an antagonism between distilled water and salts for these fish. The fact that a number of *Fundulus* can live a long time (if not indefinitely) in distilled water and that these fish, if they become landlocked, can live indefinitely in fresh water indicates that the distilled or fresh water are not in themselves toxic for these animals but that the toxic effect occasionally (but not always) observed is due to an inconstant or quantitatively varying constituent of the water. This constituent may be a parasite, or it may be a substance given off by the fish itself, *e. g.*, CO_2. Wasteneys and I have recently found that CO_2 may produce the same changes on the skin and the gills of the fish as those produced by mineral acids; and that, as in the latter case, the etching effects of the CO_2 may be counteracted through the addition of a neutral salt. The beneficial effect of the addition of some salt to the fresh or the distilled water, therefore, indicates that the salt either kills certain parasites contained or developed in the distilled water, or antagonizes the toxic effects of some electrolyte, *e. g.*, carbonic acid, if its concentration exceeds a certain limit, as it possibly did in some or all of Sumner's experiments.

JACQUES LOEB

ROCKEFELLER INSTITUTE

THE PERMEABILITY OF PROTOPLASM TO IONS AND THE THEORY OF ANTAGONISM

EVIDENCE was recently presented which showed[1] that a great variety of salts readily enter living cells and that antagonism between salts may be due to the fact that they mutually hinder or prevent each other from penetrating the protoplasm.

In these experiments plasmolysis was the criterion of penetration. Plasmolysis shows which salts enter and how rapidly, but does not indicate whether it is the ions or only undissociated molecules which penetrate the cell. To decide this question experiments were performed to test the electrical conductivity of living tissues in various solutions. The results agree in showing most conclusively that ions readily penetrate living protoplasm and that many ions which penetrate quite rapidly in pure solutions may be hindered or prevented from going in by the addition of small amounts of $CaCl_2$ or other salts.

To obtain reliable results in conductivity experiments material should be used which is not injured by weak currents or by other experimental conditions. It is desirable that the amount of space between the cells be constant so that the current which passes between the cells may be a constant fraction (as small as possible) of that which actually traverses the living protoplasm. The current should pass through a large number of thin sheets of living tissue, separated by thin films of solution. The penetration of various ions may then be studied by merely changing the solution. If the material is in thin sheets the ions are forced by the alternating current to pass in and out of a great extent of protoplasmic surface; this is of great importance, since the larger the surface the more reliable the measurement. The sheets of living tissue should be sufficiently rigid to permit manipulation and to endure without injury pressure sufficient to pack them firmly together so that the films of solution which separate them may be as thin as possible.

All these conditions are admirably fulfilled by the common kelps of the Atlantic coast (species of *Laminaria*). This material was accordingly used throughout the investigations.

Disks about 13 mm. in diameter were cut from the fronds by means of a cork-borer (the average thickness of the frond was about 0.5 mm.). From 100 to 200 of these disks

[1] SCIENCE, N. S., 34: 187, 1911.

were packed together (like a roll of coins) into a solid cylinder from 50 mm. to 100 mm. long. They were firmly held in place by glass rods arranged to make a hollow cylinder which closely fitted over the outside of the solid cylinder of tissue. Spaces between the rods allowed free access of the solution to the living tissue. At each end of the cylinder of tissue was placed a block of hard rubber containing a platinum electrode covered with platinum black; by means of a screw these blocks could be pressed with considerable force against the opposite ends of the cylinder of living tissue. The only substances which came into contact with the solution were hard rubber, glass, the electrodes and the living tissue. Details of construction will be given in a subsequent paper.

The current after leaving the electrodes traversed the solution for a very short distance and then passed directly into the living tissue. The same solution which bathed the electrodes was also present between the disks of living tissue in the form of thin films. The surface in and out of which ions were forced by the current amounted to from 26,500 to 53,000 sq. mm.

The measurements were made in the usual manner by means of a Wheatstone bridge. The solutions were brought to the same temperature before measuring except that in the case of small deviations the proper correction was made.

The usual method of procedure was to place the cylinder of disks in the solution, clamp the electrode carriers firmly against both ends of it, lift it out of the solution and read the resistance as soon as the superfluous liquid had drained from the tissue.

A preliminary series of experiments showed that the material remained to an extraordinary degree uninjured by the action of the currents employed as well as by the additional treatment involved in the experiments. In the first experiments the material was usually left immersed in sea water in the apparatus for 24 hours. During this period 12 readings were taken (the current passing for about two minutes each time) and the disks were 12 times taken out and then replaced in the apparatus. At the end of the 24 hours the resistance to the current remained unchanged (if injury had occurred the resistance would have been diminished) and there was no indication either macroscopic or microscopic that the cells were injured.

If the plasma membrane and the cell wall presented no obstacle to the passage of ions we should expect the resistance of a cylinder of living tissue to be practically that of a similar cylinder full of sea water. It was found that a cylinder of living tissue had a resistance of 1,100 ohms (all the figures given in this paper refer to readings taken between 18° C. and 18.2° C.) while that of a cylinder of sea water of equal size was 320 ohms. To ascertain whether this excess of resistance was due to living protoplasm or to cell walls the protoplasm was killed by adding sufficient formalin to the sea water to make a 2 per cent. solution. In other experiments the disks were killed by careful drying. In all cases the resistance after killing fell to about 320 ohms. These experiments demonstrated in the clearest manner that the ions penetrated very much less rapidly into living cells than into dead protoplasm or into cell walls.

Experiments were then made to determine the rate of penetration of various ions. As the treatment was the same in all cases it will suffice to describe a typical experiment dealing with NaCl and $CaCl_2$.

The material was first tested in sea water and found to have a resistance of 1,100 ohms. After remaining four hours in sea water the resistance was unchanged. The material was then transferred to NaCl .52 *M* which had the same temperature as the sea water and the same conductivity (as determined by numerous careful tests). The electrode carriers were unclamped and moved apart. Each disk was then seized in turn by the forceps and moved back and forth in the solution so as to wash out the sea water and replace it by the solution of pure NaCl. This was repeated several times. It was thus possible to rinse each disk thoroughly in the solution without removing it from the apparatus or changing

its position in the series of disks which composed the cylinder. The mere act of rinsing the disks in this way and then reclamping the electrodes made only slight changes in the reading.

After remaining five minutes in NaCl .52 *M* the resistance had dropped to 1,000 ohms; after ten minutes to 890 ohms; after fifteen minutes to 780 ohms; after sixty minutes to 420 ohms. It continued to fall steadily until it reached 320 ohms, at which point it remained stationary; it then had practically the conductivity of sea water. On replacing in sea water it did not recover any of its resistance, even after standing for several days. It should be noted that the solution of NaCl employed is nearly isotonic with sea water and that none of the observed effects could be due to osmotic action.

Further experiments showed that if the material was removed from NaCl solution and placed in sea water as soon as its resistance had fallen about one hundred ohms below the original resistance it quickly regained its original resistance and remained unchanged for a long time.

It is therefore evident that pure NaCl produces a very rapid decrease in resistance which, up to a certain point, is reversible.

A very striking contrast is obtained by placing living tissue in a solution of $CaCl_2$ having the same conductivity as sea water. The resistance then rises rapidly to a maximum (very often in the first fifteen minutes from 1,100 ohms to 1,750 ohms) and remains practically stationary for some hours. After this it slowly sinks and finally reaches about 320 ohms, which is the resistance of an equal amount of sea water. If, however, it be returned to sea water shortly after it has reached its maximum it soon regains its original resistance and remains for a long time (in sea water) practically unchanged. The rise in resistance caused by $CaCl_2$ is in no way due to its action on the cell walls, for dead tissue shows no rise.

It is therefore evident that $CaCl_2$ produces a very rapid *increase* in resistance, which is reversible.

What is the effect of combining NaCl and $CaCl_2$ in the proportions in which they exist in sea water? This question has great theoretical and practical interest in view of the fact that $CaCl_2$ is known to antagonize the toxic action of NaCl in the most striking way. To answer this question the following experiment was performed. To 1,000 c.c. NaCl 1 *M* there was added 15 c.c. $CaCl_2$ 1 *M;* the mixture was then diluted until it had the same conductivity as sea water. On placing living tissue in this mixture it neither gained nor lost in resistance and even after twenty-four hours had the same resistance as at the start.

It is therefore evident that the entrance of the ions of NaCl is greatly hindered by the presence of very small amounts of $CaCl_2$ and that this may explain the antagonistic action of $CaCl_2$ on NaCl.

Further experiments showed that such salts as KCl, $MgCl_2$, CsCl, RbCl, LiCl, NH_4Cl, NaBr, NaI, $NaNo_3$, Na_2So_4 and Na-acetate act in general like NaCl (though with different degrees of rapidity) while $BaCl_2$ and $SrCl_2$ act like $CaCl_2$.

It might be supposed that some of these effects are due to expansion or contraction of the cells under the influence of the salts, but microscopic observation showed that this was not the case except only that when a cell is injured by the salt a contraction (which I have elsewhere called false plasmolysis) may take place. But as the fall in resistance is already great before any such contraction begins and as the contraction is in any case too small to account for more than a small per cent. of the decrease in resistance it may be regarded as at best a secondary factor which is absent until the resistance has reached a low point and which is almost negligible beyond that point.

It might be supposed that the change in resistance is due to causes which operate in the interior of the cell rather than in the plasma membrane, but this is opposed to a variety of evidence which can not be discussed here.

Two hypotheses may be formed regarding

the increase of resistance which is observed when the tissue is transferred from NaCl + $CaCl_2$ to pure $CaCl_2$ of the same conductivity. On one hypothesis the plasma membrane would retain its normal properties after the transfer but would show increased resistance because it is normally less permeable to the ions of $CaCl_2$ than to the ions of NaCl.

On the other hypothesis the plasma membrane would suffer a change in its properties as the result of the transfer. The facts strongly favor this hypothesis. I will mention only a few. Visible changes in the outer layer of the protoplasm are produced by $CaCl_2$ (and many other substances) and this makes it probable that the plasma membrane suffers change. Alum, which is known to alter the properties of many colloids (*e. g.*, in tanning), when added in solid form to the sea water greatly increases the resistance of the protoplasm although it greatly decreases the resistance of the sea water. In this case the only explanation is that the permeability of the plasma membrane is altered. On the other hand it is clear that the large number of substances which produce irreversible decrease of resistance must also alter the plasma membrane.

It seems probable therefore that a great variety of substances alter the plasma membrane so as to increase or decrease its permeability.

It may be pointed out that these results are precisely what should be expected if the antagonistic action of salts is due, as Loeb has suggested, to the fact that they hinder each other from penetrating the protoplasm. It is quite clear from the experiments that $CaCl_2$, $SrCl_2$ and $BaCl_2$ in small amounts are able to hinder very greatly the entrance of the ions of NaCl. The mechanism of this action is not fully understood, but I may state that $CaCl_2$, $BaCl_2$ and $SrCl_2$ bring about visible changes in the plasma membrane which are entirely different from those produced by such salts as NaCl. It is hoped that a further study of these visible changes may throw light on this question.

Previous experiments on plasmolysis have shown essentially similar phenomena and the complete confirmation of the results of one method by those of the other form the most striking proof possible of the facts outlined above.

It may be asked how merely delaying the entrance of a salt can protect the protoplasm against its toxic action. In this connection it may be pertinent to recall the familiar phenomenon of colloid chemistry that a salt which produces marked effects when added suddenly may produce little or no effect when added slowly. It should be noted that there is good evidence to show that the NaCl does not enter the cell alone but is accompanied by $CaCl_2$. It is possible that these salts may wholly prevent each other from penetrating internal membranes (*e. g.*, the nuclear membrane) which are of importance in this connection.

The chief conclusions are as follows:

1. Quantitative studies of permeability may be made by a simple and accurate method.

2. Slight changes in the rate of penetration may be observed and accurately measured at very brief intervals.

3. A great variety of anions and kations readily penetrate living protoplasm.

4. Inasmuch as these ions are insoluble in lipoid it would appear that Overton's theory of permeability can not be correct.

5. The plasma membrane is readily altered by a variety of substances in a fashion which is easily understood on the hypothesis of a colloid (probably proteid) plasma membrane but which can not be explained on the hypothesis that the plasma membrane is a lipoid.

6. The antagonistic action of salts is largely or entirely due to the fact that they hinder or prevent one another from entering the protoplasm.

W. J. V. OSTERHOUT
LABORATORY OF PLANT PHYSIOLOGY,
HARVARD UNIVERSITY

NOTES ON THE DISTRIBUTION OF THE SOUTHEASTERN SALAMANDERS (GEOMYS TUZA AND ALLIES)

A CHARACTERISTIC feature of many parts of the pine forests of the coastal plain of Georgia,