EFFECTS OF pH AND THE COMPONENTS OF BICARBONATE AND PHOSPHATE BUFFERED SOLUTIONS ON THE METABOLISM OF POTATO DISCS AND THEIR ABILITY TO ABSORB IONS[1]

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(WITH NINE FIGURES)

Introduction

This paper consists of two parts. The first concerns the effect of certain variables on the absorption of bromide by potato discs and experiments which were made by the senior author in the Division of Plant Nutrition, University of California, in 1933–1934. The second part describes the effect of similar treatments on the metabolism of potato discs and experiments carried out with the assistance of the junior author at Birkbeck College, London, in 1937–1938. Since the results of the later work are essential for a full interpretation of that which preceded it, the two investigations are described together.

Investigation of the effects of external pH presents an obvious approach to the problem of salt accumulation. It is, therefore, an essential part of any survey of the variables which affect this important process (**18, 19, 20**). Despite the great emphasis which others have placed upon the rôle of pH in salt absorption, detailed discussion of the effect of this variable on bromide absorption by potato discs has been long postponed. For some preliminary observations see (**18**). This delay was due to the knowledge that until the effects of a wide range of variables—including the nature and concentration of neutral salts—upon the behavior of cells had been investigated, phenomena due to hydrogen ion *per se* could not be segregated from those due to other variable components of the system used to control pH. Knowledge of the metabolic processes of potato tissue and the way these are affected by neutral salts (**21, 22, 23**) is now adequate to permit investigation of the problem.

Of the buffer systems which can be used to regulate the pH of solutions in contact with plant cells, that which depends upon the proportion of bicarbonate to free carbonic acid is of outstanding interest since its components are metabolic products of cells. This paper contains a survey of the effects of pH, carbon dioxide, and potassium carbonate concentration. Though somewhat incomplete, it enables us to visualize the trend of the effect of these variables upon absorption and metabolism of potato discs and prepares

[1] This paper is the fourth of a series of papers on the biochemistry of salt absorption by plants. The writers are indebted to Prof. D. R. HOAGLAND for proofreading this paper.

the way for the rather formidable task which a still more complete investigation entails. A similar survey made with phosphate—buffered solutions shows the extent to which the effects obtained in bicarbonate solutions are peculiar to a particular buffer system.

Methods

Potato discs were used under the standard conditions which have proved conducive to salt accumulation. Bromide was used as an indicator of anion absorption and was supplied as potassium bromide at the same equivalent concentration throughout a series of experiments. All known variables, other than salt concentration and pH, which affect salt absorption were standardized as follows: temperature 23° C., mean disc thickness, 0.75 mm., number of discs in two liters of solution which was stirred (100 r.p.m.) and aerated (total gas flow 15 liters per hour) to maintain equilibrium with the oxygen tension in the gas used. This facilitated comparisons between a series of experiments which, though not run concurrently, were carried out in rapid succession using tissue from the same uniform stock of tubers. A standard experimental time of 70 or 72 hours was used.

The experiments were of two general kinds: experiments at constant salt (potassium bicarbonate or phosphate) concentration but embracing a range of pH values; and experiments at constant pH in which the total salt (potassium bicarbonate or phosphate) was the variable. At constant potassium bicarbonate concentration, the range of pH values was obtained by changing the partial pressure (composition by volume at constant pressure) of carbon dioxide in the gas stream which flowed through the culture vessels. For the comparable procedure in the phosphate series free acid (H_2SO_4 or H_3PO_4) was added to solutions of potassium phosphate (K_2HPO_4) as required. To reveal the specific effects due to these buffer salts (potassium bicarbonate and phosphate) without confusion with those due to pH, the total salt concentration was varied at *constant pH.* To eliminate confusion between the effects due to the free acid or anion and those merely due to the cation (K), certain experiments were also carried out at constant potassium concentration. In the latter case a low concentration of the experimental salt (potassium bicarbonate or phosphate) was raised to the desired potassium level by the addition of the required amount of a salt (potassium sulphate) in which the anion has been shown (**22**) to be without conspicuous effects on the metabolism of potato discs.

The required partial pressure of carbon dioxide was obtained by combining streams of oxygen and carbon dioxide at known rates of flow. Oxygen was used in order that even the richest mixture (26.6 per cent. CO_2) did not contain an oxygen pressure so low that behavior of the tissue would be limited thereby. Manifolds were connected to a high pressure source of

oxygen which delivered the *total* flow required at a constant rate which was regulated by a reducing valve. A similar device was also used for carbon dioxide. For each gas mixture, the flow of oxygen and carbon dioxide, drawn off at convenient points on the manifolds, was regulated by separate needle valves which were adjusted in accordance with the readings of a calibrated flow meter (**15**) through which the mixture passed. Thorough mixing occurred in the large pressure stabilizers which were described with the original apparatus (**15**). The final adjustment of the needle valves was so made that the gas mixture passing through a wash bottle which contained the required bicarbonate concentration, gave the requisite pH as seen by the indicator which was added for the purpose. The final record of the pH of the salt solutions actually in contact with the tissue was made by a glass electrode, but the indicator in the wash bottle gave warning if the mixing apparatus was not working faithfully. In this way the reaction of solutions was kept within narrow limits throughout the period of experiments. The volume percentage of carbon dioxide in the gas mixture used was recorded by gas analysis in a modified type of HEMPEL'S apparatus.

Results

THE EFFECT OF pH, POTASSIUM BICARBONATE, AND DISSOLVED CARBON DIOXIDE ON BROMIDE ABSORPTION

The combined results of three experiments are assembled in table I. The choice of the concurrent treatments was determined partly by convenience in operation and partly by considerations which need not concern us here; it is the impression which the *combined* results convey of the effect of pH, added bicarbonate, and carbon dioxide on the accumulation of bromide which is of interest.

At constant bromide concentration, in the absence of added bicarbonate and in solutions in equilibrium with a gas free of carbon dioxide, the bromide uptake was not significantly affected by the extreme range of total potassium concentration which these experiments incurred. This factor can thus be ignored. It is very clear, however, that the components of the system which did affect bromide absorption profoundly were the concentration of hydrogen ions, bicarbonate, and carbon dioxide. It remains to be seen which of these is the most important.

At any given pH (pH 7.23 and 6.8) the effect of a simultaneous increase of bicarbonate and dissolved carbon dioxide is to greatly reduce the bromide absorbed; so much so, in fact, that by such increases alone the *accumulation* of this ion *was almost entirely suppressed* even though all other variables were favorable. Ignoring for the moment the differences in pH (7.2 and 6.8) at which two different experiments were conducted, it can be shown that over the range of added bicarbonate concentrations (0.0 to 0.020 equiv.

TABLE I

EFFECT OF pH, [$KHCO_3$] AND [CO_2] ON ACCUMULATION OF BROMIDE BY POTATO DISCS FROM 0.001 EQUIVALENTS PER LITER KBr AT 23° C. DURING 70 HOURS

EXPERIMENT*	[$KHCO_3$] EQUIV. PER LITER	PERCENTAGE CO_2†	MEAN pH OF EXTERNAL SOLUTION	[Br] SAP. PER LITER	EXPERIMENT	[$KHCO_3$] PER LITER	PERCENTAGE CO_2†	MEAN pH OF EXTERNAL SOLUTION	[Br] SAP. PER LITER	EXPERIMENT	[$KHCO_3$] PER LITER	PERCENTAGE CO_2†	MEAN pH OF EXTERNAL SOLUTION	[Br] SAP. PER LITER
		%		*mg. equiv.*		*mg. equiv.*	%		*mg. equiv.*		*mg. equiv.*	%		*mg. equiv.*
1	–	26.6	4.75	7.88	1	0.001	26.6	5.3	5.26	1	–	0.0	6.90	17.3
										3	–	0.0	7.70	17.3
2	–	7.8	5.92	14.3	3	0.001	.....	6.8	15.3	2	0.0050	4.2	7.23	11.5
										2	0.0100	7.8	7.23	6.8
2	–	4.2	6.12	16.1	2	0.001	0.8	7.3	15.8	3	0.0025	2.2	6.81	14.1
2	–	0.8	6.58	16.5	3	0.001	.....	7.7	15.9	3	0.0150	12.5	6.76	2.9
1	–	0.0	6.90	17.3	1	0.001	0.0	8.3	16.1	3	0.0200	16.5	6.81	1.7
3	–	0.0	7.70	17.3	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....

* Expt. 1. Total [K] = 0.002 equiv. per liter
Expt. 2. Total [K] = 0.011 " " "
Expt. 3. Total [K] = 0.021 " " "

† Volume percentage CO_2 in gas phase.

per liter) the relationship between bromide absorbed by the discs and either the external potassium bicarbonate concentration (fig. 1) or the volume percentage of carbon dioxide in the gas phase, is smooth. At pH 7.2 and 6.8 the curves are almost coincident and are linear over much of the range of bicarbonate (0.0 to 0.010 equiv. per liter) or carbon dioxide concentrations (0 to 8.0 per cent. by vol.) although they do deviate at higher bicarbonate concentrations from the straight line as they tend to approach somewhat asymptotically the bromide concentration of the external solution. It is, therefore, clear that some component of the system which is increased proportionally to the added bicarbonate or the dissolved carbon dioxide depresses the bromide uptake. To fully map out the depressant effect of bicarbonate and dissolved carbon dioxide at each pH value would be a considerable task. It suffices for present purposes, however, to identify by a straight line the slope of the curve which expresses this relationship and, extrapolating this to zero bromide concentration, slight error will be encountered only at the highest concentrations of bicarbonate.

In the absence of added bicarbonate, increased pressure of carbon dioxide in the gas phase caused lower pH in the solution; when it became more acid than pH 6.9, the bromide absorption was decreased—especially so below a critical value of approximately pH 6.0—when the decrease was rapid (fig. 1). As shown by paired cultures which received the same gas mixture, the

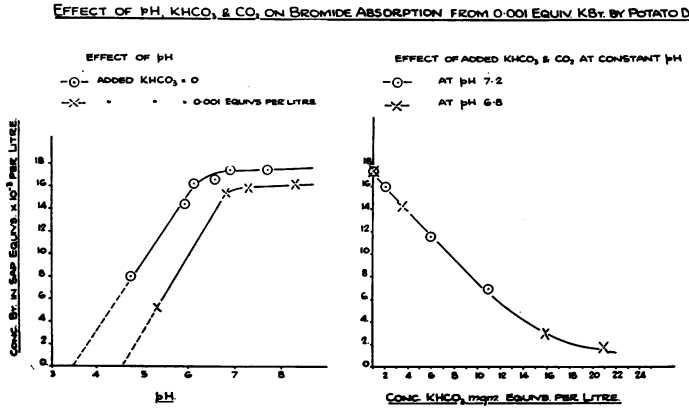

FIG. 1.

dissolved carbon dioxide, free to exert its unbuffered effect on pH, depressed bromide absorption much less than did the combined effect of dissolved carbon dioxide and added bicarbonate at more alkaline reactions. To appreciate the full range of effects of bromide absorption (70 hr. at 23° C. from 0.001 equivalents KBr per liter), the effect of pH and, at each pH, of increased bicarbonate and carbon dioxide concentration must be visualized. This can be done if three co-ordinates (bromide concentration in sap after

70 hr., pH of external solution, bicarbonate concentration in the solution or percentage by volume of carbon dioxide in the gas) are plotted simultaneously (fig. 2). This method of presentation will be used throughout, as it permits the effects of several variables to be visualized simultaneously.

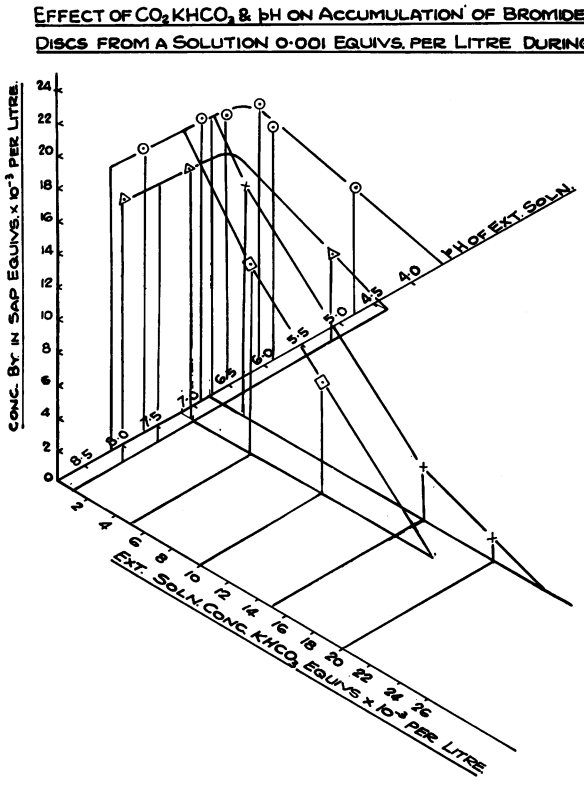

FIG. 2.

To specify completely the relationships of bromide uptake to pH in bicarbonate buffered solutions the surface of a solid model is required. By making the fullest use of the data available, it is possible to visualize the kind of surface which expresses these relationships. The solid model may be constructed in two different ways and each serves a useful purpose.

THE EFFECT OF pH AND ADDED $KHCO_3$ ON BROMIDE UPTAKE.—In the simplest procedure, the two variables plotted are those which were set at arbitrary values in the actual experiments; namely, pH, and the concentration of potassium bicarbonate added to the solution. The third is the concentration of bromide observed in the sap after a 70-hour treatment. One surface of such a model (fig. 3) can be specified at once from the data at zero concentration of added potassium bicarbonate. At each pH, a section

through the model parallel to the bicarbonate concentration axis is bounded by the line, assumed for this purpose straight, which records the rate of *decrease* of bromide uptake with increased bicarbonate and carbon dioxide concentration. A sufficient number of these lines enable the surface of the complete model to be visualized.

EFFECT OF CO_2 $KHCO_3$ & pH ON ACCUMULATION OF BROMIDE BY POTATO DISCS FROM A SOLUTION 0·001 EQUIVS PER LITRE DURING 70 HOURS AT 23°C.

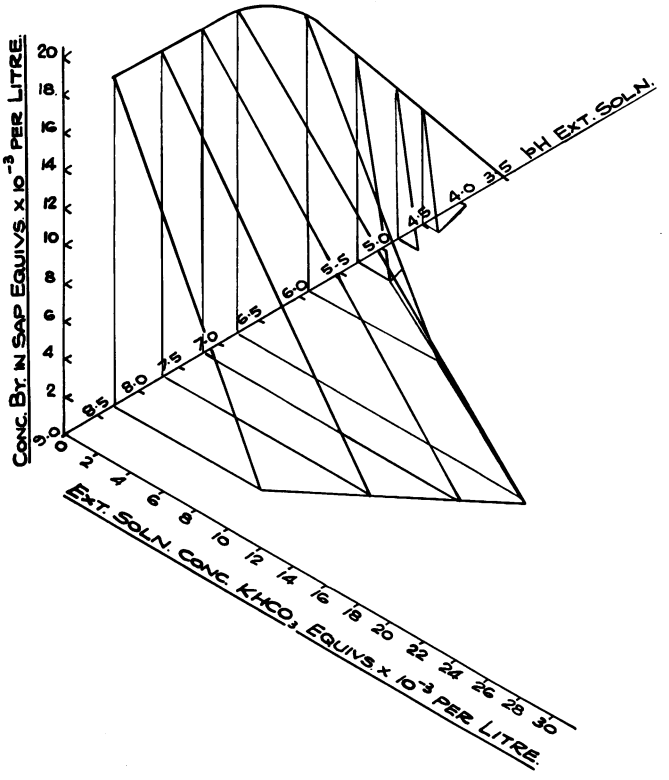

FIG. 3.

Figure 3 shows that, in the absence of added bicarbonate there is a considerable range (pH 8.0 to 6.0) in which pH and dissolved carbon dioxide concentration have but little effect on bromide absorption. That a hundredfold range of hydrogen ion concentration is without conspicuous effect on bromide uptake is in agreement with other results (**6, 18,** p. 1012) which show that salt absorption may be less dependent upon pH than upon other

variables. At reactions more acid than pH 6.0, bromide absorption was reduced and its decline with acidity implies that at *about pH 3.5 bromide absorption should vanish* (fig. 3). At increased bicarbonate concentration (0.001 equiv. per liter) bromide uptake was decreased at all reactions (table I; figs. 2, 3) and from the graph (fig. 2) it is clear that bromide uptake should vanish at a much less acid reaction (pH 4.5) in presence of 0.001 equivalents of bicarbonate.

Having fixed the reactions (pH 3.5 and 4.5) at which bromide uptake vanishes at zero concentration of bicarbonate and at 0.001 equivalents per liter, it can be estimated with sufficient accuracy for the intermediate concentrations. The bromide concentration attained in the sap from solutions at pH 5.3 and two different bicarbonate concentrations can be found, and these values fix the steep slope of the bromide uptake-bicarbonate concentration curve which intercepts the plane of zero bromide uptake (fig. 2) at a bicarbonate concentration of 0.002 equivalents per liter. At pH 6.8 and 7.2 the data available fix the slope of the curve which represents the effect of bicarbonate and carbon dioxide on bromide uptake (fig. 2) with some certainty; and by joining points at which bromide absorption vanishes, the probable boundary of the model is thus ascertained. At each of the two reactions (pH 7.7 and 8.3) data on bromide uptake are available for one bicarbonate concentration only. It can be obtained, however, at the other by reference to the well defined curves of figure 2. Although these two points represent the minimum to establish the slope of the line passing through them, these lines have been prolonged in figure 3 to the plane of zero bromide uptake. It will be shown later that the resultant figure and the curve in which it cuts the plane at zero uptake—a curve which specifies the conditions of pH and bicarbonate concentration which suppress bromide absorption—can be correlated with the metabolism of potato discs under a similar set of conditions.

The characteristics of this model which are of interest are as follows: At constant pH the depression in the bromide absorption which is due to an increase in potassium bicarbonate concentration is a minimum at a pH of 6.8; *i.e.*, uptake is at a maximum. This reaction is near to strict neutrality and also to the pK value of carbonic acid as a monobasic acid. At pH = pK, (pH = 6.4) the ratio $\frac{\text{salt}}{\text{free acid}} = 1$. At reactions more acid than this the amount of free acid becomes rapidly much greater than the salt added; at reactions more alkaline than pH = 6.4 the free acid decreases until at pH 8.3 it is virtually zero. Since there is a greater effect of added bicarbonate at constant pH at *both* more acid and more alkaline reactions than pH = 6.4, the effective component of the system cannot be immediately selected from all those which are affected by increased bicarbonate concentration. It is

suggestive that at acid reactions where the unneutralized carbonic acid exceeds the salt present, the effect of increased bicarbonate concentration at constant pH is particularly great.

THE EFFECT OF pH AND TOTAL CARBONIC ACID ON BROMIDE ABSORPTION.— In figure 4, the variables plotted are bromide concentration in the sap (mg. equiv. per liter), pH of the external solution, and total carbonic acid in the solution; *i.e.*, that which is present as salt ($KHCO_3$) *plus* the uncombined acid. For the bicarbonate solutions the free acid can be calculated from the relation $pH = pK_1 + \log \frac{[salt]}{[free\ acid]}$ where $pK_1 = 6.4$ and in the absence of potassium bicarbonate the total dissolved carbonic acid is given by the relationship: mols H_2CO_3 per liter $= 3.645 \times 10^{-2} \times P$, where P = partial pressure in atmospheres (volume percentage of $CO_2 \times$ total barometric pressure in atmospheres) of carbon dioxide in the gas phase. The data are given in table II.

In figure 4, data from experiments at the same concentration of *added*

EFFECT OF CO_2 $KHCO_3$ & pH ON ACCUMULATION OF BROMIDE BY POTATO DISCS FROM A SOLUTION 0·001 EQUIVS. PER LITRE DURING 70 HOURS AT 23°C.

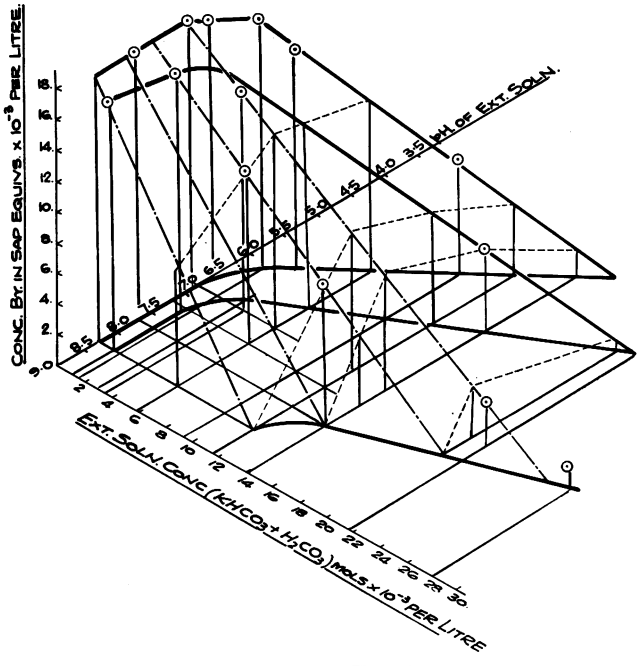

FIG. 4.

potassium bicarbonate all lie in a curved surface which cuts the basal plane of the model in a curve. This curve which traces out the increase in the total carbonic acid as a given concentration of bicarbonate (initially at pH 8.3), is brought to more acid reactions by dissolved carbon dioxide. Such a curve, at zero added potassium bicarbonate, defines one boundary of the model; another, at 0.001 equivalents of added $KHCO_3$ per liter, has been drawn. At points on these curves, indicated by the thin construction lines, ordinates have been erected which correspond with the experimentally observed bromide concentration in the sap. The several data at pH 7.2 and 6.8, respectively, define two vertical sections cut through the model at these pH values[2] and the edge of the section thus exposed (which can be treated as linear) represents the *depressing effect at constant pH of total dissolved carbonic acid on bromide uptake.* In this way two curves and two straight lines, which lie in the surface of the model, can be defined. At any given *total* carbonic acid concentration vertical sections[3] can be cut through the model (at right angles to the sections at constant pH) and ordinates erected to intercept the curves which lie in the surface and have been defined above. Observed data at pH 7.5 and 8.3 were insufficient to establish the section along these planes of constant pH value but, by prolonging to pH 7.5 and 8.3 the slope of the lines which connect points in the surface of the model at the same total acid concentration, the experimental data can be supplemented; thus sections through the model at these pH values may be established with sufficient accuracy for present purposes.

This second model now corrects a possible misconception gained from the first. Both models show that at pH 8.3 (where free carbonic acid can be neglected) the depressing effect of added bicarbonate (total carbonic acid present in the system) on bromide uptake is great. This effect must be due either to $[HCO_3^-]$ or to this supplemented by $[OH^-]$ since the only free undissociated acid is that which arises from hydrolysis. The effect of increased concentration upon bromide absorption, *per unit of total carbonic acid present,* at *constant pH,* is a maximum at pH 8.3. It decreases progressively at more acid reactions at which relatively more of the acid present is in the uncombined and undissociated form. This must mean that, as between bicarbonate ion and undissociated free acid, the *most effective component of the bicarbonate buffer system which suppresses the uptake of bromide is the bicarbonate ion itself.* It is equally clear, however, that other factors are involved. Were it not so, all of the data could be fitted to a smooth curve of bromide uptake plotted against bicarbonate ion concentration and this is not possible. Solutions which are acid due to carbon

[2] Where such sections cut the surface of the model is shown by a chain-dotted line in the figure (.-.-.-).

[3] Where such sections cut the surface of the model is shown by a broken line in the figure (--------).

dioxide, unbuffered by potassium bicarbonate, exert on bromide uptake a retarding effect which is out of all proportion to the bicarbonate ion which they contain; this suggests that the free carbonic acid also has its specific effects on the bromide absorbed—either due to the undissociated free acid or the H^+ *per se*.

In the absence of added bicarbonate the bromide uptake is but little affected by increased concentration of dissolved carbon dioxide between pH 7.7 and 5.9; but at more acid reactions it is depressed and, in the pH range 7.7 to 4.7, the data fall on a straight line, the empirical equation of which is $[Br] = 17.3 - \frac{17.3}{17.0} \times [\text{dissolved } CO_2]$ where the concentrations of bromide in the sap and carbon dioxide in the solution are expressed in milligram equivalents and millimols per liter, respectively.

In the presence of added bicarbonate the effect of increased carbon dioxide on bromide accumulation is due to the combined action of the bicarbonate and the free acid. Although the concentration of salt and free acid is either known or can be calculated at the given pH values, the data are hardly adequate to derive a satisfactory empirical relationship between dissolved carbon dioxide, bicarbonate, pH or $[H^+]$, and bromide absorption which covers the full range of conditions. Such a relationship could not do more than describe concisely the models shown—it would not alone explain the effects of bicarbonate and carbon dioxide on bromide uptake, since these factors clearly operate through the metabolic processes which are involved in absorption and which have yet to be described.

It happens that all of the data in tables I and II lie on a smooth curve of bromide uptake plotted against total carbonic acid in the external solution. This relation is not general, however, and would probably not hold at high concentrations of total carbonic acid and reactions more alkaline than pH 7.7.

At any given pH value, a simultaneous increase of potassium bicarbonate and dissolved carbon dioxide retarded, and eventually suppressed, bromide uptake. Both salt (bicarbonate ion) and free undissociated acid (H_2CO_3) contributed to this effect which was clearly not due to the H and OH ions. If the effect of bicarbonate is additive to that of carbonic acid, then simple calculations show that at constant pH, near neutrality, the drop in bromide concentration in the sap due to the external bicarbonate was 1.1 milligram equivalents per liter of sap per milligram equivalent of bicarbonate in 1 liter of external solution.

The retardation of bromide uptake which was due to increase of potassium bicarbonate concentration at constant pH is the more interesting because increased concentrations of other potassium salts (with anions Cl, Br, NO_3) stimulate all those processes which are concerned in salt accumu-

lation (**22**). Therefore, the processes which are deemed essential to salt uptake should be suppressed by increased concentrations of total carbonic acid at constant pH and evidence to be described shows that this is, in fact, the case.

SPECIFIC EFFECTS OF pH ON BROMIDE UPTAKE.—An obvious difficulty is to ascertain from the evidence whether H^+ and OH^- have any direct effect upon bromide uptake. In solutions enriched with bicarbonate, the bromide uptake falls off more at alkaline reactions than near neutrality and this result is evident from the models (figs. 3 and 4) whether one compares cultures of constant potassium bicarbonate concentration or of the same *total* carbonic acid concentration. If the bromide uptake was influenced only by the bicarbonate ion and the free acid then, since their relative effects are almost equal at pH 7.0, the effect of increased carbonic acid in the system should be independent of pH provided the *total* (bicarbonate and dissolved carbon dioxide) remained constant. Figure 4 shows that such is not the case. At reactions more acid than pH 5.9 and more alkaline than 7.2, bromide uptake is less than at the intermediate reactions; this effect becomes the more conspicuous as the total capacity of the tissue to absorb is retarded by carbon dioxide and bicarbonate (see vertical section through model of figure 4 along 0.014×10^{-3} mols total CO_2 per liter). Such effects obtained in the more acid and more alkaline reactions *are attributable only to acidity and alkalinity per se* and, therefore, to specific effects of H^+ and OH^-. This statement is made only after a full attempt to account for the effects observed *solely* on the basis of the calculated concentrations of bicarbonate ion and undissociated carbonic acid without reference to H^+ and OH^-.

There is thus *a range, relatively broad in the absence of bicarbonate, within which bromide uptake is not much affected by hydrogen ion concentration and its attendant variables. Reactions near to neutrality, however, are at any given total concentration of added carbonic acid, more favorable to bromide uptake than either more acid or more alkaline solutions.* The investigation of the effects of carbonic acid and bicarbonates on metabolism shows that metabolic processes now known to be closely associated with bromide uptake are similarly affected and show optima near pH 7.0. The view that these are, in fact, effects due to H and OH ions is strengthened because similar results have been obtained in solutions of other buffer salts; *e.g.*, phosphates.

These data predict the acid reactions at which bromide uptake should vanish. In solutions unbuffered by bicarbonate there are no data above pH 8.3. The form of the models suggests that at still more alkaline reactions bromide uptake might also decline and eventually vanish. This problem, upon which it is interesting to speculate, can be solved only if experiments are carried out with other buffer systems, or in a range of pH where the second dissociation of carbonic acid operates.

TABLE II

MOLS OF ADDED BICARBONATE AND TOTAL ACID (COMBINED AND UNCOMBINED H_2CO_3) IN SOLUTIONS USED

pH	Added $KHCO_3$	Total acid	pH	Added $KHCO_3$	Total acid	pH	Added $KHCO_3$	Total acid	pH	Added $KHCO_3$	Total acid
	mols.	*mols.*		*mols.*	*mols.*		*mols.*	*mols.*		*mols.*	*mols.*
4.75	–	0.00940	5.3	0.001	0.01400	7.23	0.005	0.00574	6.81	0.0025	0.00349
5.92	–	0.00270	7.3	0.001	0.00113	7.23	0.010	0.01148	6.76	0.0150	0.02150
6.12	–	0.00149	8.3	0.001	0.00101	.....	.....	.....	6.81	0.0200	0.02780
6.58	–	0.00028	.....	.....	.....	.....	.....	.....	.....	.....	.....

TABLE III

EFFECT OF INCREASED [$KHCO_3$] AND [CO_2] AT pH 6.5 ON THE NITROGEN FRACTIONS OF POTATO DISCS AT 23° C. AND 72 HOURS*

Sample	[$KHCO_3$] per liter	Protein N per gram fresh weight	Soluble N per gram fresh weight	Protein N	Soluble N	Amino N	Amide N		Ammonia* N
							Stable	Unstable	
	equiv.	*mg.*	*mg.*	%	%	%	%	%	
Initial	–	0.58	1.44	28.7	71.3	56.5	8.4	7.4	.....
Final	0.005	0.90	1.16	43.7	56.3	45.6	4.9	5.8	.....
"	0.020	0.65	1.35	32.5	67.5	51.0	6.0	12.0	.....
"	0.040	0.65	1.40	31.7	68.3	50.8	6.3	10.7	.....

* Absolute units milligrams nitrogen per gram initial fresh weight. Results on a percentage basis are relative to total nitrogen. Negligible—0.001 mg. per gm. fresh weight.

SUMMARY OF THE EFFECTS OF THE COMPONENTS OF THE HCO_3^-/H_2CO_3 BUFFER SYSTEM ON BROMIDE UPTAKE.—The effect of bicarbonate and dissolved carbon dioxide on bromide uptake is complex. The components of the carbon dioxide buffer system *depress absorption.* Combined and free carbonic acid both depress bromide uptake and at pH 7.0 their relative effects are approximately equal. The indications are that the undissociated free acid in the system is less effective than the bicarbonate ion in retarding bromide uptake. A full interpretation of all the facts cannot evade effects which must be ascribed specifically to H and OH ions—effects which operate in such a fashion that reactions near neutrality become especially conducive to bromide uptake. This is particularly true when the total absorption is already reduced by the effects of added carbonic acid.

THE EFFECTS OF pH, DISSOLVED CARBON DIOXIDE, AND POTASSIUM BICARBONATE ON THE METABOLISM OF POTATO DISCS

THE EFFECT OF INCREASED $KHCO_3$ AND CO_2 CONCENTRATION AT pH 6.5.—The most striking feature of the effect of the components of the CO_2/HCO_3^- buffer system upon bromide uptake is that at a constant pH, bromide accumulation is progressively decreased, and eventually suppressed, by the simultaneous increase of potassium bicarbonate and dissolved carbon dioxide. This is true even though all other variables remain at values conducive to a high degree of salt accumulation. If bromide absorption is to be correlated with a particular aspect of metabolism, then this should be a process which is retarded by these treatments. Therefore, investigation of the metabolic processes so affected will go far to identify those which are essentially concerned in bromide absorption.

Discs exposed to aerated solutions of potassium bicarbonate at constant pH do not brown as much in the strong as they do in the dilute solutions or in distilled water. Usually (**21, 22**) increased concentrations of potassium salts tend to accentuate the surface browning reaction. Since bicarbonates are unusual in this respect, it is to be expected that the specific effects of the bicarbonate and dissolved carbon dioxide on metabolism would operate through processes which are linked with the activity of the oxidase (phenolase) system of the potato tuber. Experiments which were carried out at pH 6.5, show that this is the case; this is the pH at which the buffer effect is at its maximum.

From table III and figure 5, it is apparent that the protein synthesis which normally occurs in potato discs in distilled water or in dilute potassium bicarbonate solution is *almost completely suppressed* by an external concentration of 0.02 mols per liter and its attendant carbon dioxide concentration. This result, as well as much other evidence not given here (**21, 22, 23**) suggests that protein synthesis plays an indispensable part in the accumulation of bromide by potato discs.

Further analysis of the soluble nitrogen fractions shows that, as previously recorded (**21, 22**), the nitrogen used in synthesis is mainly amino-N.[4] The treatments which in the experiment of table III depressed synthesis (compare cultures at 0.005 and 0.020 equiv. $KHCO_3$) also retarded the utilization of amino-acid but *not to the same degree.* When there was little gain of protein in the tissue (cultures at 0.020 and 0.040 equiv. $KHCO_3$) more amino acid disappeared than reappeared as protein and the excess was accounted for by an increase in the unstable amide-like compound which has previously been noted (**21**) and which was then regarded as a possible reactive intermediary between amino-acid and protein. It thus appears that the combined effect of bicarbonate and carbon dioxide is not exerted exclusively upon the deamination of the amino-acids, but even more upon the

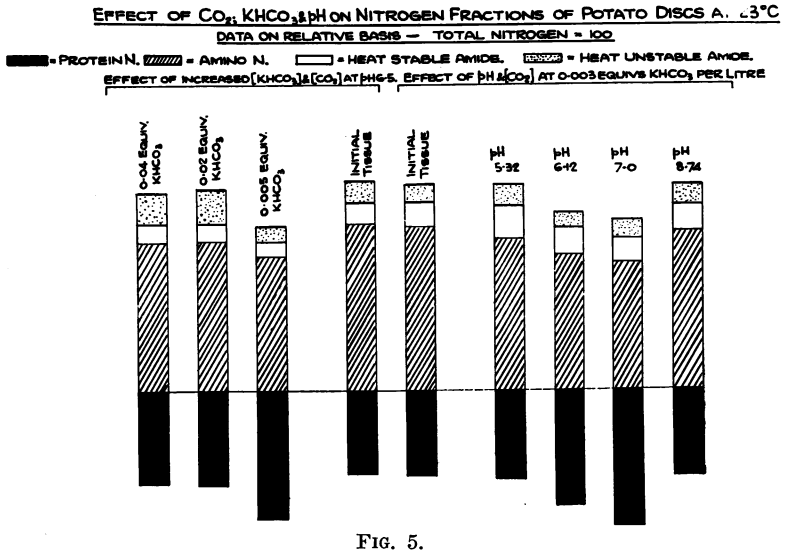

FIG. 5.

later stages of protein synthesis in which the reactive intermediaries are converted to protein. As the intermediates accumulate in bicarbonate cultures which depress synthesis they, in turn, tend to depress the deamination of the amino-acids. It will be recalled that when potassium salts stimulate synthesis they do so by affecting both the deamination of amino acid and the use of the reactive amides which are supposed to be intermediates in synthesis. When calcium salts depress synthesis, however, the unstable amides do not accumulate.

THE EFFECT OF pH AT CONSTANT BICARBONATE CONCENTRATION.—Superimposed upon the effect of bicarbonate and dissolved carbon dioxide, which at constant pH does not concern the H and OH ions, there is at any given

[4] Amino-N free from confusion with heat unstable amides which react both in the amide and the VAN SLYKE determination.

concentration of $KHCO_3$, an effect on protein synthesis which must be due primarily to these ions. This is shown by the data of table IV which are

TABLE IV

EFFECT OF pH IN $KHCO_3$ BUFFERED SOLUTIONS (0.003 EQUIV. PER LITER) ON THE NITROGEN FRACTIONS OF POTATO DISCS AT 23° C. AND 72 HOURS*

SAMPLE	pH	PROTEIN N PER GM. FRESH WT.	SOLUBLE N PER GM. FRESH WT.	PROTEIN N	SOLUBLE N	AMINO N	AMIDE N		AMMONIA† N
							STABLE	UNSTABLE	
		mg.	*mg.*	%	%	%	%	%	
Initial	.....	0.62	1.46	29.8	70.2	55.0	8.6	6.2	.....
Final	5.32	0.63	1.42	30.7	69.3	51.7	11.2	6.3	.....
"	6.12	0.82	1.25	39.6	60.4	46.3	9.2	4.9	.....
"	7.00	0.95	1.09	46.6	53.4	43.6	8.4	6.4	.....
"	8.74	0.59	1.39	29.8	70.2	54.0	9.0	6.5	.....

* Absolute units, milligrams nitrogen per gram initial fresh weight. Results expressed on a percentage basis are relative to total nitrogen.

† Negligible—0.001 mg. per gm. fresh weight.

illustrated in figure 6. It is clear that *protein synthesis is at a maximum at pH 7.0* and that *it declines both in more acid and more alkaline solutions.* It will appear later that similar results are obtained in phosphate buffered solutions although specific effects of phosphate and bicarbonate on protein synthesis are quite different. This effect of pH must, therefore, be due to H and OH ions specifically.

The detailed analyses of the soluble nitrogen fractions simply show that the effects of pH are exerted mainly upon the utilization of the amino-acid fraction. The changes in total soluble nitrogen and amino nitrogen run parallel throughout and they are complementary to the observed change in protein nitrogen (table V).

Combining now the results at constant pH and at constant bicarbonate concentration in figure 6, it is impossible to ignore the similarity between the effects of these treatments on protein synthesis and on bromide accumulation. In both cases the process in question is retarded and eventually suppressed by increased concentrations of bicarbonate and dissolved carbon dioxide; at a given salt concentration, it exhibits an optimum at a pH value of 7.0. It is to be concluded, therefore, that the specific effects of the components of the H_2CO_3/HCO_3^- buffer system and of pH on bromide absorption are linked with their effect on protein synthesis—a process with which bromide uptake in potato discs is closely associated.

EFFECT OF pH, BICARBONATE, AND DISSOLVED CARBON DIOXIDE ON THE RELATIVE ABSORPTION OF ANION AND CATION

The absorption of potassium in the experiments of table I presented a

TABLE V

EFFECT OF pH, [$KHCO_3$] AND [CO_2] ON ACCUMULATION OF POTASSIUM BY POTATO DISCS AT 23° C. DURING 70 HOURS

EXP. NO.	[K] IN EXTERNAL SOLUTION	[$KHCO_3$] IN EXTERNAL SOLUTION	MEAN pH	GAIN OF K IN SAP	EXP. NO.	[K] IN EXTERNAL SOLUTION	[$KHCO_3$] IN EXTERNAL SOLUTION	MEAN pH	GAIN OF K IN SAP	K ABSORBED IN EXCESS OF BR
	equiv./l.	*equiv./l.*		*mg. equiv./l.*		*equiv./l.*	*equiv./l.*		*mg. equiv./l.*	*mg. equiv./l.*
1	0.001	.....	4.75	− 9.6	2	0.011	0.001	7.30	11.0	.....
2	0.011	.....	5.92	− 5.7	2	0.011	0.005	7.23	20.0	8.5
2	0.011	.....	6.12	+ 0.2	2	0.011	0.010	7.23	14.5	7.7
2	0.011	.....	6.58	+ 5.5	3	0.021	0.025	6.81	19.1	5.0
1	0.001	.....	6.90	+17.4	3	0.021	0.015	6.76	32.9	30.0
3	0.021	.....	7.70	+ 9.4	3	0.021	0.020	6.81	22.5	21.0

more difficult problem than that of bromide. It is complicated by the effect of the treatment on the high concentration of potassium which existed in the initial tissue (76 mg. equiv. per liter of sap) and which, under conditions unfavorable for absorption, may leave the cells. It is necessary to examine the results obtained to see whether these variables affect potassium and bromide absorption in a similar fashion and to ascertain whether the evident connection between protein synthesis and bromide uptake applies equally to potassium absorption.

The effect of pH on the absorption of potassium and on the potassium content of the initial tissue can be seen in the first part of table V.[5] The different experiments were conducted at various total potassium concentrations which are shown, though they were all at constant bromide concentration and in the absence of bicarbonate. It is clear that the concentration of the only mobile anion (Br), which was constant, had a greater control over the uptake of cations than the varying concentration of potassium sulphate in the range shown. If potassium sulphate concentration had any tendency to increase potassium absorption, allowance for this would merely accentuate the effect of pH to which reference will now be made.

At neutrality (strictly pH = 6.9) absorption of potassium and bromide was in equivalent amounts—a fact which has often been observed for potato discs in potassium bromide solutions. At more acid reactions, due to increased concentrations of carbon dioxide, there was an apparent decrease in the potassium absorbed. This was due to the effect on the absorption of potassium bromide but also to the effect of carbon dioxide on the loss of potassium which was present initially in the tissue. This loss was so great at acid reactions (pH $<$ 6.0) that it entirely masked the potassium absorbed along with bromide. Although the data are limited at reactions more alkaline than pH 7.0, there is a strong suggestion that these also caused loss of the potassium previously stored in the cells. The obvious conclusion is that the tissue retains its stored potassium best under those conditions most suitable for protein synthesis.

Reference may be made here to a similar, though even more striking, case to which a similar conclusion applies. After prolonged storage of tubers at low temperatures, potato discs no longer retain their salts against aerated distilled water (**17**, p. 536); they lose the capacity to grow as shown by meristem formation in moist air, and *it is now known that they also lose their ability to synthesize protein.*[6] Though as yet unpublished, these results strengthen the conviction that the observed effect of pH and carbon dioxide concentration on the loss of potassium from the cells is causally connected with their effect on protein synthesis (fig. 6). This suggests that,

[5] For these potassium analyses we have to thank members of the laboratory of Plant Nutrition, University of California, Berkeley, California.

[6] Data in thesis of T. K. RAMAMURTI, University of London.

in order to retain their solutes against distilled water, the cells cannot merely remain static; they must also be capable of synthesizing protein. The evident connection between protein synthesis and the ability of cells to retain potassium in their sap, recalls previous observations that the discs must maintain an unexpectedly high rate of respiration in order to retain

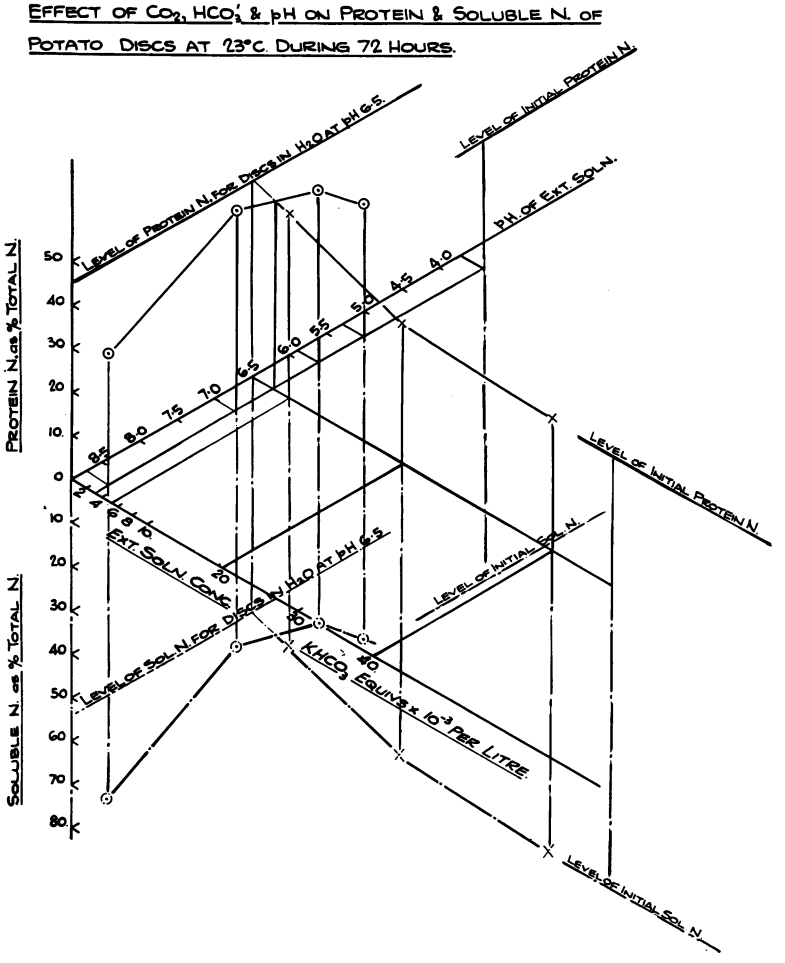

FIG. 6.

their salts (**16**, pp. 215–234). These results stand in contrast to the long-cherished conception of a passive "semi-permeable" structure by which cells retain the solutes contained in their vacuoles. It must be recognized that the metabolic processes of respiration and protein synthesis are equally necessary to retain salts *after they are accumulated* as they are to produce the high internal salt concentration *de novo*.

The effect of bicarbonate and dissolved carbon dioxide concentration at constant pH is revealed by two series of data from experiments at different total potassium concentrations. In the presence of added bicarbonate, the absorption of potassium always greatly exceeded the absorption of bromide, even at reactions close to neutrality (pH 7.23 and 6.8). At contant pH, the potassium absorbed in excess of bromide increased with bicarbonate concentration up to an optimum concentration above which, presumably owing to the effects of dissolved carbon dioxide, further increase reduced the total absorption of potassium as well as its excess over the bromide absorbed. There can be little doubt that the *potassium absorbed which was unaccompanied by bromide was absorbed along with bicarbonate ion* and that this process occurred under conditions such that *protein synthesis and bromide uptake were reduced almost to zero* (culture at 0.020 mol. $KHCO_3$ and pH 6.8, table V). The absorbed bicarbonate, however, did not remain as such in the sap; when this was acidified, it yielded little more carbon dioxide than did the normal sap. The presumption is that potassium bicarbonate was absorbed as such and reacted with the organic acids of the sap. This process represents at least a transitory means by *which the cells accumulate potassium independently of protein synthesis and of growth* although it can persist only so long as the stored reserves of organic acids remain or are replenished. The final result would be the same if potassium exchanged for hydrogen ion supplied by the organic acid. Some may prefer this interpretation but it leaves the rôle of bicarbonate in the solution without explanation.

Further work on this point is necessary but there seem to be grounds for homologizing this uptake of potassium by potato discs with the brief but rapid uptake of potassium by "low salt" barley roots during which, if it occurs from potassium bicarbonate, organic acids increase in the cell sap. Potassium may be absorbed, therefore, by distinct mechanisms which bear different relationships to metabolism. The absorption of potassium from bicarbonate solutions presents a very special case—it is clearly less dependent upon certain aspects of metabolism (protein synthesis and processes linked with it) than the absorption of potassium bromide. The extent to which it is conditioned by oxygen in the external solution remains an interesting problem for the future.[7]

The unequal absorption of anion and cation may be a contributory cause of the metabolic effects which obtain in bicarbonate solutions. HOAGLAND'S observation that barley roots respond to bicarbonate absorption by actual increase in their organic acid content has been noted. Indications that the centers of protein synthesis in potato discs respond to unequal absorption

[7] In barley root experiments, HOAGLAND and BROYER have shown that the absorption of potassium from bicarbonate solutions does not occur at very low oxygen concentrations (private communication to the authors).

of cation and anion as though they operate best under conditions of strict neutrality, have been noted elsewhere (**21**). Equal uptake of both ions from some potassium salts (KBr) with its concomitant synthesis and the fact that both synthesis and uptake are increased by greater concentrations, contrast with the unequal uptake of calcium salts (accentuated perhaps by the possible fixation of the cation as insoluble compounds) and the depression of synthesis which greater concentrations cause. In potassium bicarbonate solutions the final effect is as if the cation were absorbed unaccompanied by anion (or exchanged for hydrion) and this condition of ionic unbalance is associated with a markedly depressed protein synthesis. Moreover, both in phosphate and bicarbonate buffers at constant total acid the synthesis is a maximum at a neutral external reaction of 7.0. Hence the relation between protein synthesis and salt uptake is not merely that synthesis is a vital property of growing cells which makes uptake possible. It is that continued absorption, like synthesis, does not tolerate the consequences of ionic unbalance due to unequal intake of anions and cations by the cells and it is favored by external solutions with reactions close to strict neutrality. If disturbance in the metabolic machinery is the consequence of such ionic inequality, one may well look for its explanation in the behavior of the organic and amino acids. The whole tendency is to conserve the latter in the presence of bicarbonate and avert their conversion to protein.

The effect of pH, potassium bicarbonate, and carbon dioxide concentration on the respiration and metabolism of carbohydrate of potato discs

The direct determination, by carbon dioxide evolved, of the respiration of potato discs in solutions rich in bicarbonate and dissolved carbon dioxide presented too great technical difficulties to be profitable. Indirect determinations by the change in total carbon in the tissue due to respiration was complicated by the loss of carbon which the tissue sustains when it is blotted dry with paper (**23**) and by the absorption of bicarbonate from the external solution. Consequently, the effect of the treatments already described on respiration cannot be stated very precisely. It is clear, however, that respiration, like the phenolase activity and protein synthesis, was retarded at pH 7.0 in the presence of bicarbonate (0.020 N) and dissolved carbon dioxide (solution in equilibrium with 20 per cent. CO_2 by volume). This was demonstrated by using tissue of a variety different from that used in experiments 1, 2, and 3, and by comparing the total heat content (bomb calorimeter determination), starch and sugar content of initial washed discs, and comparable samples after 70 hours of contact with either bicarbonate solution at pH 6.5 or the equivalent strength of potassium sulphate.

In the tissue in the bicarbonate solution the hydrolysis of starch to sugar (final sugar, 0.24 gm.; final starch, 3.10 gm. per 40 gm. initial fresh weight) was retarded in comparison with that which received the sulphate treatment (final sugar, 0.34 gm.; final starch, 2.75 gm. per 40 gm. initial fresh weight). The total carbohydrate recovered (starch + starch equivalent of final sugar) was greater in the bicarbonate culture than in the sulphate. The still outstanding loss of carbohydrate (initial tissue contained 4.16 gm. and the bicarbonate treated 3.32 gm. total starch per 40 gm. fresh wt.) in the tissue treated with bicarbonate solution at pH 6.5 was due partly to carbon losses incurred in blotting the discs (**23**), and partly to respiration or other metabolic processes which were not separately measured. One must make the same allowance (estimated at 0.42 gm. per 40 gm. initial tissue) for loss of carbohydrate owing to the formation of the surface film of mucilage in the bicarbonate series, as that which is necessary to "balance" the carbohydrate balance sheet of the potassium sulphate series. It then appears that at pH 7.0 in contact with 0.020 N potassium bicarbonate and the appropriate carbon dioxide concentration, 40 gm. of this tissue (in which protein synthesis was depressed but not completely eliminated) respired 0.42 gm. of starch in 70 hours as against 0.64 gm. in bicarbonate and carbon dioxide-free solutions. The total heat change in the discs also indicated that respiration was depressed in presence of bicarbonate and dissolved carbon dioxide but the precise effect must be determined by other methods. Knowing the general parallelism between the effects of other salts on respiration and protein synthesis (**23**) and also the phosphate experiments in this paper, the trend of the effect of the bicarbonate and carbon dioxide treatments on respiration may be inferred from their effects on protein synthesis which are here recorded. Adopting this standpoint, the now oft-repeated parallelism between bromide absorption and respiration of potato discs would again emerge—a treatment (increased bicarbonate concentration) which depresses bromide uptake has a similar effect on respiration.[8]

Carbon dioxide has somewhat unexpected results on living cells. The observations of THORNTON (**25**) and FIFE and FRAMPTON (**3**) have given prominence to an effect of carbon dioxide on the pH of the cell and show that this is not always predictable on *a priori* grounds. FIFE and FRAMPTON showed that the explanation of the unexpected shift toward alkalinity in the sap of carbon dioxide-treated cells lies in its effect on systems in the cells which catalyze the hydrolysis and re-synthesis of amides—reactions which occur only in the living tissue in the presence of oxygen (**3, 25**). Actual tests showed that this effect did not enter appreciably into the behavior of

[8] Evidence from the researches of A. ULRICH on barley roots (in course of publication) suggest that the respiratory quotient may be altered when accumulation of K occurs from a solution of $KHCO_3$. Further investigation of this possibility with reference to potato tissue would be of interest.

immersed potato discs at pH 7.0. FIFE, at the author's request, made tests in 1934 which showed that the shift in pH toward alkalinity in the sap expressed from potato discs which have been exposed to high concentrations of dissolved carbon dioxide (equilibrium with 2 atmospheres pressure CO_2) was only slight (pH 0.26). At the same time, the detailed analysis in tables III and IV did not show the free ammonia to be expected if amide hydrolysis had occurred extensively. It will be recalled, however, that the tissue at pH 7.0 in solutions rich in bicarbonate and dissolved carbon dioxide did not show the usual symptoms of oxidase activity and it is here, through some esential property of the living protoplast, that the mechanism must be sought. From the evidence presented it is clear that the effect of bicarbonate and dissolved carbon dioxide on metabolism embraces the nitrogen compounds although mainly in a way other than that which FIFE and FRAMPTON (3) described for the beet plant exposed to carbon dioxide gas. The response of beet leaves to CO_2 is rapid and considerable (increases of pH approaching 1.0 pH unit were observed by FIFE and FRAMPTON in 60 minutes). THORNTON'S work was done on much larger masses of tissue in a gas phase and the response, though considerable, required a longer period. A possibility that thin discs would react even more than the large masses was evidently not realized. This difference is most probably due to factors incidental to the use of discs immersed in aerated solution, though the possibility that the reversibility of the effect, which FIFE and FRAMPTON observed for beets, is so accentuated in these discs that their reaction is reversed before sap can be expressed and measured may have to be considered. Even if this were the case, the clue to the metabolic effect of carbonic acid and bicarbonate solutions should be with the permanent, rather than the transient, results of such treatments.

Experiments made on the effect of pH and phosphate concentration on the respiration and nitrogen metabolism of potato discs afford an interesting comparison with the bicarbonate series from which the effects specifically due to H and OH ions may be inferred.

THE EFFECT OF pH AND PHOSPHATE CONCENTRATION ON THE METABOLISM OF POTATO DISCS

The trend of the effects of total phosphate concentration and of pH in phosphate buffered solutions is shown by the results of two series of experiments. One is conducted at constant pH and the other at constant total concentration of total phosphate and potassium; otherwise both are conducted under identical conditions of time, temperature, aeration, etc. These variables are fixed at the same arbitrary values used in the bicarbonate experiments so that direct comparisons can be made between the two series of experiments.

The effects due to varying concentration of total phosphate were determined at pH 6.9. This is a reaction close to strict neutrality and at the pKa value of phosphoric as a dibasic acid where the buffering effect of potassium dihydrogen phosphate in presence of potassium monohydrogen phosphate is at its maximum. The effects due to pH were determined at constant concentration of 0.020 mol per liter of total phosphate. To eliminate confusion due to variable concentration of potassium, the solutions had their potassium content raised where necessary, to a constant level of 0.040 equivalent per liter, by the addition of potassium since the sulphate ion has only slight influence on the metabolism of potato discs. The composition of the solutions used is given in table VI.

TABLE VI

COMPOSITION OF BUFFER MIXTURES FOR EXPERIMENTS AT CONSTANT PHOSPHATE AND POTASSIUM CONCENTRATION*

pH	Solution A to 2 liters	Solution B to 2 liters	Solution C to 2 liters
	ml.	*ml.*	*ml.*
8.04	133	137.0	.....
7.46	133	112.0	25.0
6.95	133	73.0	64.0
6.48	133	33.0	104.0
5.96	133	14.6	122.4
6.82	.....	.....	270.0

* Stock Soln. A = 0.3 M KH_2PO_4.
Stock Soln. B = 0.3 M KOH.
Stock Soln. C = 0.15 M K_2SO_4.

To arrive at these desired mixtures stock solutions of 0.3 M KH_2PO_4 and KOH were prepared and, using the glass electrode, the titration curve of the one against the other was determined and from this the volume mixtures necessary for any desired pH could be ascertained.[9]

For the experiment at constant pH a phosphate buffer mixture at pH 6.9[10] was prepared which was then diluted so that the desired total phosphate concentrations were obtained; namely, 0.002 M, 0.01 M, 0.025 M, and 0.040 M. The last was too strong and, because of the less turgid condition of the tissue in this solution, no safe conclusions could be drawn from this culture. These data are therefore omitted.

The effect of the above treatments can be represented by a method similar to that previously adopted for the bicarbonate experiments. Respiration rate and protein, or soluble nitrogen, content of the discs were plotted on isometric paper against *both* pH of the external solution *and* the

[9] See HOLT, LAMER, and CHOWN (7) for a discussion of the ionic equilibria in phosphate solutions at different pH's; also CLARK (2).

[10] One liter of 2 M/5 KH_2PO_4 + 720 ml. 2 M/5 KOH, the mixture diluted to 2 liters.

total phosphate concentration. The data obtained identify two sections cut through a solid model, the surface of which describes the inter-relationships of the three variables concerned. Sufficient data to identify such surfaces completely would involve a rather formidable investigation which it might be difficult to complete before the changes which ensue during storage affected the behavior of the tissue. The experiments were so planned, however, that the more limited data available permit one to discern the general trend in the effects which were obtained.

RESPIRATION.—The time curve of respiration of tissue in phosphate buffered solutions at pH 6.9 is identical in form to that already shown for tissue in distilled water or certain neutral salt solutions ($CaCl_2$). It consists of an initial period during which the respiration rises to a value which is subsequently maintained for long periods. Phosphate cultures at acid or alkaline reactions introduced further complications into the initial period and it is not proposed to deal with these at length here. It will suffice to state that the carbon dioxide removed by the air stream from the cultures in the first period increased progressively from the more alkaline to the more acid solution. This was doubtless due to the bicarbonate which remained in the more alkaline solution. Later in the time drift cultures, both more acid and more alkaline than pH 6.9, it produced a transient increase in respiration; this had, however, elapsed before the period of 26 to 72 hours to which particular attention will be directed.

The effect of increased concentration of total potassium phosphate on respiration is revealed by the series at constant pH (table VII). Those

TABLE VII

EFFECT OF EXTERNAL pH ON RESPIRATION* OF POTATO DISCS IN POTASSIUM PHOSPHATE BUFFERS (0.020 M) OF CONSTANT K CONTENT (0.040 EQUIV.) AT 23° C.

External pH	5.96	6.48	6.95	7.46	8.04
Respiration rate	0.207	0.299	0.325	0.302	0.234
Relative respiration rate	63.8	92.0	100.0	93.0	72.0

* Respiration rates = mean rates in mg. CO_2 per gm. initial fresh wt. per hour for period 26 to 72 hours of exp.
Relative respiration rate = culture at pH 6.95 as 100.

effects are due either to potassium or to the simultaneous and proportional increase in $H_2PO_4^-$ and HPO_4^{--}; the relative amounts of these are equal in solutions of pH equal to the pKa value of the second dissociation of phosphoric acid (pH = 6.9). Total phosphate concentration increased respiration at pH = 6.9 (table VIII, fig. 7); this might be expected from the prevalent belief that hexose phosphate plays a prominent rôle in respiration.

It is well to emphasize, however, that the increase observed is not conspicuously greater than the response obtainable with potassium nitrate and

TABLE VIII

EFFECT OF TOTAL CONCENTRATION OF POTASSIUM PHOSPHATE BUFFER ON RESPIRATION RATE* OF POTATO DISCS AT pH 6.9 AND 23° C.

Molar concentration phosphate	0.0	0.002	0.010	0.025
Respiration rate	0.193	0.204	0.245	0.367
Relative respiration rate†	100.0	106.0	127.0	190.0

* Respiration rates = mean rates in mg. CO_2 per gm. initial fresh wt. per hour for period 26 to 72 hours of exp.

† Relative respiration rate = culture in distilled water = 100.

it is actually less than the response which has been obtained in ammonium sulphate and nitrate solutions (unpublished experiments). The interpretation of the mechanism of the phosphate response can be deferred, but it is clear that the doubtful specific effect of phosphate, unlike that of bicarbonate, is to increase respiration.

Despite the contrast in the effects due to the two anions, the effects of pH on respiration in phosphate buffered solutions (table VII, fig. 7) are similar to those of pH on protein synthesis (and presumably also on respiration) in bicarbonate buffered solution. After the respiration had attained somewhat steady levels during the last 36 hours of the treatments, the maximum respiration occurred at pH 6.9. When data from the two experiments are combined (fig. 7) the probable form of the surface which depicts the effect of phosphate concentration and pH on the respiration of potato discs can be visualized.

THE GENERAL EFFECT OF pH ON RESPIRATION.—The literature contains no other record of the effect of phosphate concentration and pH on the respiration of potato discs similar to figure 7. Reported attempts to show the effect of pH suffer from inadequate appreciation of the variables which affect the behavior of potato discs. LEMMON (**8**) and BOSWELL (**1**), both working with potato tissue, used the WARBURG manometric method—a technique the full implications of which have not been adequately investigated relative to the behavior of potato discs. LEMMON was primarily concerned with the effects of pH on respiration but used buffer solutions of such varied composition that the effect of pH *per se* could not be segregated from that due to other variable components of the solutions. This was doubtless responsible for the erratic behavior observed.

BOSWELL though not primarily concerned with the effects of either salts or pH on respiration, used such short time periods (0 to 3 hours) that it is questionable if the tissue had fully responded either to the salt treatment or to the oxygen content of the solution if this was indeed in equilibrium with air. BOSWELL did not state the full composition of his buffer solutions; *e.g.*, the nature and concentration of the cation. He remarks that his results

show that for the pH range of 5.29 to 8.04, the oxygen uptake is unaffected between pH 5.59 and 6.81 "falls away slightly below 5.9 and rises above 6.81." No final conclusion can be drawn from his figures which merely show that in his experiments the effect of pH, if indeed pH was the causal factor, was erratic. The pH effects described in this paper, whatever their ultimate explanation, are clearly consistent with the other results of this

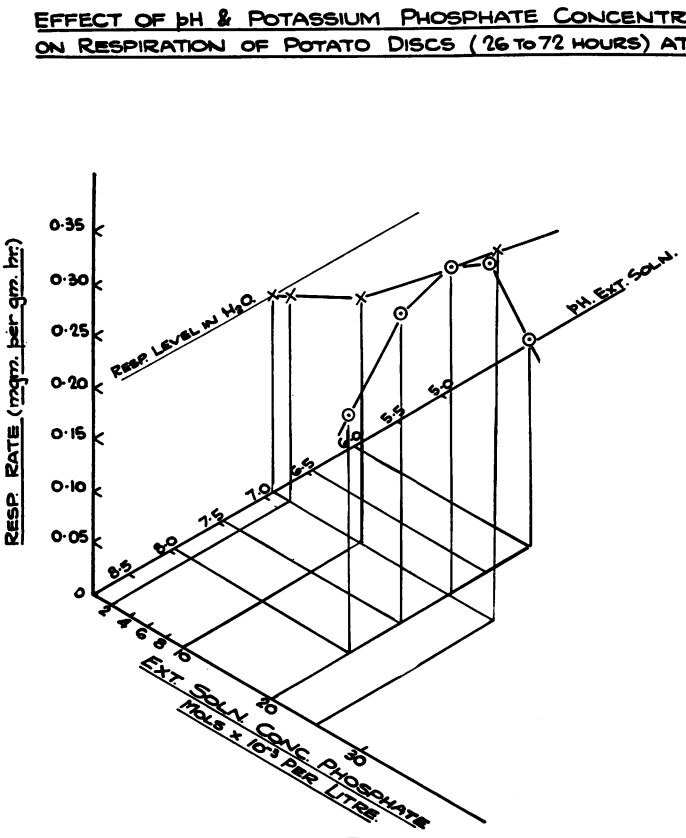

FIG. 7.

series of investigations and they form part of a comprehensive, general picture of the behavior of potato tissue.

With respect to other tissues there are inconsistencies in the relation of respiration to pH. To cite but two cases: LYON'S (**10**) results with Elodea showed greater respiration with increasing alkalinity beyond pH 7.0; whereas THOMAS (**24**) claims that respiration increases with acidity in carrots.

Even the effect of phosphate concentration on respiration is not free from contradictions. LYON (**9**) using Elodea treated with phosphate solu-

tions at a "neutral reaction" for one hour—treatment which if prolonged to 30 hours caused death—showed that increased phosphate concentration stimulated respiration although preceded by a very brief depression. He ascribed this effect to the PO_4 ion and related the effect to the "p PO_4" (**11**). BOSWELL's data on the potato are different but difficult to interpret. The cation content of the buffer solutions was not specified, and during 3 hours the effect of increased phosphate concentration at pH 5.5 was to decrease respiration. Whenever a preliminary decrease in respiration of potato discs, due to phosphates of sodium or potassium, has been observed in our investigation it has always been succeeded by a prolonged period in which phosphate increased respiration. The general conclusion that phosphates decrease the respiration of potato discs should not be drawn from BOSWELL's data. LYON (**11**) sees in his well-known observation that inorganic phosphate will stimulate the activity of potato oxidase preparations (aqueous extracts of pulp) so that—*in presence of phosphates only*—they can oxidize sugar, evidence that the mechanism of the phosphate effect on aerobic respiration is a catalytic effect on the oxidases. Recognizing that LYON's tissue pulp extracts may have also contained nitrogen compounds, and broadening the usual range of oxidase substrates to include their secondary effects on amino-acids, such an explanation would be consistent with the standpoint of this paper.

NITROGEN METABOLISM.—Using the initial and final tissue from the experiments of tables VII and VIII, the changes which occurred in the protein nitrogen, soluble nitrogen, and the various components of the soluble nitrogen were determined by the methods which have been described. The data in absolute units are to be found in tables IX and X. The recov-

TABLE IX

EFFECT OF CONCENTRATION OF POTASSIUM PHOSPHATE BUFFER SOLUTION AT PH 6.9 ON THE NITROGEN FRACTIONS OF POTATO DISCS AT 23° C. DURING 72 HOURS*

SAMPLE	CONCENTRATION POTASSIUM PHOSPHATE IN EXTERNAL SOLUTION	TOTAL N PER GRAM	PROTEIN N PER GRAM	PROTEIN N	SOLUBLE N	AMINO N	PERCENTAGE AMIDE N		AMMONIA† N
							STABLE	UNSTABLE	
	M	*mg.*	*mg.*	%	%	%	%	%	
Initial tissue	.....	2.02	0.59	29.2	70.8	58.4	9.4	6.4	.....
Final	0.0	2.02	0.90	44.6	55.4	44.6	5.4	7.4	.....
"	0.002	2.09	0.93	44.6	55.4	46.7	6.2	1.0	.....
"	0.010	2.05	0.99	48.3	51.7	42.9	6.8	0.5	.....
"	0.025	2.03	1.20	59.2	40.8	34.5	6.4	0.5	.....
"	0.040	2.03	1.08	53.2	46.8	38.4	7.4	0.2	.....

* Absolute units = mg. N per gm. initial fresh weight. Results on percentage basis are relative to total nitrogen.

† Negligible, order of 0.001 mg. per gm. fresh wt. No effect of conc.

TABLE X

EFFECT OF pH IN POTASSIUM PHOSPHATE BUFFERED SOLUTIONS (0.020 M PHOSPHATE) ON THE NITROGEN FRACTIONS OF POTATO DISCS AT 23° C. DURING 72 HOURS*

SAMPLE	pH	TOTAL N PER GRAM	PROTEIN N PER GRAM	PROTEIN N	SOLUBLE N	AMINO N	AMIDE N		AMMONIA† N
							STABLE	UNSTABLE	
		mg.	*mg.*	%	%	%	%	%	
Initial tissue	.....	2.02	0.59	29.2	70.8	57.4	8.9	6.44	.....
Final	5.96	2.02	0.68	33.6	66.4	53.5	8.4	5.45	.....
"	6.48	2.00	0.83	41.5	58.5	50.0	6.5	0.55	.....
"	6.95	2.03	1.14	56.2	43.8	36.9	6.4	0.59	.....
"	7.46	2.00	0.78	39.0	61.0	54.0	8.0	0.50	.....
"	8.04	2.01	0.60	29.9	70.1	51.8	8.0	4.98	.....

* Absolute units = mg. N per gm. initial fresh weight. Results on a percentage basis are relative to total nitrogen.

† Negligible, order of 0.001 mg. per gm. fresh wt. No effect of pH.

ery of the initial total nitrogen of the tissue was quantitative and the changes which occurred are shown relative to the total nitrogen in figure 8. In this figure, protein nitrogen is represented by histograms below the line and the absolute nitrogen fractions by histograms above the line. In analyzing tissue from phosphate cultures it is essential to use the trichloracetic acid method for the determination of protein nitrogen. The alcohol method gives anomalous results since some protein is soluble in hot alcohol in the phosphate treated cultures.

The outstanding fact is that the salt or pH treatment caused respiration wherever it induced greater protein synthesis. This is shown clearly by a comparison of the histograms which represent the respiration rate (relative to standard treatments to which the value 100 is assigned) and those which represent the protein nitrogen content of the tissue (fig. 8). This is yet another, though very striking, example of the parallelism which exists between protein synthesis and respiration in potato discs. It has more than usual interest since it suggests that *even in its response to phosphate, the respiration of potato discs is modified through that same respiratory component which is linked with nitrogen metabolism.* This component is not controlled by sugar concentration, and is responsible for those other effects of inorganic salts on the respiration of potato discs that have been described in earlier papers.

The full implications of an investigation by RICHARDS (**14**) cannot be discussed here. It should be noted even though it treats of a problem apparently somewhat remote from the present one, since it correlates phosphorus deficiency during the growth of barley seedlings with low protein content and low respiration.

An explanation of the phosphate response without recourse to the hexose

phosphates and their reactions may occasion some surprise. At this stage finality is clearly impossible but the implication is that even potassium phosphates act upon metabolic processes which, as yet, appear to be somewhat remote from the metabolism of glucose via hexose phosphates. It is true that out of the increasing knowledge of the specific enzymes which

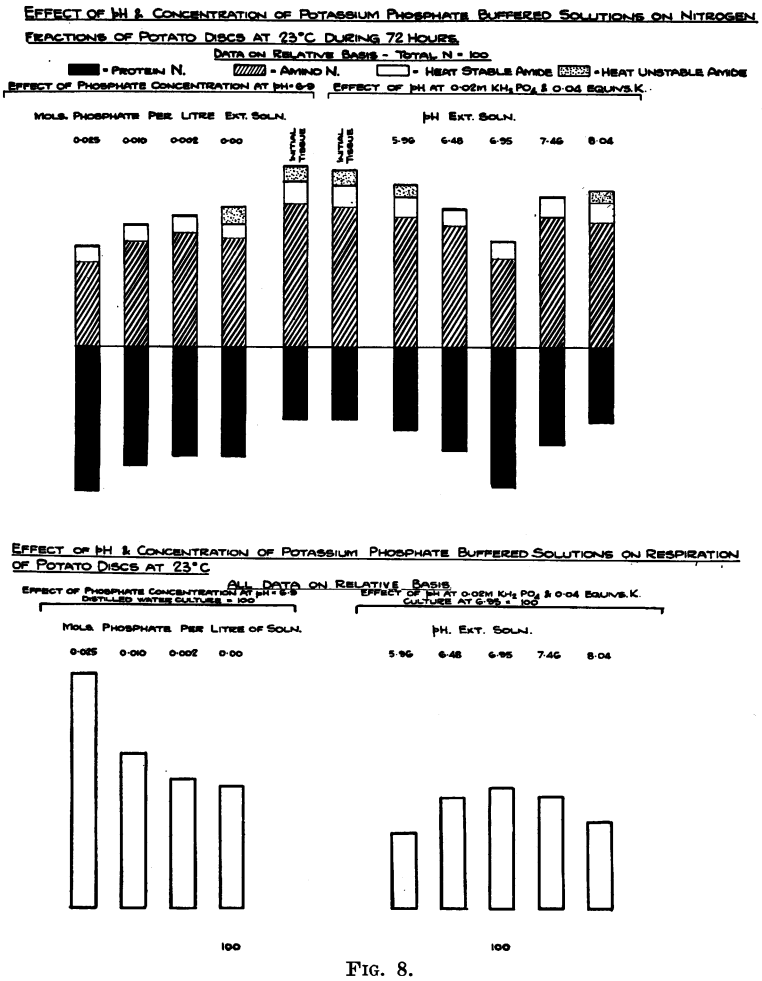

FIG. 8.

catalyze the reactions of the hexose phosphates an explanation of the effect of pH on respiration may be forthcoming. A notable achievement is that of HANES (**4, 5**) who has isolated a phosphorylase from crude potato sap which will reversibly convert starch to glucose-l-phosphate. This enzyme has a pH optimum at 6.4, but the enzyme machinery (phosphoglucose conversion system of HANES) which converts this substance to hexosediphos-

phate and thence catalyses the splitting of the carbon chain, has apparently a pH optimum at a reaction more alkaline than pH 7.0. It is, therefore, not inconceivable that processes dependent upon the consecutive action of such enzymes might appear to be favored by a reaction of 7.0. The possible connection of the phosphorylation of sugar with deamination of amino acids and protein synthesis is remote. It still seems, however, that phosphates and pH must exert some direct effect upon nitrogen metabolism in potato discs apart from the effects exerted upon the main line of carbohydrate breakdown by the route which the latter is commonly believed to take.

In the presence of phosphate, as of other salts, the bulk of the nitrogen used in protein synthesis was drawn from the amino-nitrogen fraction; it will be seen from figure 8 that the changes in amino nitrogen are closely parallel to the increase in protein nitrogen. An outstanding feature is, however, the effect of phosphate upon that part of the total soluble nitrogen which has been designated "heat unstable amide." In the initial tissue this fraction usually comprises about one-third of the soluble nitrogen other than the true amino nitrogen. The stable amide usually decreases, but the unstable amide fraction usually increases when the tissue metabolizes in aerated distilled water (table IX, fig. 8). In all phosphate solutions with reactions between pH 6.5 and 7.5 the content of "unstable amide" in the tissue was reduced to a low level.

Comparing the tissue which was subjected to distilled water and to 0.025 M phosphate (table IX, fig. 8), it is clear that of the nitrogen for the *extra* synthesis of protein which was stimulated by the salt, approximately 70 per cent. was derived ultimately from *amino nitrogen.* The remainder, however, is accounted for by the utilization of the unstable amide fraction; this was so stimulated by phosphate that, although normally increasing in the aerated discs, it was used more rapidly than it was produced and thus the amount present in the tissue decreased. It will be recalled that the unstable amide fraction (**21, 22**) has previously been regarded as a possible reactive intermediary between amino acid and protein and this further evidence that the reserves of this substance are depleted by yet another salt treatment which stimulates synthesis and respiration is suggestive in this connection. Conversely, at those reactions (pH 6.0 to 8.0), protein synthesis and respiration were both depressed, and the stimulating effects of phosphate were no longer evident because the inherent capacity of the tissue tended to maintain its initial store of unstable amide even in contact with phosphate.

The effects of pH on protein synthesis in phosphate and bicarbonate buffered solutions can be compared by reference to figures 6 and 9. The contrast in the specific effects due to phosphate and bicarbonate concentration at constant pH is evident; and also the fact that, *under each buffer*

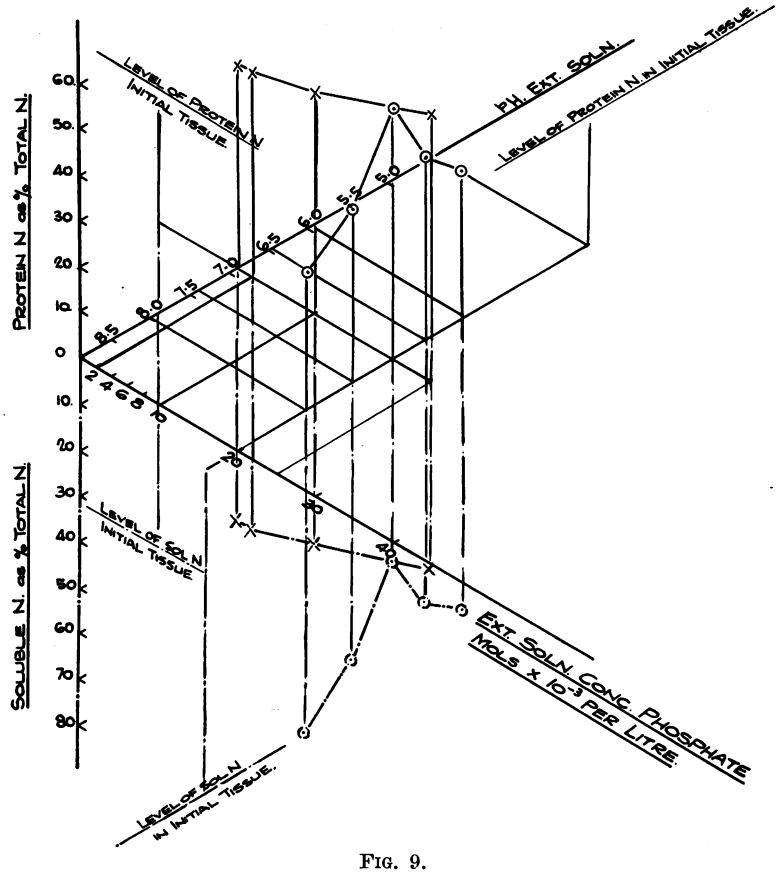

FIG. 9.

system, when the salt and other treatments were conducive to active protein synthesis, this reached a maximum at pH 7.0 and decreased to zero at more acid and more alkaline reactions. This effect can be attributed only to the direct effect of H^+ and OH^- upon the mechanism of protein synthesis. The obvious similarity between figure 7, which depicts the effect of pH and phosphate concentration on respiration, and figure 9 which shows the effect of similar treatments on protein synthesis, needs no further comment.

Figures 6 and 9 are reminiscent of the effect of pH upon the enzymatic synthesis of protein in concentrated protein hydrolysates. It was shown by WASTENAYS and BORSOOK (**26**) that the synthesis reached a maximum at a

pH of 4.0 in the case of egg albumin and the curve of synthesis as affected by pH was symmetrical about this reaction. They considered the possibility that this was due to the dissociation of an amphoteric component of the system although no component had an appropriate iso-electric point. The protein synthesis pH curves for potato discs are of a similar type. It is unlikely that the isoelectric properties of any amino acid present in potato determines the pH optimum at 7.0 and this appears to be attributable to some effect of the properties of water on the synthesis.

Salient features of the effect of pH, bicarbonate, dissolved carbon dioxide, and phosphates on the behavior of potato tissue

At pH 6.9, increased concentration of potassium phosphate increased both the respiration rate and protein synthesis of potato discs. In this respect phosphates behaved in a manner similar to potassium salts, in their reactions with other anions (Cl, Br, NO_3). It is thus clear that the stimulating agent is the potassium ion although its effect may be modified by the accompanying anion. Despite the prevalent idea that phosphates play an essential rôle in respiration, the stimulating effect of phosphates on the respiration of potato discs was not greater than that due to equivalent concentrations of other potassium salts. There is strong reason for the belief that the extra respiration stimulated by potassium phosphate was primarily due to that same component of respiration which is stimulated by the potassium ion as affected by other potassium salts.

At constant potassium and phosphate concentration both respiration and protein synthesis were at a maximum at an external reaction close to strict neutrality; at reactions somewhat more alkaline than pH 8.0 and more acid than pH 5.9 protein synthesis vanished. It is noteworthy that in potato discs the maximum protein synthesis does not occur in solutions at the same external reaction as that at which the extracted tissue proteins are iso-electric, *e.g.*, pH 4.4. [See Pearsall and Ewing (**13**) as suggested by Pearsall and Priestley (**12**) on theoretical grounds only.]

Potassium bicarbonate solutions in the presence of free carbonic acid and at pH 7.0 have a quite different effect upon metabolism since they depress protein synthesis and oxidase activity progressively as the salt concentration is increased. This is the *only case yet encountered in these investigations in which an increased concentration of a potassium salt decreased the respiration and metabolism of potato discs. It is evident that this effect is due specifically to* HCO_3^- *and* H_2CO_3. Although the available data are limited, it is clear that the metabolism of carbohydrate and respiration were also depressed by these salt treatments; the trend of the effect of bicarbonates and pH upon these processes should be similar to that found, and more fully investigated, for protein synthesis.

Increased concentrations of potassium bicarbonate and free acid at constant pH depress, and eventually suppress altogether, *both protein synthesis and also the accumulation of the bromide ion.* These facts again imply that the processes of *respiration, protein synthesis, and bromide uptake in potato cells are all closely linked together.* The data also show, however, that potassium was absorbed even from bicarbonate solutions which suppressed protein synthesis and bromide uptake. The presumption is that this was accompanied into the tissue by bicarbonate, which then reacted with the organic acids of the tissue since the bicarbonate concentration of the sap did not increase. Such absorption of potassium seems not to be as essentially related to growth as the simultaneous uptake of potassium and bromide. Treatment with solutions of bicarbonate and free carbonic acid affects the potassium previously stored in the potato cells in a manner which suggests that they *retain their salts most effectively under conditions which are optimum for protein synthesis.* All the evidence, therefore, reinforces the view that protein metabolism plays an essential rôle in the production and maintenance of ionic accumulation gradients.

The depressing effect of bicarbonates upon metabolism and bromide uptake cannot be ascribed wholly to the combined carbonic acid or to the free acid but both play a part. It seems that the bicarbonate ion present is more potent than the undissociated free acid. Three-dimensional models portray the inter-related effects of pH, bromide absorbed in the sap, protein synthesis, and potassium bicarbonate (total carbonic acid concentration in the solution). These show that the influence of added bicarbonate and free carbonic acid on bromide uptake or protein synthesis reaches a maximum at pH 7.0. Similarly, protein synthesis is, at any given salt ($KHCO_3$) concentration, at a maximum at pH 7.0 and declines to zero at reactions as alkaline as pH 8.7 and as acid as pH 5.3.

The almost identical effect on protein synthesis of solutions of different pH but constant salt concentration which were obtained with the different buffer systems employed, although the constituent salts (bicarbonate and phosphate) have distinct and opposed effects on metabolism, is evidence that the effect of pH at constant potassium concentration which is in question *is mainly due to the H and OH ions* and not to other ions or molecules in the buffer systems concerned. The importance of the reaction pH 7.0, at which protein synthesis is at a maximum and the depressing effect of added bicarbonate a minimum, is an indication that the process is limited in some way by the properties of water; the physico-chemical properties of plant amino-acids and proteins (iso-electric points) do not show maxima or minima at a pH of 7.0.

The effects of both phosphate and bicarbonate upon the nitrogen metabolism of the discs ultimately become apparent upon the utilization of the

amino-acid reserves. In both cases, the color reactions which the treatments induce, suggest that the activity of the phenolase in the tissue is a potent part of the mechanism of the salt effects. In both cases, however, there is evidence which suggests that the effect of the salts extends beyond the possible rôle of the phenolase in the deamination of amino compounds. The unstable, amide-like substance, to which previous reference has been made, is so affected by phosphate and bicarbonate treatments that a relative increase in protein synthesis caused by the salt results in a relative decrease of unstable amide and vice versa. On the highly probable assumption that the substance in question is formed from sugar and the products of deamination of amino acids and is an intermediate in protein synthesis, it becomes clear that *the effects of phosphate and bicarbonate extend to the later stages of the conversion of such intermediates to protein and are not confined to the deamination mechanism alone.* Of course, phenolase may also be involved in these stages.

Despite the fact that metabolic processes (respiration and protein synthesis), which are clearly connected with the ability of the tissue to absorb salts, are favored by a neutral reaction of pH 7.0 there is, nevertheless, a wide range of pH (6 to 8) within which the bromide uptake is not conspicuously affected by pH.

The investigation emphasizes again the ramifications of the biochemical effects due to ions in the external solution which extend to all phases of the metabolism of the tissue investigated and which are seen to be characteristic of all the ionic species that have been considered in this and preceding papers.

Summary

1. The effect of pH and the concentration of a $KHCO_3/H_2CO_3$ buffer on the absorption of bromide and the metabolism of potato discs has been investigated using thin discs in aerated solutions at 23° C.

2. Throughout, the effects of these treatments on the uptake of bromide, on protein synthesis, and on oxidations in the tissue catalyzed by oxidases (phenolase) are closely parallel.

3. At constant pH, increasing the external concentration of $KHCO_3$ and dissolved carbon dioxide depresses (and eventually suppresses completely) both protein synthesis and bromide accumulation. To this effect both combined and free carbonic acid contribute; at pH 7.0, their relative effects are about equal. Of HCO_3^- and undissociated H_2CO_3, the former appears to have the greater effect on the tissue.

4. There is a relatively broad pH range, in the absence of bicarbonate, within which bromide uptake is not much affected by $[H^+]$ and its attendant variables. In the presence of bicarbonate, protein synthesis (though depressed by the salt) is at its maximum at pH 7.0 and is less active in a more

acid or alkaline solution. These effects are specifically due to H^+ and OH^- and find a parallel in bromide uptake which is depressed by bicarbonate solutions and is more favored under these conditions by a pH of 7.0 than by reactions more acid or more alkaline.

5. The effect of HCO_3^- and H_2CO_3 on nitrogen metabolism is also shown by their effect on the utilization of amino acids but it extends to subsequent reactions; *e.g.,* reactions in which unstable amides are involved as intermediaries of protein synthesis.

6. The effects of pH and the concentration of a bicarbonate buffer on the behavior of the tissue (bromide uptake, nitrogen metabolism, etc.) can be represented by 3-dimensional figures. Comparison of these figures shows that bromide uptake and protein synthesis from soluble nitrogen reserves, are similarly affected by these variables.

7. The evidence shows again that in neutral solution (strictly pH 6.9) K^+ and Br^- are equally absorbed by potato discs. At more acid reactions (by CO_2), conditions which are also less favorable for protein synthesis, the tissue loses potassium. The best conditions for protein synthesis are conducive to the maintenance of existing concentrations in the cell sap.

Tissue, however, in which neither bromide uptake nor protein synthesis occurred, absorbed potassium from relatively strong bicarbonate solutions. This special case of potassium absorption (accompanied by HCO_3^- which apparently reacts with the organic acids of the cell sap) is less dependent on the processes of growth than the case in which a cation and a nonreactive anion are absorbed together.

8. Indirect evidence shows that the bicarbonate solutions which retard bromide absorption and protein synthesis also depress respiration and the metabolism of carbohydrate.

The mechanism of the effect of HCO_3^- and H_2CO_3 on metabolism is briefly discussed.

9. The effect of pH and phosphate concentration on respiration and nitrogen metabolism has been investigated using aerated phosphate buffers of constant potassium content.

At pH 7.0, increased concentration of a phosphate buffer increases both respiration and protein synthesis in potato discs.

At constant phosphate concentration *both* respiration and protein synthesis are at a maximum at pH 7.0 and decrease in solutions more acid and more alkaline.

The similarity in the effects of these variables on both respiration and nitrogen metabolism is seen by comparing figures which show simultaneously the variation of the property in question (respiration, protein synthesis, or loss of soluble nitrogen) in relation to two variables (pH and phosphate concentration).

10. Phosphate treatments which stimulate respiration deplete the tissue of the unstable amide, which is normally present in discs of potato in aerated solution and which appears to be a reactive intermediary in protein synthesis.

11. Despite the different specific effects attributable to the phosphate and bicarbonate the parallelism between protein synthesis and respiration is consistent throughout. Although phosphates increase and bicarbonates decrease respiration the effect of pH is similar in both buffer systems. These effects must be ascribed specifically to H^+ or OH^- and, if not due to specific enzymes with definite pH optima, their symmetry about pH 7.0 suggests that they are determined by properties of water and not of the ampholytes (amino acids, proteins) concerned.

Brief reference is made to the general problem of the effect of pH on respiration and to discrepancies in the literature.

12. Phosphates seem to influence metabolism of potato discs through their effect on nitrogen metabolism (protein synthesis and use of amino acids); *i.e.*, processes which appear remote from the accepted rôle of hexose phosphates in carbohydrate breakdown.

13. Respiration, protein synthesis, and bromide absorption are again seen to be closely linked. They are similarly affected by external variables, the effect of which on the tissue is consistently shown by reactions stimulated by the oxidase (phenolase) it contains.

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