LI. THE ACTION OF SALTS AND NON-ELECTROLYTES UPON BUFFER SOLUTIONS AND AMPHOTERIC ELECTROLYTES AND THE RELATION OF THESE EFFECTS TO THE PERMEABILITY OF THE CELL.

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(*Received March 15, 1921.*)

INTRODUCTION.

THE presence of buffer solutions in living protoplasm is generally assumed by physiologists, although it is only in a very few instances that direct chemical evidence of their presence has been obtained. The mixture of phosphates and carbonates which exists in the blood is well known and has been the subject of much examination, both theoretical and experimental, and it has recently been shown [Hempel, 1917] that certain vegetable saps contain buffer mixtures of a different type—mixtures of the acid and neutral salts of organic acids. The existence of buffer mixtures as a normal constituent of the living protoplasm of the cell is however a hypothesis which it is impossible at present to submit to experimental verification. It is nevertheless supported by much indirect evidence and gives a simple and entirely satisfactory explanation of the way in which the organism is able to maintain the reaction of its protoplasm very near to absolute neutrality in spite of the continuous production of carbon dioxide and other acidic substances.

It is perhaps in consequence of the great importance of the neutralising action of buffer solutions that attention has been focussed upon this property to the exclusion of others which may have an equal physiological significance. The direct action of salts upon the proteins of the protoplasm has been the subject of much study, but little attention has been paid to the indirect action which they may exert by modifying the reaction of the buffer solutions which bathe the protoplasm, although this effect is well known to take place. The whole trend of modern research is however to lay stress upon the importance of the reaction of the medium in which physiological processes occur, and the modifications of reaction which buffer mixtures undergo, not only by the addition of salts, but also by the addition of non-electrolytes and by changes of concentration and temperature, cannot fail to be of importance. Most of these changes can be deduced very simply from the theory of buffer solutions and they can also be demonstrated by experiment. The theory of buffer solutions will therefore be very briefly outlined here in order to emphasise these neglected elements, and the results will be given of some series of experiments which have been undertaken to confirm the theoretical deductions and to obtain some estimate of the order of magnitude of the effects upon which it is desired to lay stress. Finally an attempt will be made to discuss the bearing of these results upon the theory of certain physiological processes.

THEORY OF BUFFER SOLUTIONS.

Although the complete series of equations which determines equilibrium in a buffer solution is usually complex, since a number of ions and undissociated molecules may be present, it is generally possible to neglect a number of these equations without introducing any considerable error and to regard the properties of the mixture as due to the balanced dissociation of two substances—weak acid and salt—having a common ion. The equations which determine the properties of the mixture may then be generalised in the form

$$H_2X \rightleftarrows H^{\cdot}+HX'$$
$$MHX \rightleftarrows M^{\cdot}+HX'$$

in which case the dissociation and hydrolysis of HX' are neglected as well as any formation of undissociated M_2X.

Henderson [1908] has worked out the theory of buffer solutions in this form and has applied it to mixtures of carbonates and phosphates. He points out that H_2X, being a weak acid, is very slightly dissociated in the presence of its salts, and that MHX may therefore be regarded as the sole source of HX' ions. The concentration of hydrogen ions is therefore determined by the equations

$$[H^{\cdot}] = k\frac{[H_2X]}{[HX']} = k\frac{[H_2X]}{a[MHX]}$$

where k is the first dissociation constant of the acid H_2X and α is the degree of dissociation of the salt MHX.

The concentration of hydrogen ions is therefore a function of

(1) the concentration of H_2X;
(2) the concentration of MHX;
(3) the value of α;
(4) the value of k.

The effects of changes in the relative concentrations of acid and salt has been very fully dealt with by Henderson and others. It is proposed here to enquire into the effect of changes in the other factors which determine acidity—the degree of dissociation of the salt, and the dissociation constant of the acid—factors which up to the present have been almost completely ignored. Since $[H^{\cdot}]$ varies inversely as α, the acidity of the solution will vary with any change in the degree of dissociation of the more alkaline constituent of the buffer mixture, whether this change be due to an alteration in the active mass of salt present or to any change of external conditions which affects its dissociation constant. In this latter case the value of k, the dissociation constant of the acid, will also tend to change—probably in the same direction. It is possible however that in certain cases, which will be touched upon later, the dissociation constants of acid and salt may vary in opposite directions, in which case the effect of the variations will be greatly increased.

It is possible from the equation

$$[H^{\cdot}] = k \frac{[H_2X]}{a[MHX]}$$

to predict the general effect of many of the changes which can be produced in a given buffer mixture. Unfortunately this is not always the case. Conditions are frequently so complex that to unravel their effects becomes a very formidable task, while in other cases the data necessary for calculation are altogether lacking. It will be worth while, however, before proceeding to detail experimental results, to enumerate the more important conditions affecting the values of k and a and hence of $[H^{\cdot}]$, and to estimate as far as possible the bearing of theory upon them.

(1) *Change of concentration.* Decrease of concentration will increase the value of a and will leave k untouched, consequently the acidity of a buffer mixture will decrease with dilution. This effect is small but it seems doubtful whether it is so entirely negligible as has been assumed by Henderson and by Bayliss [1915]. The order of magnitude of the effect will be seen in the experiments on phosphate and acetate mixtures given below.

(2) *Addition of neutral salts.* The addition of a salt MX' to the mixture MHX, H_2X will increase the concentration of the cations and thus reduce the dissociation of MHX. Increase of acidity will therefore follow. If the salt added has a different cation, M', the conditions are rather more complicated. If however M' is univalent the mixture $M'HX$, MHX will have properties very similar to those of the simple salt. A bivalent cation is in a different category; it produces a far greater increase of acidity. This is due primarily to the formation of the less dissociated salt $M'(HX)_2$, but other salts may also be formed as *e.g.* $M'X$. A comparison of the effect of magnesium chloride in phosphate and acetate mixtures will serve to show that the possibilities which polybasic acids afford for the formation of compounds of this type is not to be ignored.

The effect of the anion has hitherto been disregarded. When the salt $M'X'$ is the salt of a strong monobasic acid both $M'X'$ and HX' may be regarded as completely dissociated and the anion X' will be without effect. Where however HX' is not completely dissociated, a second mixture of acid and salt having buffer properties will be formed and the net result may be either to increase or decrease the value of $[H^{\cdot}]$. A particularly interesting case is that of the polybasic organic acids. Acids of this type form neutral salts which are not hydrolysed in solution and which have a relatively large first dissociation constant. They may therefore be looked upon as moderately strong acids, but their dissociation is of a peculiar type which becomes of great importance when they form one of the constituents of a buffer mixture. Ostwald has shown that the carboxyl groups in these acids affect one another in such a manner that it is only when the first stage of dissociation has progressed to a considerable extent that the second stage begins [cf. Lewis 1918, p. 267]. The peculiar behaviour of citrates may be attributed with great probability to the dissociation of the third carboxyl group suffering a steric hindrance of this nature. Thus a mixture of sodium citrate with acetic acid produces a displacement in the alkaline direction owing to the formation of a non-dissociated acid citrate.

(3) *Addition of non-electrolytes.* It is well known that when non-electroytes are added to solutions of electrolytes the conductivity of the solution is found to decrease. This is largely due to increase of viscosity, but partly also to a decrease in the degree of dissociation of the electrolyte. Arrhenius [1892] has shown that this latter effect becomes far more marked as the degree of dissociation of the electrolyte decreases, and although the effect is somewhat complicated and depends upon the nature of both electrolyte and non-electrolyte, certain broad generalisations can be made, based upon the observations which he records.

(*a*) The effect of alcohols, ether and acetone on solutions of acids is similar, but the effect of methyl alcohol tends to be rather smaller, of acetone rather greater, than the average; the effect of cane-sugar is markedly less.

(*b*) Salt solutions show different behaviour according to type. In most cases the effect of cane-sugar is again small.

Unfortunately the effect of non-electrolytes on weak bases has not been determined.

(*c*) The influence of degree of dissociation is very marked. Arrhenius has shown that the effect of non-electrolytes upon the conductivity of acids increases with the concentration, and it is generally true that the less an electrolyte is dissociated the greater is the effect of non-electrolytes upon its degree of dissociation. There is, however, also a specific effect, as has been pointed out, the nature of which is unknown. The effect of adding non-electrolytes (which produce a decrease in the value of k) is evidently similar to that of increasing concentration (which is equivalent to a decrease in the value of v) and it is important to notice that this similarity of effect may be inferred from the symmetry of the equation

$$\alpha^2 = kv$$

in respect to these quantities, this being the form that Oswald's dilution law assumes when $1 - \alpha$ does not differ appreciably from unity.

Where k is constant we have:

$$\frac{d\alpha}{dv} = \frac{1}{2}\sqrt{\frac{k}{v}}.$$

Where v is constant: $\frac{da}{dk} = \frac{1}{2}\sqrt{\frac{v}{k}}$.

The rate of change of a with change of either v or k is therefore inversely as the square root of these quantities.

It is evident from what has been said above that the addition of non-electrolytes to buffer mixtures will produce a differential effect upon the constituents of these mixtures where these are unequally dissociated unless there is a compensating difference of specific effect. This differential action of non-electrolytes will show itself by a change in the reaction of a buffer solution to which alcohol or some similar substance is added. The slightly dissociated acid constituent will undergo a large reduction of its degree of dissociation, while the highly dissociated salt will be little affected; the value of $[H^{\cdot}]$ will therefore decrease. This is fully borne out by experiment.

(4) *Change of temperature.* The effect of change of temperature is difficult to predict. Noyes, Melcher, Cooper and Eastman [1910] have shown that the dissociation of neutral salts decreases very slightly with rise of temperature; salts of the type common to buffer mixtures have not been investigated, but it is probably safe to assume that it is very small. The change in the value of k is the determining factor, and though this can be calculated in certain cases the calculation usually rests on a somewhat doubtful basis.

Henderson[1] [1908] has attempted to calculate the change of reaction which takes place in a H_2CO_3–$NaHCO_3$ mixture with change of temperature, but, as he points out, the results of the calculation must be regarded as giving a not very close approximation to the true result. He however deduces from the general result the important consequence that while a small increase of $[H^{\cdot}]$ is produced in the mixture with rise of temperature, the alkalinity of the solution will rise owing to the larger temperature coefficient of dissociation of water. It is worthy of notice that this effect may possibly be greatly exaggerated in some buffer mixtures owing to the fact that the dissociation of weak electrolytes is sometimes decreased by rise of temperature.

No attempt has been made here to investigate the change of reaction which the buffer solutions of phosphates and acetates under examination experience with change of temperature. The change is probably small for most buffer solutions, but it can hardly be negligible, especially if the temperature coefficients of acid and salt should prove to be opposite in sign.

THEORY OF AMPHOTERIC ELECTROLYTES.

It is well known that solutions of ampholytes have many of the properties of buffer mixtures. Proteins are present in considerable quantities in the blood and undoubtedly help to maintain the constancy of its reaction, and it is quite probable that proteins and similar substances form a normal constituent

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[1] It may perhaps be useful to point out that in Henderson's calculation of log k 38°, the number 0·491 is given in mistake for 0·481, the logarithm of 3·04. He thus obtains for k 38° the value of 4·2 instead of 4·0. The difference is probably immaterial in so approximate a result, but it leads to some confusion in his argument.

of the buffer mixtures which regulate the reaction of the protoplasm of the cell. This role must, however, be a somewhat subordinate one, and it can hardly be doubted that proteins—which may be taken as typical of colloidal ampholytes in general—have other and more important functions to fulfil in the living cell; a theory of their regulative action will be put forward later. As a preliminary it is necessary to extend this enquiry to the case of amphoteric electrolytes and to examine how these also are affected by a change of conditions.

Since ampholytes have the character of weak acids, the degree of dissociation of the acidic group which they contain will decrease as the concentration of hydrogen ions increases in the solution. This has long been recognised and it is this behaviour which is the cause of any buffer effect which they may exercise, for their capacity for forming salts with weak acids is probably very small, and ampholytes may be considered as existing in buffer solutions in the free state.

Although it is not probable that the buffer effect of ampholytes is of great general importance, it is worthy of notice that the behaviour of an acid ampholyte in respect of changes of concentration is opposite to that of the ordinary buffer solution. Walker [1904] has shown that as the concentration of an ampholyte is increased, its acidity increases, decreases, or remains constant according as k_a, the acidic dissociation constant, is less than, equal to, or greater than k_b, the basic dissociation constant. This theory of ampholytes based on the electrolytic dissociation theory was worked out for substances of the class of simple amino-acids; the application to colloidal ampholytes will be justified at a later stage. If this be assumed it is evident that the presence of proteins such as albumins or globulins in a buffer solution will tend to moderate any changes of reaction induced by changes of concentration.

It will be suggested in the sequel that the behaviour of ampholytes in the presence of non-electrolytes and at different temperatures may be a matter of very far-reaching importance. It has been shown above that $\frac{da}{dk} \propto \frac{1}{k}$; if therefore $dk_a = dk_b$ the effect of adding a non-electrolyte to the solution of an ampholyte will be to repress the dissociation of the less dissociated group relatively to that of the more dissociated and thus to remove the ampholyte further from its iso-electric point. In the immediate neighbourhood of the isoelectric point, since $d_a = d_b$, this effect will vanish.

There is however the possibility of a differential effect upon k_a and k_b, which will produce a change in the ratio k_a/k_b, therefore, k_b will be reduced in greater measure than k_a. Now Michaelis [1909][1] has shown that at the isoelectric point

$$\frac{[H^{\cdot}]}{[OH']} = \frac{k_a}{k_b}$$

i.e.,

$$[H^{\cdot}] = \sqrt{\frac{k_a}{k_b}}\,K$$

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[1] For modifications of this formula see Michaelis and Rona [1919].

where K is the dissociation constant of water. A change in the ratio $\frac{k_a}{k_b}$ will therefore displace the iso-electric point.

The effect of adding a non-electrolyte to an acid ampholyte for which $k_a > k_b$ will thus be to displace the iso-electric point in the acid direction if $dk_a < dk_b$ since dk is negative.

A difference in temperature coefficients will also displace the iso-electric point, but in this case it is impossible to estimate either the magnitude or the direction of the effect.

EXPERIMENTAL.

The following measurements of hydrogen ion concentrations in buffer solutions have been made on the lines indicated in the preceding sections.

The apparatus used was of the ordinary type, but the usual capillary electrometer was replaced by a sensitive high resistance galvanometer. This was found to give sharp readings within 0·5 millivolt when the solutions under examination were good conductors.

The hydrogen electrodes were of Walpole's [1913] pattern; rubber stoppers were used. These electrodes, being in contact with the connecting solution, allow a slow diffusion of hydrogen through the tube of the electrode vessel. It is therefore only the surface layer that is completely saturated with hydrogen. Walpole accordingly makes contact at the meniscus. It was found more convenient in the present instance to allow the platinum point to dip just below the surface; results can be obtained by this method which agree within 1 millivolt, if a uniform procedure is adopted for saturation and sufficient time is allowed for equilibrium conditions to establish themselves. The final readings were in each case taken not less than half an hour after saturation.

The calomel electrode was of the saturated type [Michaelis and Davidoff, 1912]. This gave a reading of 0·5165 against standard acetate. Michaelis found at the temperature of experiment (25°) 0·518. The difference may be attributed to the incomplete saturation already mentioned.

The hydrogen was obtained from zinc and sulphuric acid and was purified by the usual reagents—lead nitrate, potassium permanganate and mercuric chloride. As an additional precaution it was allowed finally to bubble through $N/10$ silver nitrate, sodium hydroxide and water.

Calculations were made from the formula [Michaelis and Davidoff, 1912],

$$P_H = \frac{E - \beta}{f(t)}$$

where E is the observed E.M.F. against the calomel electrode

$$f(t) = 0{\cdot}0591 \text{ (at } 25°\text{)}$$

$$\text{and } \beta = \alpha - \gamma f(t)$$

where α = E.M.F. against standard acetate

γ = value of P_H for standard acetate = 4·62,

hence β = 0·2435, and the formula becomes

$$P_H = \frac{E - 0{\cdot}2435}{0{\cdot}0591}.$$

All measurements were made at the temperature of 25°. Diffusion potentials were found to be very small and have been neglected. Three standard buffer solutions were used in these estimations: (1) potassium phosphate mixture ($P_H = 6{\cdot}88$) made by mixing 5 parts of $M/15$ KH_2PO_4 (P_H 4·58) with 6 parts of $M/15$ K_2HPO_4 ($P_H = 8{\cdot}38$); (2) sodium phosphate mixture ($P_H = 6{\cdot}86$) made by mixing 5 parts of $M/15$ NaH_2PO_4 ($P_H = 4{\cdot}56$) with 6 parts of $M/15$ Na_2HPO_4 ($P_H = 8{\cdot}38$); (3) sodium acetate mixture ($P_H = 4{\cdot}70$) made by mixing 5 parts of $M/5$ acetic acid with 6 parts of $M/5$ sodium acetate.

The acid phosphates which were used in the preparation of these solutions were twice re-crystallised. The solution of sodium acetate was prepared from salt obtained with the "A.R." guarantee, and once re-crystallised. As di-potassium and di-sodium phosphates are not easy to purify, the solutions of these substances were made up by mixing a weighed quantity of the acid salt with an equivalent quantity of standard hydroxide solution and diluting to the correct volume.

The neutral salts were either re-crystallised or obtained with the "A.R." guarantee, except in the case of magnesium acetate, which was tested by analysis after drying *in vacuo*, and of potassium acetate and potassium tartrate, the solutions of which were prepared by neutralising standard acid solutions until they were just alkaline to phenolphthalein. As a check on this procedure a comparison was made between solutions of $M/6$ potassium citrate prepared in this way and by solution of the salt; this gave the following results:

	P_H
By neutralising acid	6·96
From salt	6·94

Weaker solutions were generally made up by dilution from the normal. In the case of calcium and magnesium chlorides the semimolar solutions were standardised by diluting to $M/20$ and titrating with silver nitrate.

In order to carry out the comparative estimations, the results of which are shown below, the standard solutions of phosphate or acetate were diluted with their own volume either of water or salt solution. Usually 5 cc. of each were taken. In the case of the alcohols, 25 cc. of standard solution and a given volume of alcohol were made up to 50 cc. Three sets of results were thus obtained—for mixtures containing (1) potassium phosphates, (2) sodium phosphates and (3) sodium acetate and acetic acid—showing the effect of changes of concentration and addition of salts and non-electrolytes. The phosphate solutions were chosen both on account of their physiological importance and because their normal reaction is very near to absolute neutrality. The acetate solution was chosen on account of the large number of soluble acetates which allowed a more complete investigation of the action of salts.

The readings given represent in each case the mean of not less than two determinations with different hydrogen electrodes.

Table I. *Effect of Concentration.*

Vol. of water added	Potassium phosphate P_H	Sodium phosphate P_H	Sodium acetate P_H
0	6·88	6·86	4·70
1	6·96	6·96	4·71
2	7·02	7·00	4·73

The slight effect of change of concentration on the acetate mixture is to be ascribed to the fact that *M*/5 sodium acetate is already very highly dissociated; dilution can therefore produce only a slight effect.

Table II. *Effect of potassium salts of various acids upon potassium phosphate.*

	Water	Acetate *M*/4	Citrate *M*/12	Chloride *M*/4	Sulphate *M*/8	Tartrate *M*/8
P_H	6·96	6·91	6·94	6·48	6·74	6·79

Table III. *Effect of potassium citrate upon potassium phosphate.*

Concentration of salt	*M*/60	*M*/30	*M*/12	*M*/6	*M*/3
P_H	6·94	6·91	6·94	6·97	7·08

From Table II we have the series: citrate > acetate > tartrate > sulphate > chloride; but it is evident that the relative effect will vary with the concentration. In the case therefore of the other solutions the comparison has been carried over a considerable range of concentration for all the salts. The results of this comparison are shown graphically and figures are also given showing the effect of additions of metallic chlorides and of ethyl and methyl alcohol to the standard buffer mixtures. These graphs (Figs. 1–6) are self-explanatory and require little comment[1].

The addition of magnesium salts to potassium phosphate mixture produces, at concentrations above *M*/20, a labile solution which deposits magnesium phosphate on standing. A large rise of $[H^\cdot]$ is to be observed in the solution when this has taken place, but some of the previous effect must also be due to the formation of undissociated magnesium phosphate. A comparison of the effects of magnesium chloride and magnesium acetate shows that in concentrated solution the nature of the acid which replaces phosphoric acid largely determines the effect.

The alcohol measurements confirm the theoretical deduction of a decrease of $[H^\cdot]$ in the presence of non-electrolytes. In the case of the alcohols this is very marked. It was hoped to carry out similar observations on mixtures containing sugar, but the present apparatus proved quite unsuitable for the treatment of highly viscous solutions, and the observations had therefore to be abandoned. There is however very little doubt, in the light of Arrhenius' work, that these mixtures would show a similar decrease of hydrogen ion concentration, but that the effect would be much less marked.

[1] The concentrations of salt solutions in these figures are expressed in terms of normality. By a normal solution is here meant a solution containing one equivalent of base per litre, *i.e.* *M*/3 sodium citrate, *M*/2 calcium chloride, etc.

PHYSIOLOGICAL EFFECTS.

The effect of buffer solutions and ampholytes in determining reaction has been demonstrated in the preceding sections, and some consideration must now be given to the physiological consequences which may ensue when these changes take place in the living cell. The whole trend of modern physiology is to emphasise the preponderant importance of the reaction of the medium, in determining the rate and direction of the processes of metabolism, but the processes of metabolism which take place in living protoplasm are themselves processes of chemical change which tend to modify reaction, *e.g.* by production of carbon dioxide and changes in the concentration of salts, and non-electrolytes in the cell may also produce changes in the reaction of the buffer solutions of the protoplasm. A regulative process is thus at work which it will be necessary to examine more closely.

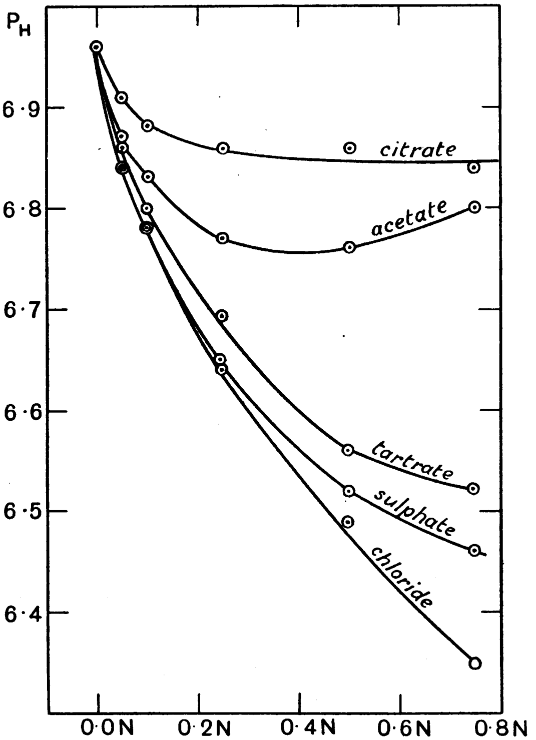


Fig. 1. The effect of the sodium salts of various acids upon the reaction of sodium phosphate mixture.

Before proceeding to more definitely physiological considerations, it is desired to lay some stress upon the action of the anions of the neutral salts. It has been shown that the salts of various acids differ in the effect which they produce upon the reaction of buffer mixtures; consequently we have here an anionic series similar to the well-known Hofmeister series and to those obtained in various physiological reactions. It is to be noticed that the order of the series of anions expressing the effect of salts upon the reaction of buffer mixtures varies to some extent with the nature of the buffer mixture and also with the concentration at which the salts are compared.

It has usually been the custom in physiological and biochemical work to make comparison between the molar concentrations necessary to produce a given effect. In the graphs (Figs. 1–4) equivalent concentrations have been compared. It is interesting to notice that the Hofmeister series falls (with the exception of chloride) into two well-defined groups, if equivalents rather than molecular concentrations are compared. Thus:

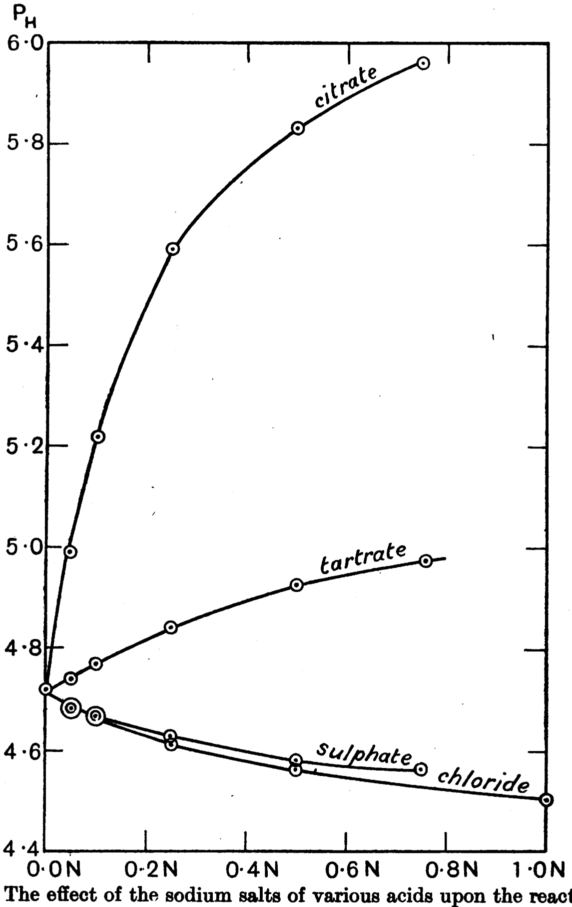
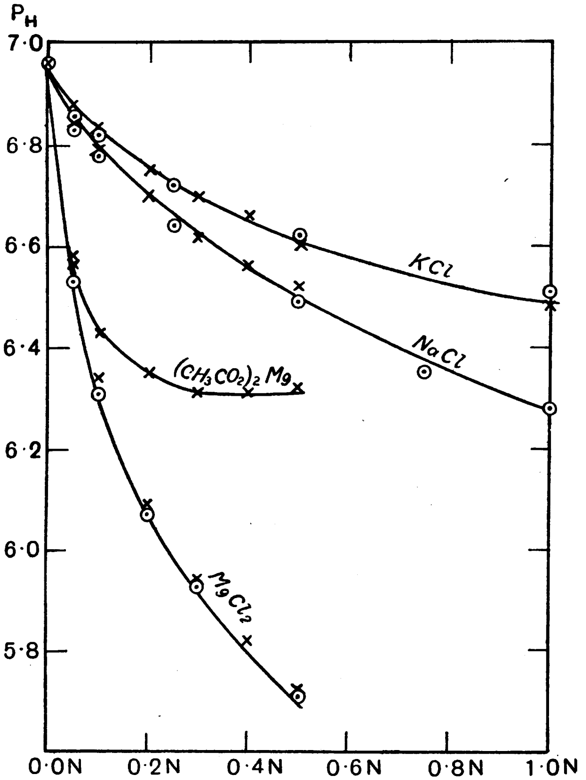


Fig. 2. The effect of the sodium salts of various acids upon the reaction of sodium acetate mixture.

Concentration of salt necessary for the precipitation of egg albumin.

	Mols.	Equivalents
Citrate	0·56	1·68
Tartrate	0·78	1·56
Sulphate	0·80	1·60
Acetate	1·69	1·69
Chloride	3·62	3·62
Nitrate	5·42	5·42
Chlorate	5·52	5·52



F g. 3. The effect of various chlorides upon the reaction of potassium phosphate mixture (×) and sodium phosphate mixture (⊙) and of magnesium acetate upon potassium phosphate mixture.

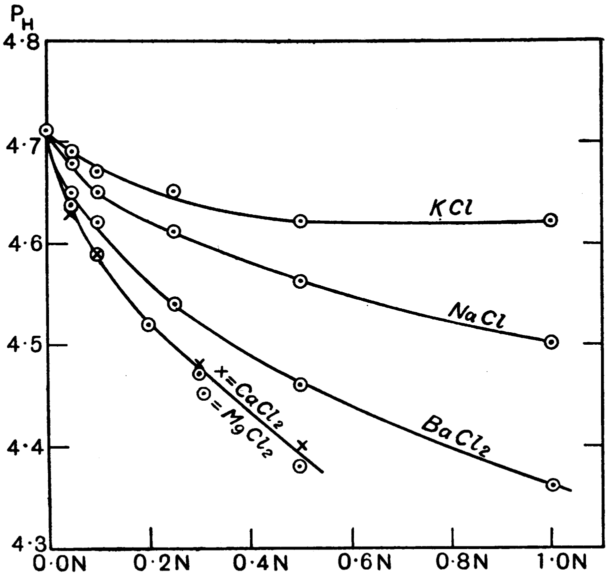


Fig. 4. The effect of various chlorides upon the reaction of sodium acetate mixture.

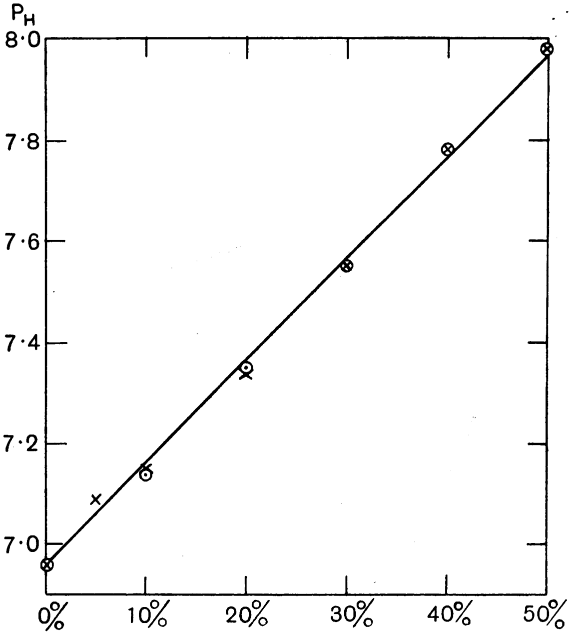


Fig. 5. The effect of methyl alcohol (×) and ethyl alcohol (⊙) on the reaction of potassium phosphate mixture.

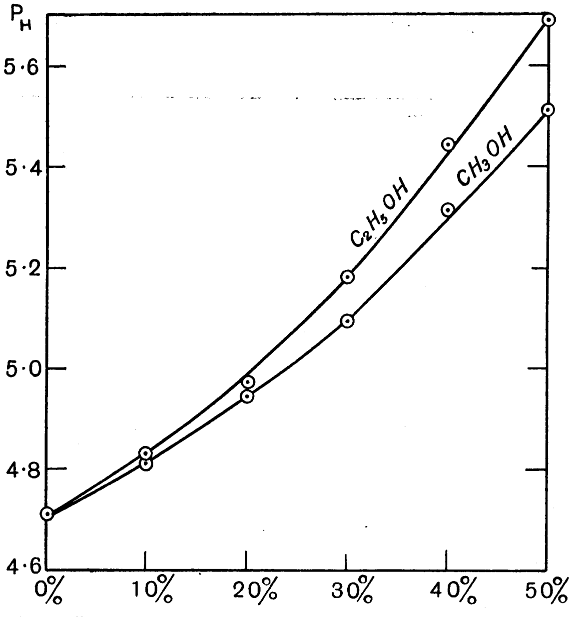


Fig. 6. The effect of methyl alcohol and ethyl alcohol on the reaction of sodium acetate mixture.

It is probable that neither in physiological nor in biochemical work has sufficient attention been given to the effects of changes of reaction arising from the double decomposition of substances of weakly acidic nature, which form ions of the type HX' or undissociated molecules and thus immobilise hydrogen ions.

THEORY OF PERMEABILITY.

It has been shown above that the effect of mineral salts upon buffer solutions is to increase acidity, and that salts having bivalent cations are far more active in this respect than those whose cations are univalent. These chemical differences, together with the small but definite differences of behaviour which are shown by similar salts of sodium and potassium, are clearly of importance as indicating a possible explanation of such physiological differences as are exhibited in the phenomena of "the antagonism of ions." Loeb has attempted to explain antagonism as a consequence of the colloidal properties of the cell and more particularly of the plasma membrane. He applies the rules worked out by Hardy and Schulze for the precipitation of suspensoid colloids, and regards the action of salts upon the living cell as effecting an incipient coagulation of the plasma membrane [see Höber 1914, pp. 529, 530]. There are however considerable difficulties in accepting this explanation as it stands, especially as it is doubtful whether the precipitating action of salts upon suspensoids and emulsoids is due to similar causes. Moreover Loeb's theory gives no explanation of the differences of behaviour which are shown by ions of equal valence. If, however, as is now suggested, buffer solutions are called in to play a part in these phenomena, the range of possible explanation becomes greatly widened. The normal reversible effects of salts upon protoplasm may then be looked upon as indirect and due to modifications in the reaction of the fluid bathing the protoplasm, while direct action upon the proteins or similar substances of the protoplasm itself will produce abnormal and usually irreversible effects. These considerations are of especial importance in relation to the plasma membrane, and it is proposed to consider in some detail the effect which changes in the reaction of the fluid medium may produce upon the proteins of the membrane, and to suggest a theory of permeability based upon these changes. The main outlines of this theory are as follow:

(1) The semi-permeable membrane is assumed to be a gel; the more solid phase of which consists principally of emulsoid colloids having an amphoteric character, while the liquid phase is of the nature of a buffer mixture.

(2) There is maximum permeability at the iso-electric point. Permeability to salts falls off very rapidly on either side of the iso-electric point.

A few words are necessary in reference to these assumptions.

There has been a considerable change of opinion in recent years as to the nature of emulsoid solutions, but most chemists are now agreed in regarding them as partaking very largely of the nature of true solutions from which they differ chiefly in virtue of the size and complexity of their particles [see Höber 1914, p. 304; Sörensen 1917, p. 4].

The recent work of McBain [1920] has shown that these particles are usually aggregates of both ions and non-dissociated molecules which contain large quantities of water, and that their composition "varies continuously with changes in concentration and temperature or upon the addition of salts." There is also much evidence that the hydration of the particles is a function of their charge; indeed the varying viscosity of protein solutions has long been interpreted as due to variations in the number of the heavily hydrated ions into which protein dissociates. This conception of "micellae[1]" capable of undergoing continuous changes of charge and water content is evidently of great value to the biologist in enabling him to form some picture of the changes which protoplasm may undergo, and in particular of the variations which may take place in the state of aggregation of the plasma membrane of the cell.

The theory of protein dissociation and of the iso-electric point in relation to ampholytic colloids has been the subject of much controversy and there is little doubt that the dissociation of proteins differs considerably from that of the simple amino-acids. There is however much evidence that proteins are ampholytes whose acidic and basic characters depend upon the dissociation of hydrogen and hydroxyl as do those of other weak acids and bases, and that their dissociation is at a mimimum at the iso-electric point[2]. Sörensen, Höyrup, Hempel and Palitzsch [1917] have recently measured the iso-electric point of highly-purified egg albumin in solutions containing sulphuric acid and ammonium sulphate, using the principle of Michaelis[3], that if an ampholyte is added to a solution the P_H of which is that of the ampholyte at its iso-electric point the reaction of the solution will be unchanged. They obtain a constant value for the iso-electric point of egg albumin under very varying conditions, thus affording strong support to Michaelis' theory of the iso-electric point.

Little direct evidence exists as to the chemical nature of the membrane, but there is a very strong probability that proteins are present in it and there is also much reason to believe that it contains lipoid substances. It is therefore legitimate to assume that it consists largely of ampholytic colloids. The properties of ampholytic colloids have been principally studied in the case of proteins, and although lecithin is known to possess an ampholytic character, no detailed investigation of its behaviour has been made. It will therefore be necessary, pending the results of further investigation, to assume the properties of protein as typical of the ampholytes of the semi-permeable membrane.

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[1] It seems convenient to follow the current botanical use and to write micellae as the English plural of Nägeli's term.

[2] A very complete summary of the evidence relating to the dissociation of proteins and the theory of the iso-electric point is given by Robertson [1918] and Pauli [1914, 1920].

[3] For a recent amplification of Michaelis' theory see Michaelis and Rona [1919] in which references to the earlier developments of the theory are given.

It has been shown that the plasma membrane may be regarded as composed of particles undergoing continuous changes of charge and water content according to variations in the reaction of the surrounding medium. We have now to consider the conditions for permeability in such a membrane. Substances soluble in the continuous phase of the gel—lipoid-soluble substances—will obviously pass through with more or less ease in all circumstances and will experience no regulative action. Water-soluble substances on the contrary will have to pass through pores, the size of which will vary with the state of hydration of the colloidal particles, and will therefore pass through most easily when those pores are at their maximum size, *i.e.* at the iso-electric point. It is moreover to be expected that this variation in permeability will be greatly exaggerated in the case of charged ions; at reactions above that of the iso-electric point the membrane will carry a positive charge and will repel cations; at reactions below this point it will carry a negative charge and repel anions; in each case it will also impede the movement of the oppositely charged ion, on which it will exercise an attraction. It is only in the immediate neighbourhood of the iso-electric point that the membrane will be permeable to ionised salts as a whole. A membrane of this nature will thus possess a variable permeability, especially to electrolytes, and will exercise a regulative function conditioned largely by changes in the reaction of the plasma medium, since by these changes the ampholytes of the membrane will necessarily be brought nearer to the iso-electric point or will be removed further from it. It is clear therefore that neither salts nor non-electrolytes can be without effect upon its permeability, and this effect must now be considered in detail.

To begin with the case of non-electrolytes. It has been shown above that these reduce the acidity of a buffer mixture and also that there is strong reason to suppose that if they are added to the solution of an ampholyte they may displace the position of the iso-electric point. Non-electrolytes may therefore affect permeability in two different ways; action on the buffer solutions of the membrane will take place in the liquid phase, action on its ampholytes in the solid phase, but one effect will usually be negligible since most substances are relatively insoluble in one of the two phases of the membrane.

For water-soluble substances such as sugar the principal effect will be on the buffer solutions of the liquid phase, while lipoid-soluble substances such as ether, which are largely soluble in the solid phase of the membrane, will act chiefly by displacing its iso-electric point. Alcohol it may be noticed will be soluble in both phases. It will be convenient to leave the further consideration of the action of the lipoid-soluble substances to a later stage and to deal with them under the head of narcotics. Most food materials are water-soluble; the only important exception is fat, and the fats are readily hydrolysable into water-soluble substances.

It has been shown that there is much reason to suppose that a change in the concentration of sugar in the protoplasm of the cell will change the reaction of the buffer mixtures of the protoplasm and thus bring it nearer to or remove it further from the iso-electric point. If the protoplasm is below the iso-electric point a decrease in the concentration of sugar will bring it nearer to this point and thus promote permeability and diffusion; if the protoplasm is above the iso-electric point, an increase of concentration will have the same effect[1]. As, however, the effect of the sugars upon dissociation is small, it may be expected that considerable changes of concentration will be necessary to produce any marked physiological effect.

The diffusion of salts into the cell is conditioned by two factors—the concentration of the salt and the change of reaction which it produces. If the reaction of the buffer mixture of the plasma membrane is above its iso-electric point, the diffusion of salt into the cell will increase acidity, and thus an increasing resistance to the entrance of more salt will be produced. It is here that the antagonism of ions will come into play. Calcium chloride will cut down permeability at a much lower concentration than sodium chloride; therefore the addition of a little calcium chloride to a solution of sodium chloride will impede its penetration. On this hypothesis it is easy to understand such observations as those of Osterhout [1911] and Loeb [1916]. Osterhout showed that solutions of sodium chloride and calcium chloride, neither of which was capable of plasmolysing the cells of Spirogyra, effected rapid plasmolysis when mixed. Loeb found that the amount of calcium chloride necessary to prevent injury to eggs of *Fundulus* by sodium chloride solution varied with the concentration of the sodium salt. The results obtained by Stiles and Kidd [1919] are also significant. They found that the rate of absorption fell rapidly after a few hours, and that though calcium was rapidly absorbed at first, at final equilibrium bivalent ions were absorbed to a much less extent than univalent. Another important point in this connection is the antagonism between ions of a like valency, *e.g.* sodium and potassium, calcium and magnesium. It seems probable that this also can be explained by the different effect which they produce on the reaction of the medium. It has been observed that both phosphate and acetate mixtures show a definite difference of response to potassium and sodium salts; the effect of calcium and magnesium could be compared only on sodium acetate mixture, and in this case their reactions were alike, but since the chemical differences between the salts of calcium and magnesium are frequently considerable, this case cannot be general and it is probable that the antagonism may find a general explanation in differences in the properties of the products of reaction of the salts in question with the constituents of the buffer mixture of the protoplasm. It should however be remarked that the striking physiological effect of very small traces of certain substances appears to indicate a direct action upon the plasma membrane in some cases. The foregoing considerations show clearly the necessity for main-

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[1] The excretion of sugar from plant cells when the concentration of sugar becomes high is thus explained. It is suggested below that in leaves the plasma membrane is normally maintained above the iso-electric point.

taining a definite ratio in the concentration of the various ions present, since any modification will affect the reaction of the protoplasm. The "balanced" nature of such mixtures as Ringer's solutions, sea-water, and the water-culture solutions used for the growing of plants is therefore explained.

No definite hypothesis has hitherto been made in this paper as to the reaction of the protoplasm and the plasma membrane in respect to the iso-electric point. Electrical phenomena which will be discussed later suggest that the membrane of many cells is normally permeable to cations. This indicates that the plasma membrane of such cells is below the iso-electric point.

In the case of leaves, changes in the concentration of carbon dioxide due to alteration of the balance between respiration and assimilation must lead to changes of permeability, and a variation in the supply of salts would produce a like effect. If the reaction is above the iso-electric point, permeability and diffusion will be at a maximum during periods of active photosynthesis. This is in accordance with the observations of Lepeschkin [1909], Tröndle [1911], and Blackman and Paine [1918], who all found a marked increase of permeability as the result of increased illumination. It is possible too that a falling off in the supply of salts is one of the causes of the death of leaves, since death is known to be preceded by increased permeability.

Equilibrium in the cell. It is well known that the living cell can maintain permanent differences of concentration on either side of a membrane which it is impossible to regard as completely impermeable (cf. Abderhalden's analyses of blood corpuscles and serum [1909]; also Stiles and Kidd [1919]). The question of equilibrium is very complex and a number of factors are involved, of which permeability may not in many cases be the most important. It seems probable however that there is true dynamic equilibrium in certain cases, and this must be due to the properties of the membrane. As an example reference may be made to the exclusion of organic acids from the protoplasm by the action of the vacuole membrane, such as is known to occur in the leaf of Oxalis. When the cell sap is highly acid it is probable that the vacuole membrane is maintained at a reaction far above that of its iso-electric point. In this case it is probably impermeable to ions of either sign. Equilibrium may thus be maintained between the non-dissociated fraction on either side of the membrane. Overton [1902] has shown that the plasma membrane is in general in some degree permeable to undissociated molecules which thus behave like any other non-electrolyte, and it may be supposed that the membrane of the vacuole has similar properties. Any acid present in the vacuole will of course be dissociated on passing the membrane, but in presence of its salts the hydrogen ion concentration will be kept very low. We have thus the possibility of equilibrium between acid present in the vacuole and in the protoplasm, while at the same time the hydrogen ion concentration of the latter is at a minimum. In the case of permanent differences of salt concentration, such as are found between blood corpuscles and serum, it may be objected that such an equilibrium could not be maintained, since salts are only completely dissociated at infinite dilution. This is of course true, if an undissociated fraction is really present, but the existence of strong electrolytes in the undissociated state has recently been called in question. Ghosh [1918] has found that the abnormalities of strong electrolytes can be satisfactorily explained on the supposition that they are completely dissociated at all concentrations.

The question of differential absorption, as *e.g.* by such an organ as the root, is closely related to that of equilibrium, and here again a number of factors are probably concerned; it is nevertheless probable that a true differential permeability exists, of which the difference between sodium and potassium ions affords the most striking instance. Potassium ions are highly diffusible; whether this is to be ascribed to a low degree of hydration, to a small atomic volume, or to any other difference of physical conditions, is at present undetermined (see *Faraday Soc. Trans.* [1919]), but on almost any hypothesis potassium salts will continue to penetrate the membrane in virtue of their diffusibility, under conditions which completely exclude sodium salts. Moreover, the effect of potassium ions upon the reaction of buffer mixtures is small and increases slowly with increasing concentration; they may therefore reach a considerable concentration before being also in their turn cut off by the lessened permeability of the membrane.

Stimulation. There is very little doubt that stimulation is accompanied by changes of permeability. It is therefore necessary that any theory of the plasma membrane should take account of the phenomena of stimulation. These phenomena have chiefly been studied on nerves or muscles. It will be assumed here that the protoplasm in such tissues is below the iso-electric point, in which case the membrane will be permeable to cations. In this case a slight excess of hydrogen ion concentration within the cell will produce a diffusion potential and the current of injury can be explained on Bernstein's hypothesis as due to a migration of anions from the interior of the cell at the point where the membrane is pierced. The current of rest is susceptible of an explanation on similar lines, for salts will produce an increase of hydrogen ion concentration at the place at which they act and will bring the membrane nearer to the iso-electric point—the point of permeability.

It is not proposed to consider the general theory of stimulation here; it may however be pointed out that on Nernst's hypothesis stimulation is accompanied by an increase in the concentration of salt in the immediate neighbourhood of a membrane: on the present hypothesis the production of increased permeability follows as a necessary consequence.

Narcosis. If stimulation is to be explained by the production of permeability at a point of the membrane, it is reasonable to suppose that the effect of narcotics is to reduce permeability. This explanation accords well with observations on the effect of narcotics on the production and transmission of excitation, and there is some direct experimental evidence in its favour; but direct experiments on permeability present very special difficulties both in execution and interpretation, and the results cannot be considered conclusive (for a critical review of the evidence see Katz [1918]). The general result of different lines of enquiry is however to confirm the conclusion that true narcosis is the result of a decrease of permeability of the membrane, and that increase of permeability is only produced by narcotics at relatively high con centrations in which case the effects are irreversible and injurious.

It has been shown that the alcohols produce a decrease of hydrogen ion concentration in buffer solutions, and that their action is thus the reverse of that of salts. This accords well with the theory that narcotics act by reducing permeability. Alcohols cannot however be taken as typical of narcotics in general, for these are normally very slightly soluble in water. Such substances act directly on the solid phase of the membrane, and as already pointed out there is reason to suppose that their action may consist in a displacement of the iso-electric point. If so narcotics may be regarded as lipoid-soluble substances which by their differential effect on the dissociation constants of the ampholytes of the membrane remove them further from their iso-electric condition.

A few words remain to be said in conclusion. No attention has been given to the possible effects of adsorption, but it is very probable that buffer solutions are adsorbed by the membrane, and it is not unlikely that excitation may in some cases be due to changes in the concentration of these salts produced by changes of surface tension. Theories relating to tropisms have also been left unconsidered, but it may be worth while to remark that on the present hypothesis the relation of the membrane to the iso-electric point will determine whether the reaction to the tropic stimulus is positive or negative. Small [1920] has already made the suggestion from a different point of view that the tissues of the root may be acid and those of the stem alkaline.

It is fully recognised that the experimental basis of certain of the theories which have been put forward in the present paper is very incomplete. It is hoped in the future to carry out experiments on the effect of non-electrolytes and of temperature on the iso-electric point of amphoteric electrolytes. It is also intended to study the specific effect of non-electrolytes on dissociation. In the meantime it is desired to point out that the present theories relating to permeability are based very largely on hypotheses which have been found necessary in the study of other phenomena. The theory of the buffer solutions present in protoplasm, of the existence of protein and lipoid substances in the plasma membrane, and of their ampholytic character, are all well established on other grounds, and the present theories consist almost entirely of necessary deductions from these assumptions. That it is possible by their means, not only to explain differences of permeability, but to correlate such well-established physiological facts as the antagonism of ions and the various phenomena of electric stimulation and the effect of narcotics, is strong evidence in their favour, especially in view of the fact that they show how the regulation of permeability can be a direct result of cell metabolism.

SUMMARY.

The theory of buffer solutions shows that an increase of hydrogen ion concentration normally results from the addition of neutral salts, a decrease from the addition of non-electrolytes. These deductions have been confirmed by experiments on buffer mixtures of phosphates and acetates.

There is a marked difference of effect between univalent and bivalent cations, and a small but distinct difference has been observed between sodium and potassium salts.

It is shown on theoretical grounds that the addition of non-electrolytes to the solution of an ampholyte may displace its iso-electric point. In the case of an ampholyte for which $dk_a < dk_b$, the iso-electric point will be displaced in the acid direction, since dk is negative.

A theory of permeability, based on the foregoing observations and deductions, is suggested. The semi-permeable membrane is assumed to be a gel, the more solid phase of which consists principally of emulsoid colloids having an amphoteric character, the liquid phase of a buffer mixture. Such a membrane will have a maximum permeability at the iso-electric point, since at this point the continuous phase will not only be in a state of minimum hydration and will thus occupy a minimum volume, but will also be without charge. Above the iso-electric point the membrane will carry a positive charge and will repel cations; below this point it will carry a negative charge and repel anions. The membrane will therefore be peculiarly impermeable to charged ions, except in the immediate neighbourhood of the iso-electric point. Additions of salts and non-electrolytes will modify the permeability of the plasma membrane by the changes which they produce in the reaction of the buffer mixture of the protoplasm.

The phenomena of antagonism can be explained as resulting from the different effect of different cations on the reactions of the buffer mixture of the protoplasm.

The bearing of the present theory upon stimulation and narcosis is outlined.

The above investigation has arisen out of work which has been carried out for the Food Investigation Board of the Department of Scientific and Industrial Research on the physiological changes which apples undergo in cold and ordinary storage.

The writer is indebted to Professor V. H. Blackman for valuable criticism and suggestions.

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