SIMILARITY IN THE BEHAVIOR OF PROTOPLASM AND GELATINE

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In the course of experiments on salt antagonism in gelatin, it occurred to the writer that it would be possible to find an analogy in gelatine for the processes which occur in protoplasm when it is acted upon by antagonistic salts. These processes may be illustrated by figure 1, which shows changes in the electrical resistance of living protoplasm produced by NaCl and CaCl₂. If these changes are due to the diffusion of salts into the protoplasm (and the resulting increase of their concentration) it is possible that similar changes would occur in gelatine if it were divided up into small portions corresponding in size to the masses of protoplasm which form the individual living cells. The increase in the concentration of the salts as they diffused into the gelatin would produce changes in the precipitability (and other properties) of the gelatine. The manner in which the precipitability varies with the concentration of salts has been explained in the previous article.

The experiments already reported indicate that the favorable action of a physiologically balanced solution is due to the fact that such a solution produces a precipitate (or some condition accompanying precipitation) in the protoplasm, the maximum precipitate being found at the isoelectric point. By varying the amount of this precipitate, variations in electrical resistance might be brought about.

If, however, the normal condition of protoplasm is isoelectric, the addition of any electrolyte would change this balance and decrease the precipitate, thus increasing permeability. It is well known, however, from Osterhout's experiments on the electrical resistance of Laminaria, that salts like CaCl₂ cause a decrease of permeability at first, but this is later followed by an increase, while NaCl causes only an increase. To make the analogy between gelatine and protoplasm more complete, therefore, we should assume that the normal condition of protoplasm is somewhat on the anion side of the isoelectric point, i.e., protoplasm must bear a negative charge. The addition of CaCl₂ would neutralize this charge, increase the amount of precipitate or aggregation in the protoplasm and thereby decrease the permeability. In excess of Ca, however, the precipitate disappears and the permeability increases.
Figure 1 shows a set of Osterhout's electrical resistance curves for *Laminaria*, in which resistance is plotted against time. In his experiments resistance was used as a measure of permeability. From what has been said, it is to be expected that the *Laminaria* curves can be duplicated in alkaline gelatine which bears, of course, a negative charge. This is done in the following way.

To a series of test tubes containing gelatine made alkaline (by adding enough NaOH to make the concentration 0.005M) is added NaCl, CaCl₂, or mixtures of the two, in increasing concentrations. Each tube is then titrated with 95% alcohol until an opaque precipitate is produced. Figure 2 shows the results of such an experiment. Inspection of the curves shows that low concentrations of CaCl₂ cause a decrease in the amount of alcohol needed for precipitation while higher concentrations cause an increase. NaCl causes only an increase. Mixtures of NaCl and CaCl₂ take intermediate positions. A comparison of these gelatine curves with the *Laminaria* curves in figure 1 shows a striking resemblance between the two.

In spite of the difference in the methods of obtaining the curves in protoplasm and in gelatine, the two are comparable on the assumption, that the effect of time in the *Laminaria* experiments is to increase the concentrations of the salts in the cells of the tissue. We might then suppose that variations in the concentration of the salts produced corresponding variations in the amount of a precipitate (e.g., at the plasma membrane) which offered a resistance to the passage of ions and so increased electrical resistance. This assumption would be rendered more probable if evidence were obtainable that the electrical conductivity of gelatine varies directly with the amount of alcohol required to produce precipitation. Some evidence on this point is already available.

Samec⁴ working on a starch gel, measured the electrical conductivity and the alcohol precipitability simultaneously in lots of starch which had been heated for different lengths of time, and found that the two ran parallel to each other. Pauli⁵ and his pupils state that the conductivity is a measure, other things being equal, of the number of electrically neutral particles present. Conductivity would therefore be least at an isoelectric point where, also, least alcohol is needed for precipitation.

There are of course quantitative discrepancies in this analogy, but one could hardly expect gelatine to be as sensitive to electrolytes as protoplasm. Qualitatively, however, the resemblance between the two curves is remarkably close, even in the following details.
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RESISTANCE

PROTOPLASM
- CaCl₂ 100%; NaCl 0%
- " 67 " 67
- " 0 " 100

GELATINE
- CaCl₂ 100%; NaCl 0%
- " 67 " 67
- " 0 " 100

FIG. 1

Curves from Osterhout's experiments showing the changes in the electrical conductivity of protoplasm when discs of Laminaria are transferred from sea water to NaCl 0.52M or to CaCl₂ 0.278M or to mixtures of the two (all having the same conductivity as sea water.) Ordinates represent per cent of resistance (the resistance of the discs in sea water being taken as 100%). When the resistance has fallen to 10% of the control, the death point is reached. Here the protoplasm has the same conductivity as sea water.

FIG. 2

Curves showing how the effects of NaCl and CaCl₂ on the electrical resistance of protoplasm may be imitated by the effects of NaCl and CaCl₂ on the alcohol precipitability of gelatine (containing NaOH 0.005M). The ordinates represent the decrease (8 minus the number of cc.) of 95% alcohol required to produce an opaque precipitate, the abscissae salt concentrations. For comparison with Laminaria it is assumed that the salts diffuse into the gelatin (as into the protoplasm of Laminaria) CaCl₂ diffusing 80% as fast as NaCl. The molecular concentration of the NaCl solution is twice as great as that of the CaCl₂ solution (which is approximately the case in the Laminaria experiments). At the end of a given time, when the concentration of CaCl₂ in the gelatin is 0.031M, the concentration of NaCl will be 0.031 × 2 × (100 + 80) = 0.078M. This point is indicated in the figure. The concentrations in mixtures at this point can easily be calculated (e.g., in NaCl 50% + CaCl₂ 50% they would be half of those just mentioned).

It is therefore evident that we may regard the abscissae as representing time as well as concentration and that from this point of view the figure becomes comparable with figure 4, showing the changes in alcohol precipitability and in resistance which would be expected to occur with time if the salts were diffusing into the gelatin.

The last point on each curve is obtained by interpolation between the preceding point and a later one not shown in the diagram.
1. The maximum is higher in pure CaCl₂ than in mixtures of CaCl₂ and NaCl.

2. The maximum is reached slightly sooner (or at a slightly lower concentration) in pure CaCl₂ than in mixtures of CaCl₂ with NaCl.

3. There is a slight lag at the start of the NaCl curve in both Laminaria and gelatine. In the case of gelatine, this lag appears only if more NaOH is put into the gelatine at the start than in the experiment recorded in figure 5. With sufficient NaOH, the lag may be magnified into a distinct rise. This is in agreement with the fact, that NaCl will antagonize both anions and cations. The lag in the Laminaria experiments is seen in the fact that the velocity constant of the loss of resistance in NaCl is lowest at the start.⁷

The NaCl curve falls more slowly in figure 2 than in figure 1 but this may be paralleled by the results with Rhodymenia and other plants where the NaCl curve falls slowly.

4. The final increase in conductivity (or of alcohol) in the mixtures of NaCl and CaCl₂ is less rapid than in either of them taken separately. In general, the greater the percent of CaCl₂ in the mixture, the less rapid is the increase.

In the Laminaria experiments the curves approach a death point where the conductivity of the protoplasm is the same as that of sea water. No further increase of conductivity is possible. Of course this behavior would not be expected in gelatine.

In a recent article in Science, Spaeth⁸ has presented a colloid-chemical theory of the 'vital equilibrium' of protoplasm as an explanation of Osterhout's Laminaria experiments. Spaeth's theory differs from that outlined above in the following fundamental points.

1. According to Spaeth the 'vital equilibrium' is between liquefying agents like NaCl and precipitating agents like CaCl₂. Reasoning from the gelatine analogy the equilibrium is simply between positive and negative ions.

2. Spaeth supposes that CaCl₂ and similar electrolytes increase the viscosity of the protoplasm by an irreversible coagulation, after which the viscosity becomes that of the dispersion medium, due to the complete separation of the two phases. According to the gelatine experiments, on the other hand, low concentrations of Ca cause a perfectly reversible precipitate, or increase of aggregation, of an isoelectric nature, which disappears gradually with excess of CaCl₂. Irreversible changes resulting in death appear only in much higher concentrations of CaCl₂.

It should be noted that the viscosity of a colloid (a conception which Spaeth uses extensively in his theory) is not a simple property of a
system but is dependent upon many different factors which may sometimes be opposing. Thus Pauli and his pupils find minimum viscosity at an isoelectric point in protein sols due to minimum ionization of the protein. In the writer's gelatine experiments, however, the gelation or gelation viscosity of the gel was distinctly at a maximum at the isoelectric point due to maximum aggregation. It appears necessary to distinguish between these two kinds of viscosity.

Summary.—A close analogy to Osterhout's experiments on the electrical resistance of Laminaria is found in gelatine (plus NaOH), if we assume that the effect of time in the Laminaria experiments is to increase the concentrations of the salts in the cells of the tissue.

1 These PROCEEDINGS, 2, 534 (1916).
2 For the sake of brevity the word precipitate is used throughout to denote not only an actual precipitate, but any accompanying conditions which vary with the amount of precipitate or the degree of precipitability.
3 Cf. Osterhout, Science, 41, 255 (1915) for summary of results.
4 Pauli has concluded for other reasons that protoplasm reacts much like protein soils containing alkali. Biochem. Zs., 24, 239 (1910).
6 Pauli, loc. cit.
7 Osterhout, Science, 39, 544 (1914).
8 Spaeth, Science, 43, 502 (1916).

ON CERTAIN ASYMPTOTIC EXPRESSIONS IN THE THEORY OF LINEAR DIFFERENTIAL EQUATIONS

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The nature of the solutions of a certain linear differential equation containing a complex parameter has been investigated by Prof. G. D. Birkhoff,1 who discovered the asymptotic character of the solutions when the parameter is large in absolute value. These results he employed in the study of expansion problems connected with the particular differential equation

\[ \frac{d^n u}{dx^n} + P_2 (x) \frac{d^{n-2} u}{dx^{n-2}} + \ldots + P_n (x) u + \rho^n u = 0, \]  

(1)

together with \( n \) linearly independent linear boundary conditions

\[ W_1 (u) = 0, \quad W_2 (u) = 0, \quad \ldots, \quad W_n (u) = 0. \]  

(2)

It is the aim of this paper to present asymptotic formulas for \( n \) linearly independent solutions \( y_1, y_2, \ldots, y_n \) of equation (1) which are in