SCIENCE

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CONTENTS

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ANTAGONISM AND PERMEABILITY[1]

BY antagonism we mean that one toxic substance acts as an antidote to another. A solution containing salts in the proper proportions may have none of the toxic action of the individual salts. Such a mixture has been called by Loeb a physiologically balanced solution. It is found that physiological balance is of the greatest importance not only for marine organisms, but also for fresh-water and terrestrial plants and animals: these considerations have found practical application in agriculture.

In the hope of throwing light on the cause of antagonism the speaker made experiments on the penetration of salts into the cell. It was found that while NaCl alone penetrated rapidly the addition of a little $CaCl_2$ delayed penetration. It therefore seemed as though calcium antagonized sodium by preventing more or less completely its entrance into the cell. This idea had been suggested by Loeb but had not received experimental support.

These experiments (which included a number of salts) were carried out by means of the method of plasmolysis. This method did not yield quantitative data of the desired precision, but it was found possible to obtain much more accurate results by the method of electrical conductivity. By this method we measure the resistance offered by protoplasm to the passage of ions. In sodium chloride the resistance rapidly diminishes until it becomes stationary: this means that in NaCl the permeability of the protoplasm rapidly increases until death occurs,

[1] Address delivered before Section G, American Association for the Advancement of Science, at a symposium, December 27, 1916.

after which it remains fixed. In $CaCl_2$ the permeability at first decreases until a certain minimum is reached: after this it begins to increase and finally reaches a constant value (as in NaCl), which signifies death.

Further experiments showed that all substances which affect permeability may be divided into two groups, (1) those which act like NaCl; (2) those which act like $CaCl_2$. This led to the following hypothesis: Substances of the first group antagonize substances of the second group and vice versa.

Experiments were then made to test this hypothesis. It was found that substances which behave like NaCl with respect to antagonism (in experiments on growth) behave like NaCl in their effect on permeability. Substances which behave like $CaCl_2$ with respect to antagonism also behave like $CaCl_2$ in their effect on permeability. Moreover, substances like $LaCl_3$, which antagonize NaCl more powerfully than does $CaCl_2$, are found to affect permeability more powerfully than $CaCl_2$. There is therefore a striking parallel between effects on permeability and the antagonistic effects observed in experiments in which growth and length of life are employed as criteria of antagonism.

Equally remarkable is the outcome when permeability is used as the criterion of antagonism. It is found that all solutions which permit normal growth are likewise solutions which preserve normal permeability.

These experiments which were originally made on *Laminaria* were afterward extended to other algæ, to flowering plants and to animals.

Using permeability as a criterion of antagonism, the speaker has made investigations on a great variety of substances. Time is lacking to describe these, but it may be said that the outcome in every case has supported the hypothesis. This was strikingly shown in investigations on organic substances (non-electrolytes), a number of which were found to belong to the second group. It turned out that all of these substances were able to antagonize NaCl, as is required by the hypothesis.

This result greatly strengthened the speaker's confidence in the hypothesis which seems to serve a useful purpose by enabling us to predict what substances will antagonize each other.

As the result of these investigations we seem to be justified in concluding that there is a close connection between antagonism and permeability. Conclusions concerning such fundamental relations should be tested, whenever possible, by a variety of methods. This task was undertaken by Dr. Brooks, who confined himself chiefly to the following methods: (1) diffusion through living tissue, (2) exosmosis, (3) change of curvature of strips of tissue.[2]

In the first of these methods different solutions were placed on opposite sides of a piece of tissue. The diffusion of salts through the tissue was then measured.

In the second method the tissue was placed for a short time in a salt solution and the rate at which substances subsequently diffused out of the cell was measured.

In the third method strips of the peduncle of the dandelion were placed in hypertonic salt solutions and the rate of penetration of the salt into the protoplasm was calculated from the rate at which the strips recovered their normal shape after being curved by the action of the hypertonic solution (the strips remaining in the solution during recovery). This gives the same kind of information as plasmolysis but avoids the most serious errors of that method.

[2] Brooks, S. C., *Proc. Nat. Acad.*, 2: 569, 1916.

It is a very striking fact that all three of these methods agree with those already described in showing that physiologically balanced solutions preserve normal permeability, while NaCl causes a rapid increase, and $CaCl_2$ an initial decrease, followed by an increase of permeability.

This general agreement can not but increase our confidence in the conclusion that permeability and antagonism are intimately connected.

Further studies have shown that permeability serves as a delicate indicator of what we may call the vitality of the organism. By this is meant a condition of normal health and vigor and the ability to resist unfavorable influences. An organism which has normal permeability (as shown by determining its electrical conductivity) behaves normally in all respects and lives a normal length of time under laboratory conditions, while one which has abnormally high permeability behaves abnormally and does not live the normal length of time. Hence it would appear that we can treat vitality quantitatively, for if the vitality of a large number of organisms is measured in this way we obtain a variation curve: this indicates that vitality may be treated in the same manner as any other character (as, for example, length or weight).

Moreover, since increase of permeability indicates injury, we have a method of measuring injury and of distinguishing quantitatively between temporary and permanent injury. It is found that great fluctuations of permeability are possible without permanent injury. These fluctuations may control the metabolism of the cell.

These studies show that all agencies which sufficiently alter the normal permeability of the protoplasm (such as poisons, excessive light, heat, electric shock, severe plasmolysis, mechanical shock, partial drying, lack of oxygen, etc.), shorten the life of the organism. This is a very striking fact and its significance seems to be unmistakable. It indicates that permeability is a delicate and accurate indicator of vitality.

An analysis of the factors which control permeability has been attempted in subsequent studies. The changes in the resistance of tissues placed in mixtures of NaCl and $CaCl_2$ have been carefully determined. These are shown in Fig. 1. A glance at the figure suggests that there are two processes, one of which causes a rise, the other a fall of resistance. It is natural to suppose that these are chemical in nature and we may assume that they are consecutive reactions by which a substance M, which determines the resistance of the protoplasm, is formed and broken down according to the formula

$$A \rightarrow M \rightarrow B.$$

It may be assumed that M is a substance at the surface of the cell which offers resistance to the passage of ions.

It is evident that if the first reaction $A \rightarrow M$ is more rapid than the second, M will be formed more rapidly than it is decomposed and will increase in amount. Eventually, as the supply of A becomes exhausted, the formation of M will go on more and more slowly, so that it will no longer keep pace with the decomposition. The amount of M will then diminish until it finally disappears or reaches a fixed minimum (this corresponds to the death of the tissue). It is evident that if the relative velocities of the two reactions $A \rightarrow M$, and $M \rightarrow B$ be properly varied the curves of resistance will rise and fall rapidly or slowly in the manner shown in Fig. 1. It can be shown that these assumptions enable us to account for all the experimental curves.

A point of importance is that the velocities of both reactions reach a minimum in a definite mixture of $NaCl + CaCl_2$. This mixture contains the molecular proportions 95.24 $NaCl$ + 4.76 $CaCl_2$. We can account

The extent to which these assumptions enable us to predict the behavior of the tissues in various mixtures is evident from Table I.

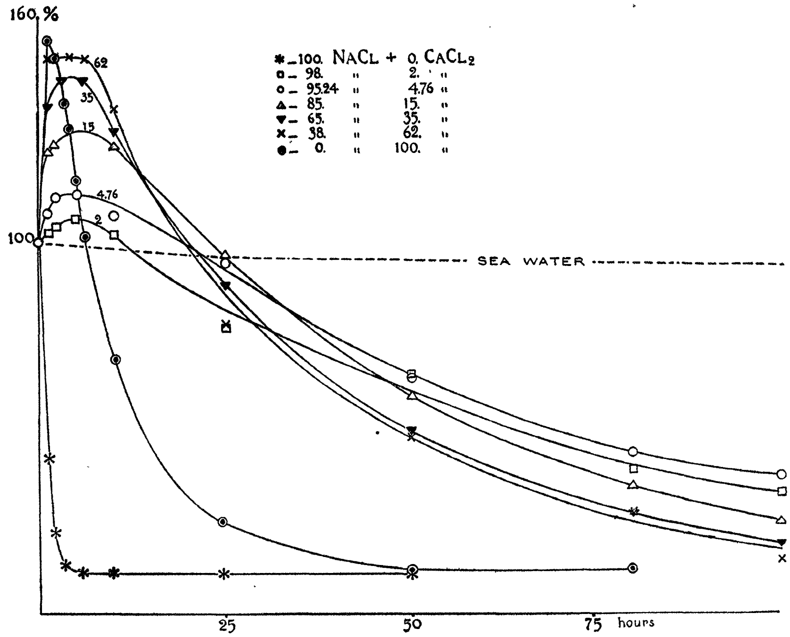

FIG. 1. Curves of electrical resistance of *Laminaria* in NaCl .52*M*, in $CaCl_2$ .278*M*, and in mixtures of these (the figures show the molecular percent. of $CaCl_2$ in the mixture).

for this if we suppose that both the reactions are inhibited by an organic salt[3] formed with a constituent X of the protoplasm according to the equation

$$2NaCl + X + CaCl_2 = Na_2XCaCl_4.$$

We may also assume that the reaction $A \rightarrow M$ is catalyzed by $CaCl_2$. This enables us to account for the fact (which is clearly evident from an inspection of the curves) that the greater the proportion of $CaCl_2$ in the mixture the higher and more rapidly the curve rises.

[3] The amount of this salt will be greatest in the mixture of 95.24 NaCl + 4.76 $CaCl_2$ if the reaction takes place in the surface and $CaCl_2$ is 10 times as concentrated in the surface as NaCl.

It is evident that the agreement between observed and calculated values is remarkably satisfactory. In regard to the theoretical procedure it should be said that in constructing equations for the curves the minimum number of constants has been employed and the attempt has been made to proceed with the fewest and the most natural assumptions. These assumptions appear to be very reasonable, for it is evident that there must be two processes in order to produce a rise and fall of resistance and that their speed must be regulated by NaCl and $CaCl_2$. It is also apparent that these salts must enter into some sort of combination with a constituent of the protoplasm and it is evident that this compound may regulate the speed of these processes.

We thus arrive at an explanation of antagonism. The theory[4] attempts to account for the following facts.

1. Why both NaCl and $CaCl_2$ are toxic.

2. Why when mixed in the proper proportions their toxicity is greatly diminished (antagonistic action).

3. Why they have opposite effects on permeability.

4. Why the decrease of permeability produced by $CaCl_2$ must be followed by an increase when the exposure is sufficiently prolonged.

5. Why all toxicity disappears in sea water. This is accounted for by supposing that in sea water A is formed as fast as it decomposes.

The theory gives a quantitative explanation of the toxicity of all the mixtures and enables us to predict the resistance (and permeability) in any mixture at any moment during exposure.

It likewise emphasizes the fact that life processes consist largely of consecutive reactions and that analysis of the dynamics of such reactions is indispensable for the solution of certain fundamental problems of biology.

Reference has been made to the suggestion that calcium antagonizes sodium by preventing it from entering the cell. This explanation encounters a difficulty in the fact that even in a balanced solution the salts penetrate the cell. This difficulty disappears if we adopt the point of view which has just been presented, for it is evident that on this basis we do not regard antagonism as due to prevention of penetration. Nor is there any reason to suppose that the penetration of salts will have an unfavorable effect provided that as they penetrate into the cell the balance between them is preserved.

There is another aspect of the subject which is of considerable interest. It is usually found that when antagonistic substances are mixed in various combinations there is one proportion which is more favorable than others. If we increase the concentration of one constituent it is necessary to increase the concentrations of the others in like proportion in order to preserve the optimum condition. This law of

TABLE I

Observed and Calculated Values of Resistance of Laminaria in Mixtures of NaCl and $CaCl_2$

Time in Hours	Per Cent. of Net Resistance in									
	98 NaCl + 2 $CaCl_2$		95.24 NaCl + 4.76 $NaCl_2$		85 NaCl + 15 $CaCl_2$		65 NaCl + 35 $CaCl_2$		38 NaCl + 62 $CaCl_2$	
	Obs.	Calc.	Obs.	Calc.	Obs.	Calc.	Obs.	Calc.	Obs.	Calc.
1	103.1	103.7	108.2	106.8	124.5	115.6	136.1	123.8	148.1	127.2
2	103.8	105.8	112.1	111.2	126.1	124.8	141.9	136.3	149.0	141.7
3	105.8	106.7	112.1	113.8	128.5	129.8	143.2	142.2	149.0	148.0
4	106.1	106.9	113.9	115.3	130.2	132.1	143.9	144.0	149.0	149.7
5	106.1	106.6	113.1	115.8	130.2	132.7	143.9	143.6	149.0	148.8
6	104.9	106.0	113.1	115.8	130.2	132.3	143.7	141.7	149.0	146.5
10	102.1	101.8	107.9	112.5	126.5	125.1	129.5	129.8	136.1	132.6
25	76.89	84.21	95.21	93.41	96.40	93.80	87.85	87.90	78.21	86.30
50	63.90	61.70	62.50	68.20	58.11	58.83	47.81	47.80	46.34	44.46
80	38.90	43.49	42.52	47.78	33.92	35.54	26.01	25.88	27.11	23.27
100	31.80	35.09	35.83	38.33	24.01	26.58	17.33	18.90	14.42	17.03

The measurements were made at 15° C. or corrected to this figure. Each experimental figure is the average obtained from 6 series of experiments.

All the mixtures had the same conductivity as sea water.

[4] *Cf. Proc. Am. Phil. Soc.*, 55: 533, 1916.

direct proportionality has been identified with Weber's law by Loeb, who says:

Since this law underlies many phenomena of stimulation it appears possible that changes in the concentration of antagonistic ions or salts are the means by which these stimulations may be brought about.

In view of the importance of these relations it seems desirable to ascertain what mechanism makes one proportion better than others and preserves this preeminence in spite of changes in concentration.

Precisely this kind of mechanism is involved in the theory just outlined. It is easy to see that such a mechanism must exist if the formation of Na_2XCaCl_4 takes place at a surface. In a surface substances usually exist in a different concentration from that which they have elsewhere in the solution. If NaCl and $CaCl_2$ migrate into the surface, so as to become more concentrated there than in the rest of the solution, their concentration in the surface must increase, as their concentration in the solution increases, up to the point where the surface is saturated. Beyond this point an increase in their concentration in the solution produces no effect on their concentration in the surface.

When this stage has been reached the formation of Na_2XCaCl_4, if it takes place in the surface, will not be affected by an increase in the concentration of the salts in the solution. It will, however, be affected by changes in the relative proportions of the salts. The number of molecules in a unit of surface will remain nearly constant, but if the proportion of NaCl in the solution be increased some of the $CaCl_2$ in the surface will be displaced by NaCl.

Below the saturation point the relative proportions of the salts will be of less importance than their total concentration: this is the case at low concentrations in the region of the so-called "nutritive effects."

It is evident that if we adopt this theory we can see why the most favorable proportion must remain approximately the same in spite of variations in concentration, and we thus arrive at a satisfactory explanation of Weber's law.

There are other ways in which permeability appears to be connected with stimulation. One of these has to do with anesthesia. Typical anesthetics decrease permeability. This accords with the idea that stimulation depends on the movement of ions in the tissue. Such movement would be checked by decrease of permeability.

Another has to do with mechanical stimulation. It is well known that the effects of certain kinds of stimuli can be referred directly to chemical changes which they produce in the protoplasm, but there are other kinds which appear to operate by physical means only. In the latter category are such stimuli as contact, mechanical shock and gravitation. While their action appears at first sight to be purely mechanical, they are able to produce effects so much like those of chemical stimuli that it appears probable that in every case their action must involve chemical changes.

The chief difficulty which confronts a theory of mechanical stimulation appears to be this: How can purely physical alterations in the protoplasm give rise to chemical changes? It would seem that a satisfactory solution of this problem might serve to bring all kinds of stimulation under a common point of view, by showing that a stimulus acts in every case by the production of chemical reactions.

An answer to this question is suggested by some observations on the cells of the marine alga *Griffithsia*. When one of the larger cells is placed under the microscope and touched near one end a change occurs in the chromatophores directly beneath the spot which is touched. The surfaces of the chromatophores in this region become permeable to the red pigment, which begins to diffuse out into the surrounding protoplasm. This change begins soon after the cell is touched. As the red pigment diffuses through the protoplasm it soon reaches neighboring chromatophores and it may be seen that their surfaces also become permeable and their pigments begin to diffuse out. In this way a wave—which may be compared to a wave of stimulation—progresses along the cell until the opposite end is reached.

The rate of propagation of this wave corresponds to that of the diffusion of the pigment. It would seem that at the point where the cell is touched, pigment, and probably other substances, are set free, diffuse out and set up secondary changes as they progress. These changes are doubtless chemical in nature.

The important question then arises: How does the contact initiate the outward diffusion of the pigment or other substances? It would seem that this may be due to a mechanical rupture of the surface layer of the chromatophores which is either not repaired at all or only very slowly. Many cases are known in which the surface layers of protoplasmic structures behave in this way. If, therefore, such structures exist within the cell, it is evident that any deformation of the protoplasm which is sufficient to rupture their surface layers will permit their contents to diffuse out into the surrounding protoplasm. A great variety of cellular structures (plastids, vacuoles, "microsomes," inclusions, etc.), possess surface layers of great delicacy and it is easy to see how some of these may be ruptured by even the slightest mechanical disturbance.

If these processes occur it is evident that purely physical alterations in the protoplasm can give rise to chemical changes. Responses to contact and mechanical stimuli may be thus explained; and since gravitational stimuli involve deformation of the protoplasm we may extend this conception to geotropism.

Further studies, which are now being made, can not be mentioned for lack of time, but it is hoped that what has been said may suffice to indicate how stimulation, vitality, injury and recovery, together with permeability and antagonism, may be brought under a common point of view and perhaps traced to similar fundamental causes.

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JOHN MUIR[1]

IT is as a human being ever striving upward that I would portray John Muir.

From his early boyhood to his old age this spirit dominated him. As a child in Scotland, at every opportunity, in spite of parental prohibitions, and notwithstanding the certainty of punishment upon his return, he would steal away to the green fields and the seashore, eagerly interested in everything alive.

Illustrating this trait, I quote his boyhood impressions of the skylarks:[2]

> Oftentimes on a broad meadow near Dunbar we stood for hours enjoying their marvelous singing and soaring. From the grass where the nest was hidden the male would suddenly rise, as straight as if shot up, to a height of perhaps thirty or forty feet, and, sustaining himself with rapid wing-beats, pour down the most delicious melody, sweet and clear and strong, overflowing all bounds, then suddenly he would soar higher again and again, ever higher and higher, soaring and singing until lost to sight even in perfectly clear days, and oftentimes in cloudy weather "far in the downy cloud" . . . and still the music came pouring down to us in glorious profusion, from a height far above our vision, requiring marvelous power of

[1] Address delivered upon the occasion of the unveiling of a bronze bust by the sculptor C. S. Pietro, at the University of Wisconsin, December 6, 1916.

[2] "The Story of My Boyhood and Youth," John Muir (Houghton-Mifflin Co., 1913), pp. 46 and 47.