THE EFFECT OF SALT CONCENTRATION UPON THE METABOLISM OF POTATO DISCS AND THE CONTRASTED EFFECT OF POTASSIUM AND CALCIUM SALTS WHICH HAVE A COMMON ION[1]

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(WITH THREE FIGURES)

Introduction

The metabolic processes which have been observed in potato discs under the conditions conducive to salt accumulation have been described (**20**) and a balance sheet of metabolites prepared (**21**). This work revealed certain relationships between the nature of the salt supplied and its effects upon the respiration and nitrogen metabolism of the cells. These results gave a new outlook upon the problem of salt absorption and, therefore, the relationship of the metabolism of potato discs to salts was intensively and systematically investigated.

The purpose of the further investigation was to identify the specific effects on metabolism of the most important inorganic anions and cations and, therefore, to provide the essential biochemical background for a fuller understanding of salt absorption. Of necessity the work was confined to cells derived from one plant and organ—the potato tuber. Broad generalizations must ultimately seek their confirmation in the behavior of other cells and tissues. No apology need be made, however, for this first restricted attention to one type of cell which was investigated under rigorously controlled conditions.

The scope of the entire work may be indicated because it demands that the results shall be described in three parts—subsections which yet represent integral parts of the whole problem.

1. The first part deals with those effects on metabolism which are caused by neutral salts. The experiments described show the effects of the concentration of different potassium and calcium salts in the external solution and the effects due to the various anions and cations, respectively, stand revealed when experiments which involved the use of bromides, chlorides, nitrates, and sulphates of potassium and calcium are compared. Wherever possible concurrent experiments employed salts with a common anion and the data not only reveal the principal effects attributable to each ion but also the interaction of the effects of anions and cations.

2. The second part deals with the more difficult problems raised by those salts which not only affect the external reaction of the solution, but which

[1] This is the third of a series of papers on the biochemistry of salt absorption by plants. The authors are indebted to Prof. D. R. HOAGLAND for proof-reading this paper.

should also have more direct effects on metabolism. The effects of phosphates and bicarbonates on the tissue are of this kind. In such cases the influence of salt concentration alone must be determined at constant pH; but at constant salt concentration (phosphate or bicarbonate) the effects attributable to the ions H and OH must also be investigated.

3. Yet a third aspect of the problem is presented by the important comparison of the effects which are due to ammonium and to nitrate. Of necessity this comparison was investigated at constant pH and to this extent involved the knowledge derived from section (2) since the solutions were appropriately buffered.

In this paper attention will be confined to those problems which fall within the scope of the section (1) referred to above.

Experimental procedure

To compare the effects due to potassium and calcium, each experiment consisted of two series, each of four cultures. In one series the external solution contained a potassium salt; in the other, the corresponding calcium salt was used. In this manner experiments were conducted with bromides, chlorides, and nitrates. When sulphates were involved the procedure was necessarily confined to the potassium salt.

All variables other than the nature and concentration of the external solution were standardized at values[2] fixed for all these experiments and maintained constant by the methods which have been described (**16**). The tissue was cut from a uniform stock of tubers (variety King Edward) and only in the case of the sulphate series was another stock used. Within one series the duration of each treatment was the same and with one exception (calcium bromide series) the duration was so nearly constant throughout all of the experiments that the effects of the salt treatments may be seen by the absolute amounts of metabolism (rather than rates) which occurred. The better procedure, however, is to compare the effects due to salt concentration within one series by reference to one culture as a standard; in this way the small differences caused by the duration of treatment and the slight change in the stock of tubers during their storage, tend to disappear.

In each series the four different salt concentrations (nos. 1, 2, 3, 4) were chosen as follows: 0.00075; 0.015; 0.050; and 0.075 gm. equivalents per liter. This choice was made upon the following considerations. The greatest concentration (no. 4) was the maximum in which the tissue, after three days contact, was apparently free from irreversible injury to the cells. The lowest concentration (no. 1) seemed so low that it was anticipated that the specific

[2] Fifty standard discs; approximately 40 gm. in two liters of solution, at 23° C., aerated by a stream of washed air at 15 liters per hour, stirred by standard stirrers at 100 r.p.m.

effects of the salts might be negligible and the metabolic behavior of the tissue was therefore not significantly different from that in distilled water. Even at 0.00075 equivalents per liter very small differences between the tissue in potassium and calcium salts were encountered. To minimize the number of replicate cultures, controls in distilled water were abandoned. An adequate estimate of the probable behavior of the tissue in distilled water can be obtained, if required, from the mean of the results obtained in 0.00075 equivalents potassium and calcium salt. The differences between the dilute potassium and calcium salt are usually very small; thus when the effects of salt concentration upon metabolism are to be expressed relative to the tissue in the dilute culture of each series the standard of reference is virtually tissue exposed to zero salt concentration.

The salt effects in question are not independent of the time and oxygen conditions to which arbitrary values were assigned in the experiments. In distilled water, potato discs eventually attain a plateau of respiration in which they do not suffer from oxygen lack when in solutions in equilibrium with air at 23° C. (**21**). It is this factor (other than oxygen supply) limiting the respiration of the discs, which is modified by salts—its limiting effect being reduced by potassium chloride and accentuated by calcium chloride. Clearly the maximum response to salts presupposes that the tissue is not suffering from lack of oxygen since specific salt effects disappear under extreme conditions of this type [see experiment at 3.80 per cent. (**21**)]; furthermore, the tissue has the requisite time to attain the maximum metabolic rate which the temperature, oxygen, and salt conditions permit. These requirements were fulfilled by the use of air as an aerating gas and experiments with a duration of approximately 60 hours.

The methods used for the analysis of the tissue have been described elsewhere (**21**) and these were applied to comparable samples of the initial, as well as the final, tissue.

Results

The effect of salt concentration on respiration

The effect of external salt concentration on respiration is shown by the calculated *relative respiration* figures which were obtained by assigning the arbitrary value 100 to the respiration rate of the tissue in the most dilute culture (no. 1) of the series (table I) and then calculating the remaining members of that series (nos. 2, 3, 4) in terms of this standard. Table I shows the *absolute* differences in the respiration rates of the tissue cut from the uniform stock of tubers and exposed to the various dilute salt solutions. These comparatively small differences were caused by the combined effects of the very dilute salt treatment, the slight drift in the composition of the tubers during about six weeks of storage, and the slight variations in the total length of the

TABLE I

RESPIRATION RATES OF POTATO DISCS IN 0.00075 EQUIVALENT SALT SOLUTION

SERIES	TOTAL HOURS	RESPIRATION RATE*	SERIES	TOTAL HOURS	RESPIRATION RATE	SERIES	TOTAL HOURS	RESPIRATION RATE
KBr	63.5	0.143	KCl	52.0	0.166	KNO_3	51.4	0.159
$CaBr_2$	69.5	0.130	$CaCl_2$	53.0	0.158	$Ca(NO_3)_2$	53.2	0.160

* Total mg. CO_2 ÷ total hours × initial fresh weight in grams.

different experiments. The calculated relative respiration figures, indicating the effects of *salt concentration* unobscured by factors which affect the absolute rates of the different series, are given in table II and their relation to external salt concentration is shown graphically in figure 1.

Clearly there are two conclusions to be drawn from table II and figure 1, which concern the effect of the cations and the anions respectively. The contrast between the potassium and calcium salts noted previously (**20, 21**) for chlorides at one concentration, is consistent throughout the entire range of salts and concentrations. Since the effect (potassium salts stimulate and calcium salts depress respiration) appears when there is a common anion it must be caused by specific properties of the cations.[3] The quantitative effect produced by the cations and therefore the contrast between potassium and calcium salts with a common anion is affected, however, by the nature of the anions. These anions apparently do not have a direct influence upon respiration but they operate inasmuch as they accentuate the effects which are clearly caused by the cations. The relative order in which the anions accentuate the effect of the cations is clearly $SO_4 < Br < Cl < NO_3$. This is shown by the "spread" between the curves of the comparable potassium and calcium series in figure 1 and, for the concentration no. 4, by the difference between the corresponding potassium and calcium cultures in table II. In the order $SO_4 < Br < Cl < NO_3$ will be recognized the relative order of the anions with respect to certain physical properties but, more particularly, their relative effect upon the absorption of a common cation[4]—a fact which constitutes strong evidence that the observed differences are not merely due to the presence of the salts in the external solution but that they also involve their absorption.[5] In order to explain how potassium and calcium salts affect

[3] It is possible that the contrasted effects of K and Ca salts may be associated with the fact that in K salts equal uptake of cation and anion usually occurs in potato discs whereas in calcium salts excess uptake of anion is to be expected.

[4] Results privately communicated.

[5] A similar conclusion follows from the fact that the specific salt effects disappear at low oxygen tension. That is, the salts do not exert their effect by mere presence in the solution.

TABLE II

EFFECT OF SALT CONCENTRATION ON RESPIRATION RATE OF POTATO DISCS

SOLUTION NUMBER	SALT* CONCENTRATION	EXPERIMENT 1	RELATIVE RESPIRATION RATE	EXPERIMENT 2	RELATIVE RESPIRATION RATE	EXPERIMENT 3	RELATIVE RESPIRATION RATE	EXPERIMENT 4	RELATIVE RESPIRATION RATE
1	0.00075	KBr	100.0	KCl	100.0	KNO_3	100.0	K_2SO_4	100.0
2	0.015	series	100.0	series	100.0	series	108.0	series	100.0
3	0.050		103.0		113.0		119.0		104.0
4	0.075		113.0		113.0		123.0		101.0
1	0.00075	$CaBr_2$	100.0	$CaCl_2$	100.0	$Ca(NO_3)_2$	100.0		.....
2	0.015	series	101.0	series	95.6	series	87.6		.....
3	0.050		98.5		77.3		79.4		.....
4	0.075		94.6		72.1		70.0		.....
4	0.075	Diff. KBr $-CaBr_2$	18.4	Diff. KCl $-CaCl_2$	40.9	Diff. KNO_3 $-Ca(NO_3)_2$	53.0		.....

* Gram equivalents per liter.

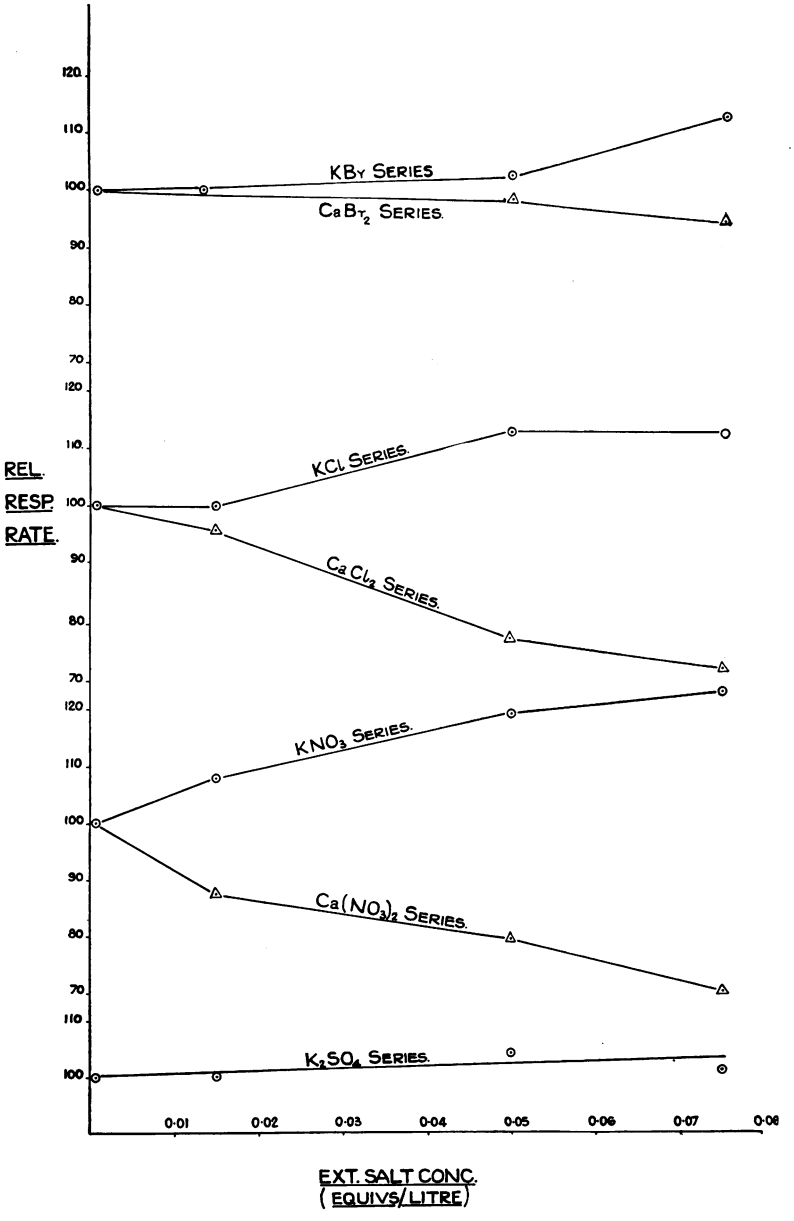

FIG. 1. The effect of salt concentration on the respiration of potato discs at 23° C.

respiration the behavior under these treatments of the principal metabolites of the tissue was investigated.

Effect of external salt concentration on carbohydrate metabolism

As for respiration and the carbohydrate fractions, the salt effects are best discerned when the data are expressed on a relative basis. The final sugar and starch contents are first expressed per gram. of initial fresh weight of tissue and then the values for the cultures nos. 2, 3, and 4 of each series expressed relative to that of no. 1, as standard, to which the value 100 is assigned. To show the absolute amounts of starch and sugar present in the initial tissue cut at various times from the uniform stock of tubers and in those final samples which served as standards, the data are given in table III; whereas, to show the effects of salt concentration, the data of table IV are calculated on the relative basis explained above.

There are two outstanding conclusions to be drawn from table IV:

Throughout all of the experiments (nos. 1, 2, 3) increased external concentration of the calcium salt caused a small but significant *increase* in starch hydrolysis. The effect of the concentration of the potassium salt was significant only in the case of the nitrate series and here the reverse effect was observed—increased external concentration of potassium salt retarded starch hydrolysis.

The final sugar content of the tissue is the resultant of the starch hydrolysis and the utilization of sugar in respiration and metabolism. The figures show that the residual sugar content of the tissue exposed to the greater potassium salt concentrations, in which greater respiration was observed (table II and figure 1), was *decreased* by the salt treatment. Conversely, in contact with calcium salts, the greater the concentration of the external salt solution the greater the residual sugar concentration and, it will be recalled, this was associated with a greatly *reduced* respiration rate.

Clearly total sugar concentration is *not* the factor which controls the respiration rate. Since it is improbable that the concentration of individual sugars would be affected in the converse manner, the cause of the effects of salts on respiration must be sought in the behavior of other metabolites.

Effects of external salt concentration on protein synthesis

The essential soluble and protein nitrogen data are presented in tables V and VI in a manner similar to that used for carbohydrates. In table V, the data for the initial tissue and those final samples which in each series served as standards, are given in absolute units. The effect of salt concentration on the balance between protein and soluble nitrogen is shown in table VI in which the data are calculated on the relative basis previously explained.

TABLE III

STARCH AND SUGAR CONTENT OF POTATO DISCS BEFORE AND AFTER TREATMENT IN AERATED SALT SOLUTIONS AT 0.00075 EQUIVALENTS PER LITER

TREATMENT	EXPERIMENT 1*	STARCH PER GRAM†	SUGAR PER GRAM†	EXPERIMENT 2	STARCH PER GRAM	SUGAR PER GRAM	EXPERIMENT 3	STARCH PER GRAM	SUGAR PER GRAM	EXPERIMENT 4	STARCH PER GRAM	SUGAR PER GRAM
		mg.	*mg.*		*mg.*	*mg.*		*mg.*	*mg.*		*mg.*	*mg.*
Initial tissue	KBr	89.6	3.53	KCl	90.8	2.99	KNO_3	87.5	3.15		.....	.....
Final tissue, salt conc. 1	series	70.1	14.05	series	67.6	8.20	series	60.0	7.80		.....	.....
Initial tissue	$CaBr_2$	84.4	2.74	$CaCl_2$	89.6	3.20	$Ca(NO_3)_2$	89.4	3.24	K_2SO_4	111.0	2.80
Final tissue, salt conc. 1	series	63.1	8.14	series	67.2	8.60	series	73.2	7.99	series	80.3	7.80

* In this experiment the two series had different duration: KBr, 63.5 hours; $CaBr_2$, 69.5 hours.
† Starch and sugar content per gram initial fresh weight.

TABLE IV

SOLUTION No.	SALT CONCENTRATION*	EXPERIMENT 1	RELATIVE STARCH CONTENT	RELATIVE SUGAR CONTENT	EXPERIMENT 2	RELATIVE STARCH CONTENT	RELATIVE SUGAR CONTENT	EXPERIMENT 3	RELATIVE STARCH CONTENT	RELATIVE SUGAR CONTENT	EXPERIMENT 4	RELATIVE STARCH CONTENT	RELATIVE SUGAR CONTENT
1	0.00075	KBr	100.0	100.0	KCl	100.0	100.0	KNO_3	100.0	100.0	K_2SO_4	100.0	100.0
2	0.015	series	101.0	74.6	series	94.5	101.0	series	107.0	88.5	series	99.0	94.8
3	0.050		101.0	46.2		98.0	53.1		107.0	80.8		103.0	92.4
4	0.075		102.0	41.4		99.0	54.1		112.0	77.2		101.0	89.8
1	0.00075	$CaBr_2$	100.0	100.0	$CaCl_2$	100.0	100.0	$Ca(NO_3)_2$	100.0	100.0		.....	.....
2	0.015	series	97.3	115.0	series	97.3	109.0	series	94.5	111.0		.....	.....
3	0.050		98.5	131.0		94.9	122.0		93.7	127.0		.....	.....
4	0.075		96.1	144.0		95.2	119.0		91.5	142.0		.....	.....

* Concentrations in gram equivalents per liter.

TABLE V

PROTEIN AND SOLUBLE NITROGEN CONTENT OF POTATO DISCS BEFORE AND AFTER TREATMENT IN AERATED SALT SOLUTIONS AT 0.00075 EQUIVALENTS PER LITER

TREATMENT	EXPERIMENT 1*	PROTEIN N PER GRAM†	SOLUBLE N PER GRAM	EXPERIMENT 2	PROTEIN N PER GRAM	SOLUBLE N PER GRAM	EXPERIMENT 3	PROTEIN N PER GRAM	SOLUBLE N PER GRAM	EXPERIMENT 4	PROTEIN N PER GRAM	SOLUBLE N PER GRAM
		mg.	*mg.*		*mg.*	*mg.*		*mg.*	*mg.*		*mg.*	*mg.*
Initial tissue	KBr series	0.77	1.26	KCl series	0.68	1.38	KNO_3 series	0.72	1.33	K_2SO_4 series	0.78	1.10
Final tissue, salt conc. 1		1.05	0.97		0.92	1.15		1.02	1.31		0.95	0.93
Initial tissue	$CaBr_2$ series	0.74	1.32	$CaCl_2$ series	0.68	1.37	$Ca(NO_3)_2$ series	0.72	1.36		.....	.....
Final tissue, salt conc. 1		1.09	0.93		0.86	1.19		0.90	1.26		.....	.....

* In this experiment the two series had different duration: KBr, 63.5 hours; $CaBr_2$, 69.5 hours.
† All N data per gram of initial fresh weight.

TABLE VI

EFFECT OF EXTERNAL SALT CONCENTRATION ON PROTEIN AND SOLUBLE NITROGEN CONTENT OF POTATO DISCS

SOLUTION NO.	SALT CONCENTRATION	EXPERIMENT 1	RELATIVE PROTEIN NITROGEN CONTENT	RELATIVE SOLUBLE NITROGEN CONTENT	EXPERIMENT 2	RELATIVE PROTEIN NITROGEN CONTENT	RELATIVE SOLUBLE NITROGEN CONTENT	EXPERIMENT 3	RELATIVE PROTEIN NITROGEN CONTENT	RELATIVE SOLUBLE NITROGEN CONTENT	EXPERIMENT 4	RELATIVE PROTEIN NITROGEN CONTENT	RELATIVE SOLUBLE NITROGEN CONTENT
1	0.00075	KBr series	100.0	100.0	KCl series	100.0	100.0	KNO_3 series	100.0	100.0	K_2SO_4 series	100.0	100.0
2	0.015		129.0	69.1		124.0	81.7		155.0	100.0		104.0	93.5
3	0.050		132.0	69.1		126.0	75.6		167.0	113.0		113.0	92.5
4	0.075		135.0	67.0		132.0	73.9		165.0	115.0		113.0	86.0
1	0.00075	$CaBr_2$ series	100.0	100.0	$CaCl_2$ series	100.0	100.0	$Ca(NO_3)_2$ series	100.0	100.0		.....	.....
2	0.015		73.3	126.0		96.5	104.0		102.0	98.5		.....	.....
3	0.050		69.7	138.0		83.7	113.0		110.0	132.0		.....	.....
4	0.075		60.5	148.0		80.3	115.0		113.0	127.0		.....	.....

TABLE VII

EFFECT OF EXTERNAL SALT CONCENTRATION ON THE SOLUBLE NITROGEN FRACTIONS OF POTATO DISCS*

SAMPLE	SOLUTION NO.	SALT CONCENTRATION	EXPERIMENT 1	TOTAL SOLUBLE NITROGEN	AMINO NITROGEN	HEAT STABLE AMIDE	HEAT LABILE AMIDE	SAMPLE	SOLUTION NO.	SALT CONCENTRATION	EXPERIMENT 2	TOTAL SOLUBLE NITROGEN	AMINO NITROGEN	HEAT STABLE AMIDE	HEAT LABILE AMIDE
				mg.	*mg.*	*mg.*	*mg.*					*mg.*	*mg.*	*mg.*	*mg.*
Initial	...	.....	KBr	1.26	1.02	0.194	0.150	Initial	...	.....	KCl	1.38	1.09	0.208	0.144
Final	1	0.00075	series	0.97	0.71	0.094	0.229	Final	1	0.00075	series	1.15	0.90	0.134	0.189
"	2	0.015		0.67	0.42	0.088	0.148	"	2	0.015		0.94	0.69	0.179	0.117
"	3	0.050		0.67	0.44	0.088	0.146	"	3	0.050		0.87	0.61	0.198	0.120
"	4	0.075		0.65	0.46	0.084	0.142	"	4	0.075		0.85	0.57	0.162	0.122
Initial	...	.....	$CaBr_2$	1.32	1.06	0.188	0.158	Initial	...	.....	$CaCl_2$	1.37	1.06	0.205	0.147
Final	1	0.00075	series	0.93	0.70	0.034	0.226	Final	1	0.00075	series	1.19	0.92	0.164	0.180
"	2	0.015		1.23	0.81	....	0.228	"	2	0.015		1.24	0.90	0.224	0.180
"	3	0.050		1.28	1.00	0.051	0.230	"	3	0.050		1.34	0.95	0.261	0.185
"	4	0.075		1.38	1.06	0.096	0.228	"	4	0.075		1.37	1.04	0.227	0.178

* All units in milligrams per gram initial weight.

The outstanding conclusion from these data is clear. All of the salt treatments (increasing concentration of external potassium salts) which increase respiration (table II, fig. 1) also increase the protein synthesis. This occurs, to some degree, at the expense of the soluble nitrogen reserves even in discs exposed to dilute salt solution or distilled water (**20**). Although time (not an absolutely constant factor throughout all of the experiments) is also involved, it is clear that the anion (NO_3) caused the greatest protein synthesis. It also accentuated the effect of potassium on respiration. Again the anion (SO_4) had but little effect on respiration; it also provided the least stimulus to protein synthesis when present as the potassium salt.

Conversely the salt treatments (increased concentration of calcium salts) which decrease respiration, also tend to suppress the synthesis of protein from the storage reserve of soluble nitrogen compounds. In the nitrate series this result apparently does not hold. Not until the nitrogen fractions are considered in greater detail can this anomaly be removed. It can be stated here, however, that the normal synthesis of protein in potato discs utilizes nitrogen mainly derived from amino compounds and *it is only when the nitrogen is derived from this source that the close parallelism between synthesis and respiration is obtained.* It has been indicated in an earlier work (**19**) that calcium tends to divert synthesis from the amino compounds to the other fractions of the soluble nitrogen. Furthermore, in the presence of nitrates, especially calcium nitrate, synthesis *may proceed directly from inorganic sources independent of nitrogen drawn from organic amino compounds.* The fact that the usual relation between synthesis and respiration apparently breaks down in the calcium nitrate series can therefore be explained.

Prior to the more detailed analysis of the nitrogen fractions, the interrelations of the effects of salt concentration on the processes thus far considered are summarized in figure 2 which is constructed from the data of tables II, IV, and VI. Respiration and sugar content are definitely affected in converse manner by salt concentration; compare the general trend, shown by the histograms, through the potassium series 4 to 1 and the calcium series 1 to 4. It is equally clear that the trend of protein synthesis (apart from the calcium nitrate series referred to above) is similar to that for respiration. In other words, figure 2 expresses the following in graphical form: *respiration and protein synthesis of potato discs are closely linked whereas there is no causal connection between respiration and sugar content* (as affected by external salt concentration).

Effects of salt concentration on the soluble nitrogen fractions

The detailed effects of salt concentration on the nitrogen metabolism of potato discs can be discerned from tables VII and VIII in which the data

are expressed in absolute units; and also from figure 3, in which the various fractions have been calculated relative to the initial total nitrogen content of the washed discs to which the value 100 was assigned for each experiment.

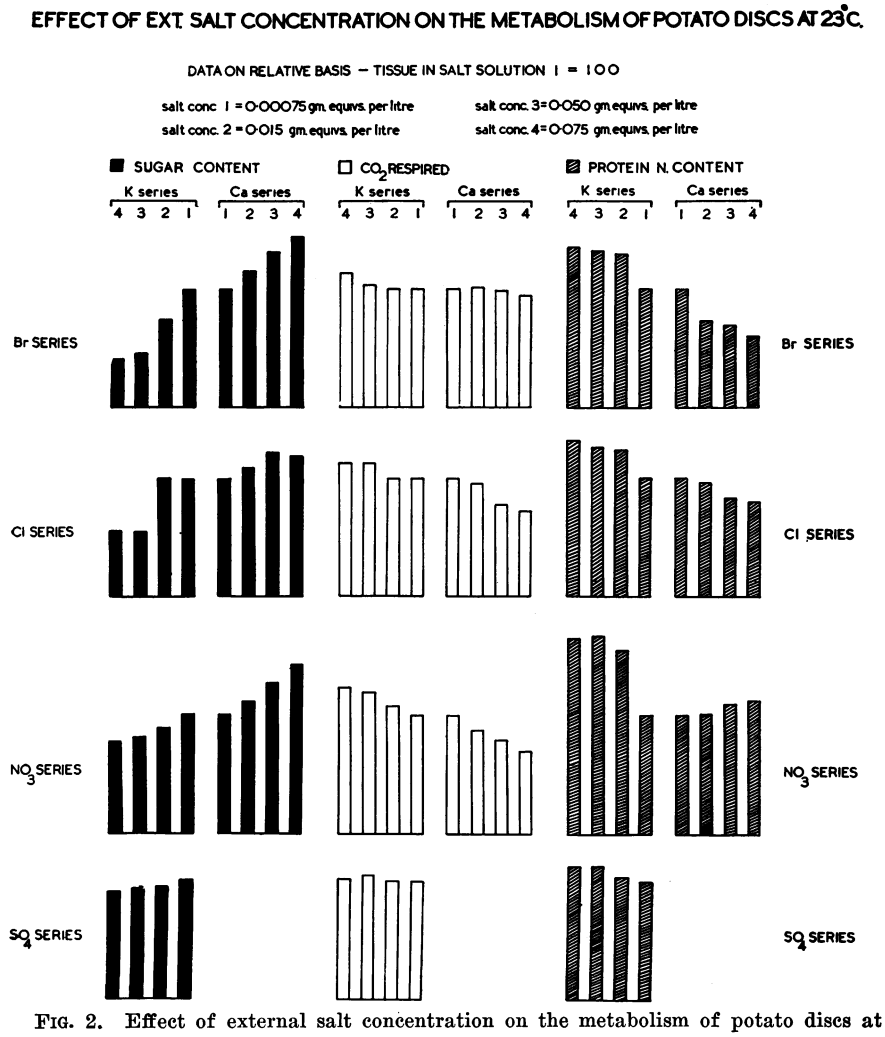

FIG. 2. Effect of external salt concentration on the metabolism of potato discs at 23° C.

From figure 3 the following conclusions, some of them already evident in the earlier data, can be verified.

(a). Protein synthesis (protein N represented by histograms below the line) occurs at the expense of the soluble nitrogen fractions (soluble N represented by histograms above the line) in potato discs exposed to dilute aerated salt solutions (see no. 1 of each series) at 23° C.

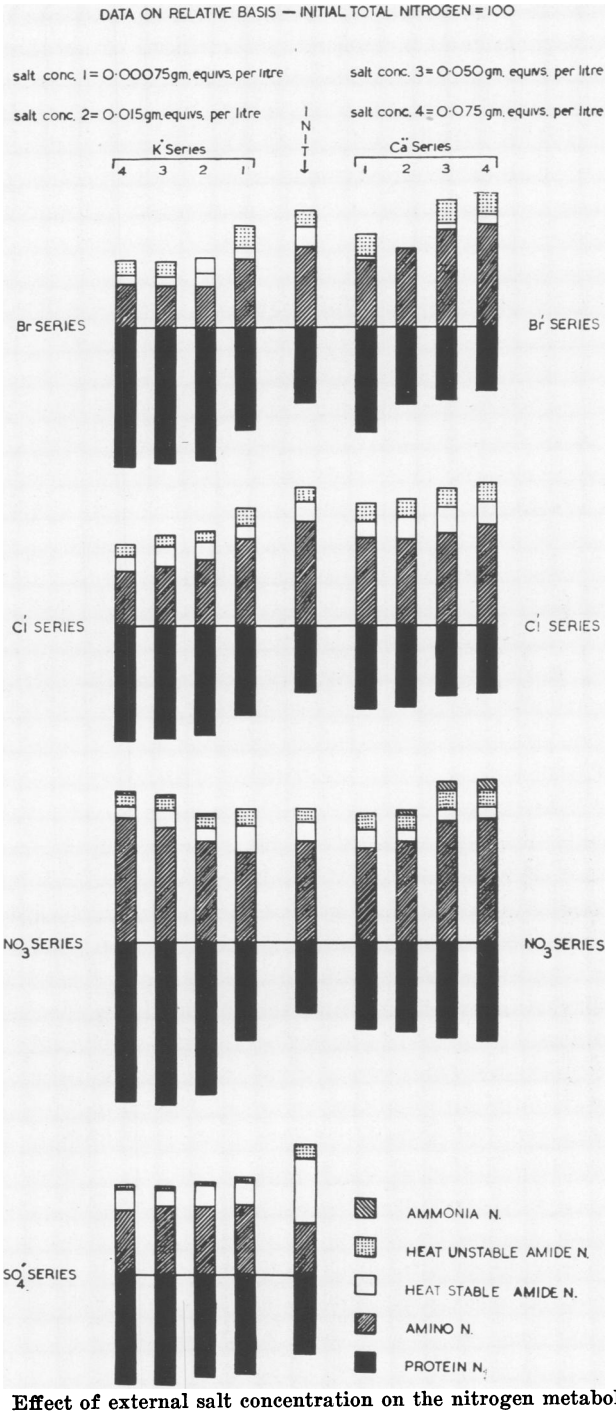

FIG. 3. Effect of external salt concentration on the nitrogen metabolism of potatoes at 23° C.

(b). In greater concentrations of potassium salts (chlorides and bromides) protein synthesis is increased. In solutions of sulphates the effect of potassium salt concentration on synthesis is slight.

(c). In solutions of nitrates the total nitrogen content is increased; more so in potassium than calcium salts of the same equivalent concentration. When nitrogen is absorbed from potassium nitrate, protein synthesis accounts for a large proportion of the *added* nitrogen (80 per cent. in solution no. 4) whereas in the calcium series the protein synthesized represents a much smaller proportion of the total nitrogen absorbed (55.6 per cent. in solution no. 4). In other words, although there is a greater total synthesis of protein in the stronger calcium nitrate than in the weaker solutions, *the effect of calcium as a depressant of synthesis is still apparent.*

TABLE VIII

EFFECTS OF EXTERNAL SALT CONCENTRATION ON THE SOLUBLE NITROGEN FRACTIONS OF POTATO DISCS*

SAMPLE	SOLUTION NO.	SALT CONCENTRATION	EXPERIMENT 3	TOTAL SOLUBLE N	AMINO N	HEAT STABLE AMIDE	HEAT LABILE AMIDE	AMMONIA N
				mg.	*mg.*	*mg.*	*mg.*	*mg.*
Initial	...	.....	KNO_3	1.33	1.03	0.192	0.150	0.002
Final	1	0.00075	series	1.31	0.93	0.243	0.166	0.002
"	2	0.015		1.31	1.05	0.126	0.144	0.021
"	3	0.050		1.48	1.19	0.175	0.151	0.037
"	4	0.075		1.50	1.29	0.119	0.149	0.041
Initial	...	.....	$Ca(NO_3)_2$	1.36	1.06	0.189	0.147	0.002
Final	1	0.00075	series	1.26	0.99	0.195	0.153	0.002
"	2	0.015		1.24	1.06	0.120	0.202	0.050
"	3	0.050		1.66	1.29	0.124	0.278	0.100
"	4	0.075		1.60	1.31	0.120	0.286	0.104
Initial	...	.....	K_2SO_4	1.10	0.51	0.605	0.141	.....
Final	1	0.00075	series	0.93	0.68	0.216	0.043	.....
"	2	0.015		0.87	0.66	0.199	0.033	.....
"	3	0.050		0.86	0.66	0.151	0.040	.....
"	4	0.075		0.80	0.62	0.201	0.041	.....

* All units in milligrams per gram initial weight.

(d). Amino nitrogen (amino acid + asparagine amino nitrogen) constitutes the largest soluble nitrogen fraction in all the tissue samples examined with the exception of the initial tissue used for the sulphate series. This contained much more amide nitrogen than the others.[6] Since this feature

[6] Some evidence obtained with potato tubers stored at low temperature (2° C.) shows that the relative accumulation of amide can be brought about by this treatment. In this extreme condition the high sugar concentration tends to produce a high rate of respiration which is different in its relations to nitrogen compounds from that observed in tissue drawn from normal tubers. The sulphate series showed a respiratory rate somewhat in excess of the normal value. There is every reason to believe that this tissue was drawn

disappeared under the experimental treatments it does not affect the comparison of the effects due to salt concentration *per se.*

(e). In the two bromide series the gain of protein in tissue exposed to the dilute solutions (no. 1) was almost balanced by the loss of amino-nitrogen (KBr loss of amino-N = 110 per cent. of gain of protein-N; $CaBr_2$ loss of amino-nitrogen = 103 per cent. of gain of protein-N) and in the dilute solution (no. 1) of the chloride series the gain of protein was also largely accounted for by loss of amino-nitrogen (79.2 per cent. for KCl and 73 per cent. for $CaCl_2$). As a result of increased potassium salt concentration (no. 4) the additional protein synthesized amounted to 0.37 (KBr series) and 0.29 (KCl series) mg. per gm. initial fresh weight and of this 0.25 and 0.33 mg. respectively could have been derived from the amino fraction. In the calcium bromide series the strong salt (no. 4) suppressed the synthesis of 0.43 mg. of protein nitrogen and of this 0.36 mg. (83.8 per cent.) remained as amino-nitrogen and in the calcium chloride series the synthesis of 0.17 mg. of protein-nitrogen was suppressed by the strong salt solution (no. 4) and 0.12 mg. of amino-nitrogen (70.7 per cent.) was conserved.

Clearly, then, the principal effect of the salt is upon the utilization of amino-nitrogen, the bulk of which is amino-acid amino nitrogen, in protein synthesis.

(f). With respect to amide the position is more complicated owing to the presence of amides of different degrees of stability—heat stable amides (asparagine) and heat labile amides (comparable to glutamine). In the dilute salt solutions (no. 1) all of the series (KBr, $CaBr_2$, KCl, $CaCl_2$) show an increase in the heat labile, easily hydrolyzable fraction which could account for a large part of the concomitant decrease in the stable amide fraction (KBr, 79 per cent.; $CaBr_2$, 44 per cent.; KCl, 61 per cent.; $CaCl_2$, 56.2 per cent). Increased concentrations of calcium salts, however, suppressed somewhat the disappearance of the amide fraction without affecting the accumulation of unstable amide appreciably. Also the increase in the concentration of potassium salts (difference nos. 1 and 4) decreases markedly the concentration of the unstable amide without a similar effect upon the reserve of stable amides.

It seems, therefore, that the heat labile, easily hydrolyzable amide fraction *does not arise directly from the more stable amide;* it accrues from ammonia which could be released mainly from amino compounds by oxidative deamination [see also (**20**)] and also in part by hydrolysis of the more stable amides. As previously noted (**20**) the unstable amide therefore appears as a reactive intermediary in the formation of protein and its final

from a stock which was in some degree affected by exposure to temperatures in storage lower than normal. There is no reason to suppose, however, that this feature determined the relationship to salt concentration which is here described.

concentration is *decreased by those salt treatments (K series) which greatly accentuate synthesis.*[7]

Certain parallelisms between the behavior of the unstable amide fraction and sugar will be apparent. The experimental conditions which cause a recrudescence of vital activity also stimulate starch hydrolysis, an increase in the concentration of sugar (culture no. 1: KBr, $CaBr_2$, KCl, $CaCl_2$ series), and a relatively high concentration of unstable, easily hydrolyzable amide.[8] Oxygen and temperature are no doubt the chief causal agents but the salt treatments (increased potassium salt concentration) which still further stimulate respiration and synthesis decrease both the sugar residue and likewise the accumulated unstable amide. The converse is not true. The salt treatment (increased concentration of the halides of calcium) which depresses respiration and protein synthesis conserves the sugar residue but the concentration of the unstable amide remains unaltered.

TABLE IX

EFFECT OF EXTERNAL SALT CONCENTRATION ON EASILY HYDROLYZABLE AMIDE CONTENT OF POTATO DISCS

SOLUTION NO.	SALT CONCENTRATION*	EXPERIMENT 1	RELATIVE CONTENT OF LABILE AMIDE	EXPERIMENT 2	RELATIVE CONTENT OF LABILE AMIDE
1	0.00075	KBr series	100.0	KCl series	100.0
2	0.015		64.6		62.0
3	0.050		63.8		63.5
4	0.075		62.0		64.5
1	0.00075	$CaBr_2$ series	100.0	$CaCl_2$ series	100.0
2	0.015		99.2		100.0
3	0.050		102.0		103.0
4	0.075		99.2		99.0

* Concentrations in gram equivalents per liter.

These points are shown in table IX in which the effect of salt concentration on the heat labile amide is shown by data calculated on a relative basis (unstable amide content in tissue of culture no. 1 for each series = 100).

[7] It is more usual to regard asparagine and glutamine as by-products of protein breakdown and somewhat off the main route of synthesis of protein from ammonia [see CHIBNALL (2, pp. 109 and 194)]—a synthesis usually supposed to reconstitute amino acids prior to protein formation. The more attractive view that the protoplast produces protein from sugar and ammonia without forming amino acids as intermediaries is admittedly devoid of direct evidence. The relations which CHIBNALL envisages between glutamine production and respiration concern the relation of glutamine to the cycle of metabolism of organic acids rather than protein synthesis.

[8] The sulphate series was done with tissue drawn from a separate stock which had an unusually high amide content in the initial washed discs. This is the only case of its kind observed in a great many such analyses and the difference between the initial and final tissue of this series is not used as the basis of any conclusion.

(g). When the cations are supplied as nitrates two factors require consideration. First, as illustrated by the data on respiration, the nitrate ion enhances the specific effects due to the cations; and, secondly, this anion can and does contribute to the soluble organic nitrogen fractions. After treatment in strong potassium nitrate the observed increase in the ammonia content of potato discs is slight. The nitrate entering from potassium nitrate forms chiefly amino nitrogen and protein and the metabolism, although much stimulated by potassium, does not cause a net loss of the amino fraction. In presence of relatively strong calcium salts the amino fraction is almost identical with that of the corresponding potassium cultures but the outstanding fact is that the other nitrogen absorbed by the tissue forms much less protein and remains much more in the form of ammonia and amide than in the corresponding potassium culture.

External potassium concentration, therefore, increases synthesis of protein from amino acid and in this the effects of potassium and oxygen are similar—the former accentuates the effect of the latter. Ammonia is not present appreciably in the tissue treated with potassium halides (and only slightly in potassium nitrate solutions) and the residual concentration of unstable amides is reduced by the greater synthesis which is stimulated by potassium. It follows that the effects of potassium salt concentration are not confined to the initial stages of the utilization of amino acids but extend to most, if not all of the reactions involved; especially those in which unstable intermediates formed from sugar and ammonia are probably concerned. Calcium salts have the converse effect on protein synthesis; they depress the use of amino-acids, conserve the heat stable amide, and (in calcium nitrate cultures) cause ammonia to accumulate appreciably. The concentration of intermediates (heat labile amides) remains unaffected by high calcium concentration at the high values (no. 1) which are attained when a low rate of protein synthesis does not consume these faster than they are formed. Inasmuch as calcium, unlike the effect of potassium, fosters the storage and preferential use of ammonia and amide in the tissue, *it diverts protein synthesis from the amino acids and encourages it to proceed* without that oxidative deamination of amino acids; the latter is now believed to form the most probable link between protein synthesis and respiration.

It is clear, therefore, that the somewhat unexpected effects of potassium and calcium salt concentration on respiration and synthesis of protein become intelligible when it is recognized that the active ions are the cations and these exert their effect through the nitrogen metabolism and especially the metabolism of amino acids. The nitrate series confirm this view, since the combined effect of nitrate and potassium increases the metabolism of amino acids whereas the combined effect of calcium and nitrate tends to divert most of the nitrogen metabolism which does occur through channels

(amide)[9] which in potato discs do not appear to be connected with carbon dioxide production. Thus the calcium series, in which the relation between protein synthesis and respiration seemed to be obscured, actually yields strong confirmation of the suggested relationship between these two processes and the way in which they are both affected by salts in potato discs.

Effects of salt concentration on the buffer constituents of the sap

The titration curves of the sap between pH 2.0–6.0 and 6.0–11.0 were recorded on all samples in the experiments here described. They contribute little, however, which cannot be derived from the tables already presented and will not therefore be given in detail. There is no discrepancy between the buffer values observed in the region pH 8.0 to 10 and the determined amino nitrogen content of the tissue—the trend of each with external concentration can be discerned from that of the other. There is, however, a curious and unexplained difference between the effects of chlorides and bromides on that part of the buffer curve in which organic acid radicals are effective.

In the bromide series increased external concentration of the potassium salt decreased the observed loss of buffer value in the range pH 2.0 to 6.0. At every concentration the calcium salt caused the tissue to lose more buffer than the potassium salt but the trend of the effect of concentration, though not great, was in the same direction for both cations. On the other hand, a perfectly clear and definite contrast between calcium and potassium chlorides was observed. Strong potassium chlorides accentuated, strong calcium chloride diminished, the loss of buffer value between pH 2.0 and 6.0. In other words, the effect of salt (chloride) concentration on organic acid radicles was parallel to the effect on amino nitrogen. Direct determinations of the organic acids are clearly necessary before the full effects of salt concentration on the metabolism of the organic acid radicles, whether free or in amino acids, can be profitably discussed.

Effects of salt concentration on oxidase activity in the cells

Reference should be made to the qualitative effects of external salt concentration on the browning reaction (**19, 20**). The chief interest of the browning reaction is that it indicates the activity of the potato oxidase system which produces, aerobically, active oxidizing agents (ortho-quinones) which can deaminate amino acids (**20**). Throughout the entire experiments described, which involved thirty different salt treatments, the same general trend was evident. Within each series increased external concentration of potassium salts increased the oxidase activity; *i.e.*, the production of brown

[9] Calcium supply tends to foster the formation of amide from inorganic sources in certain plants [(2), p. 100].

pigment and increased concentration of calcium salts decreased this activity. Within the series the gradation in the intensity of browning reflects the effect of salts on respiration—it was steepest for nitrates and least for sulphates; the great difference in browning at the same concentration between nitrate or bromide cultures has already been noted (**20**). The effects of salt concentration on browning in the cells are, therefore, parallel to those observed on respiration and protein synthesis and they give credence to the idea (**20**) that the salts affect the deamination of amino acids by virtue of their effect upon the oxidase system of the living cells.

Discussion

The effects of salt concentration upon metabolism and absorption

Experiments 1 to 4 must be scrutinized from the standpoint of the relationship between salt accumulation, protein synthesis, and respiration.

There may be confusion between two distinct types of absorption process which have been recognized in potato discs (termed for convenience "primary" and "induced") in the case of cations, especially those (Ca) which may be bound chemically to cellular components (**17, 18**). Hence the following comparisons are drawn with reference mainly to the anions, since the data do not permit an adequate distinction between the amount of the cations (Ca) which is freely accumulated in the sap and that which is chemically bound in the tissue.

The greater total absorption of bromide which occurred at the higher potassium salt concentration (exp. 1, table X) which was detected by the analysis of the external solutions, presents no new problem; the greater

TABLE X

Absorption and accumulation of bromide from potassium and calcium bromide solutions

Solution no.	Initial concentration*	Series	Final concentration† (Internal)	Accumulation ratio‡	Series	Final concentration (Internal)	Accumulation ratio
1	0.00075	KBr	0.0254	160	$CaBr_2$	0.0158	43.70
2	0.0150	series	0.109	8.66	series	0.0524	4.06
3	0.050		0.242	5.42		0.0570	1.21
4	0.075		0.239	3.42		0.0560	0.78

* Concentrations in gram equivalents per liter.
† Total equivalents absorbed per 1000 grams of water in the tissue.
‡ Internal concentration ÷ final observed external concentration.

absorption, albeit the smaller accumulation, was associated with the most intense metabolism. Both respiration and protein synthesis—the vital

processes which seem to provide the impetus for salt uptake—were stimulated by the stronger salt solutions. The case of the calcium salts is, however, a different one.

Despite the decreased respiration (fig. 1) and protein synthesis (fig. 2) caused by the stronger calcium salts, a greater *total uptake* of bromide occurred at the higher external salt concentrations.

Thus there is an apparent breakdown of an otherwise fairly general tendency according to which salt absorption and respiration are similarly affected by external variables. The discrepancy is, however, more apparent than real. It is clear that at the higher calcium salt concentration, *accumulation* (on the whole-disc basis) was not attained; even allowing for the fact that cells in the surface layers might have greater concentrations, the possibility that any of the cells attained a substantial degree of anion accumulation is remote. Hence, the emphasis should be placed upon the fact that the combined effect of the calcium salt solutions and other environmental factors depressed respiration and protein synthesis; in the strongest solutions used, they obliterated the latter altogether. Thus, increasingly at the higher salt concentration the observed anion uptake was confined to that *passive penetration of cells which may be due solely to diffusion.*

The contrast between potassium and calcium salts is evident. A hundredfold increase of external concentration produces approximately a tenfold increase of bromide absorbed from the potassium salt but only increased the bromide uptake from calcium bromide by 3.5 times.

Absorption of salt to equality of concentration with the external solution does not require metabolism but in dilute solutions the amount of salt thus absorbed is negligible. At relatively high salt concentrations the salt absorbed by mere diffusion may constitute the bulk of the salt absorbed but if additional salt is *accumulated* this is regulated by metabolism. It so happens that in potato discs relatively strong calcium solutions suppress the reactions which are apparently essential for accumulation (protein synthesis and the aerobic respiration with which it is linked) whereas in the corresponding concentration of potassium salts these processes not only persist but are stimulated. It has not yet been possible, however, to state the relationship between anion absorbed, protein synthesized, and carbon dioxide respired in more definite stoichiometrical terms than the qualitative statement used above.[10]

[10] The reason for this is no doubt that the effects of salt concentration on uptake are affected by the thickness of the discs when the data are expressed on the tissue weight basis. This indicates that the salt concentration affects both the concentration attained in the surface cells and the depth of penetration of salt in strong solutions (**15, 18**). The data available for the special case of bromides show that, in strong solutions, they may penetrate beyond the zone in which respiration is stimulated over the basal rate which occurs in the uncut tuber (**15**); *but they never accumulated there.* It is clear, therefore,

Detailed discussion of the nitrate series from the standpoint of accumulation is impossible because the ion was not accumulated. The rapid reduction and subsequent metabolism of nitrate exert a predominant influence upon the absorption of this ion. Some of the soluble products of these reactions (which are different in the case of potassium and calcium salts) might, it is true, be regarded as accumulated in the sap since they are retained against a zero concentration outside, but such processes are not commonly covered by the interpretation given to the term "accumulation."

THE NATURE OF THE SALT EFFECTS DESCRIBED

The chief results described are the contrasted effects of potassium and calcium salts which have a common anion; effects which are not nutritional as this term is commonly understood. Judged by the ability to maintain life and growth, whether of dividing parenchyma cells or of buds, the mineral content of the vacuole of potato parenchyma is already adequate. The centers in which the metabolic effects of salt concentration are exerted must be relatively inaccessible to ions already in the sap, or to ions merely present in the external solution; but they clearly become accessible under the conditions conducive to salt accumulation to cations during the actual process of absorption. Since ions have specific effects[11] on metabolism this should be considered in the comparison of their relative uptake by cells—which is usually held to be determined by their place in the ionic mobility series.

The contrasted effect of potassium and calcium salts was established under conditions such that it could not be caused by any colligative property of ions. Possible, but vague, effects due to manifestations of cell permeability or ion antagonism can be disregarded. A hypothetical effect of the salts on the permeability of the cells to dissolved oxygen might explain many of the effects observed; but these clearly involve metabolic processes with which the mere property of permeability, as it is commonly understood, has but little in common.

Antagonism—a phenomenon usually invoked to explain contrasted

that a full comparison between the effects of salt concentration and metabolism must evaluate the surface effects which *both processes exhibit* with cut discs. This demands an investigation which deals simultaneously with the salt uptake, respiration, and nitrogen metabolism of a range of disc thicknesses at different concentrations of potassium and calcium salt concentrations—a very formidable investigation which has not been made.

[11] In addition to the specific effects of ions on metabolism there may be those due to unequal uptake of anions and cations. In potassium halide solutions, anion and cation are absorbed in equal amounts whereas in calcium salts of the same anions the latter are absorbed in excess. Unequal uptake of anion and cation has been shown by others to affect organic acid metabolism. It may be that the responses described in calcium salts are not wholly specific responses to calcium *per se* but are partly or wholly due to the electrostatic inequality which would obtain in the "centers" where cations and anions are unequally absorbed.

effects of potassium and calcium—is governed by the *ratio* between the concentration of the effective ions, rather than their total concentration in single salt solutions, and comparatively small concentrations of one ion (*e.g.*, Ca) may neutralize the effect of greater concentrations of the other (*e.g.*, K). The opposed effects of potassium and calcium ions in stoichiometrically equivalent concentrations must be of quite another kind. In the concept of antagonism there is the implication that single salt solutions are injurious and it is this injury which is suppressed by the antagonistic effect of ions of another kind. The evidence is that the potato discs in aerated, single-salt solutions are free from the suggestion of injury. In the greatest potassium concentrations the most protein was synthesized—a property hardly consistent with injury to the living system. Furthermore not a single sample *lost* fresh weight during the treatment. Though not separately recorded the observed gain of fresh weight in discs which were previously washed 24 hours in running tap water was never less than 3.7 per cent., even in the strongest solutions used; in the more dilute solutions, the gains were very much greater than those recorded.

The cations and not the anions are primarily effective. The anions play, however, an undoubted rôle in so far as they accentuate the effects of the associated cation and the relative order ($SO_4 < Br < Cl < NO_3$) in which they do this is clearly that in which they also promote absorption from salts of a common cation. The cations (K and Ca) exert their effect as they accentuate or depress reactions which involve free oxygen. The connection between respiration and protein synthesis of potato discs has already been discerned in the metabolism of potato discs and the response due to salts with different concentration only becomes intelligible when this connection is appreciated. As described, the metabolic effects due to oxygen, potassium, calcium, and nitrate all fall into one scheme in which the utilization of amino acids, after oxidative deamination in protein synthesis, plays a predominant rôle.

Clearly such effects of salt concentration may not be universal. In fact, the suggestion made that the salts act through the oxidase system of the potato might suggest that similar effects would be confined to the "direct" and not given by the "indirect" oxidase (peroxidase) plants. Not all living cells are capable of such active protein synthesis as are those of potato tuber. Hence it is not to be expected that so much of the aerobic respiration will always be linked with nitrogen metabolism.[12] Nevertheless, the results

[12] The rapid salt accumulation which can proceed in "low-salt" plants, apparently independently of growth, calls for separate treatment. It happens that the cases studied have been monocotyledons low in soluble nitrogen. For various reasons, protein synthesis in the cells under the conditions of experimentation is rather improbable. Some other aerobic process, however, plays the rôle in these plants which is similar to that played in the potato by the amino acids. The details are unknown but the approach is clear.

described, which connect three of the most fundamental properties of living cells (aerobic respiration, protein synthesis, and accumulation of potassium and calcium salts) in a manner which was not hitherto evident, have widespread implications.

Salt effects on potato discs and the Lundegårdh hypothesis

The results bear on the views of Lundegårdh (**8, 11, 12**) which have since been extended (**9**).[13] Criticisms of this hypothesis arise (**6**), from a consideration of evidence drawn from a diversity of plants, materials, and the work of many investigators. That the connection between salt uptake and respiration is a respiratory component, the very existence of which depends on anion absorption, has been denied[14] (**18**). Salt uptake responds to changes in the aerobic respiration rate (brought about by variables such as oxygen concentration, temperature, etc., other than anion absorption) so as to suggest that the one (respiration) regulates the other (salt absorption). Moreover, it was stated that, apart from the anions NO_3 and PO_4, the effects of salts on respiration were due chiefly to the cations—a possibility which Lundegårdh (**8, 12**) did not allow. Lundegårdh then, as now (**9**), postulated an entirely different mechanism to account for the absorption of anions and cations.

The earlier criticisms of Lundegårdh's theory gains added force from these new results. Lundegårdh evades the detailed criticisms of his earlier work but, at least to his satisfaction, "disposes of" the views of the senior author by the mere statement that potato discs constitute "unsuitable material" in which "anion respiration" is small relative to the "basic respiration" and the experimental conditions were varied too little in relation to the salts. The latter criticism relative to these experiments is clearly void, and the former is meaningless because as conceived by Lundegårdh "Anionenatmung" has no real existence in the respiration of potato discs—the demonstrable effects of neutral salts on respiration are due primarily to the cations.

In the case of potato discs rich in soluble nitrogen compounds, these amino acids occupy the key positions. In the work of Hoagland, however, it is shown that "low-salt" barley roots (which are probably unable to synthesize protein) are rich in sugar and organic acids; the influence of the latter predominates in this case. If the deamination of amino acids in potato discs affects respiration mainly because this yields a carbon substrate (keto acids), these two cases may not be as different as one might suppose. As a result of previous nutritional conditions, the substances in question may have already accumulated in the "low-salt" barley roots.

[13] Lundegårdh attributes the relation of respiration and salt absorption ($Rt = Rg + KA$) to a special component termed "Anionenatmung, KA" which is superimposed upon the basic respiration, "Grundatmung, Rg." "Anionenatmung" has a value proportional to the equivalents of anion absorbed and the constant of proportionality (K) has the values $NO_3 = 2$, $Cl = 3$.

[14] See also Hoagland and Broyer (5) and the later exchange of views (**7, 10, 11**).

Clearly constants characteristic of each anion could not account quantitatively for the effect of potassium and calcium salts on respiration; the effects due to the same anion supplied as a potassium or calcium salt are even different in *sign*. It is true that LUNDEGÅRDH has modified slightly his earlier view and now recognizes that "at more intense anion absorption" the cation affects *slightly* the value of the "constant" from which "Anionenatmung" is calculated. The mechanism postulated is a vaguely specified colloidal effect which is not directly determined by the quantity of cation absorbed.

If the total respiration of potato discs is to be partitioned at all, then the components which should be separated are those described earlier (**21**); namely, that part of the total respiration which in its production is linked with protein synthesis (and in special cases this may constitute as much as 43 per cent. of the total[15]) and the remainder which *arises independently of a net increase in protein and of nitrogen metabolism.* Whatever the final conclusion concerning the nature of the latter component—the data in this paper establish the reality of the former. The chief interest of such a partition of the total respiration is the evident fact *that the component of respiration which is linked to protein synthesis in potato discs seems to be more closely associated with salt accumulation than even the total respiration.* Since this component is entirely aerobic there is no discrepancy between this standpoint and the previous emphasis upon the importance of the aerobic processes of metabolism in salt accumulation. The new facts indicate that the aerobic metabolism processes in question are related to the nature and concentration of the cations in the external solution.

Further discussion is not necessary regarding LUNDEGÅRDH'S views on the comparative rates of respiration of cells in distilled water and salt solution. Faced with the perhaps unexpected result (which does not follow from the theory of "Anionenatmung") that the calculated value of "Rg" is *less* than the respiration in distilled water (where anion absorption is clearly zero), LUNDEGÅRDH postulated that the concentration gradient is steeper in the absence of absorbable anions and more respiratory energy must be expended to retain the anions of the sap. He also stated that the presence of even a low external concentration of anion eliminates this necessity; in stronger solutions the "work of transport" rises and hence the increase in total respiration. In other words, the basic respiration is affected by the concentration gradient between solution and cell only when this latter is maximal (concentration difference = the concentration of the cell sap); at all other concentrations, and whenever absorption is finite, the magnitude of the respiration component "Rg" is independent of this factor. It is better to question the validity of the relationship $Rt = Rg + KA$ than to

[15] Solution 4, KNO_3 series, for example.

adopt such an argument. As it concerns absorption, LUNDEGÅRDH'S theory is not based upon the general concept that the work done in accumulation is a function of the concentration gradient against which the absorption occurs; and still less, upon any rigorous thermodynamic principles by means of which the work or energy value of the absorption can be evaluated.

The data in this paper need no such complicated assumptions regarding respiration in distilled water. For potato discs in distilled water respiration is at a level regulated solely by such variables as temperature, oxygen concentration, specific surface, etc.,—variables treated fully elsewhere—and in this condition the tissue is unaffected either by the stimulating effects of cations of one kind (*e.g.*, K) or the depressing effects of others (*e.g.*, Ca). In other words "respiration in distilled water" falls into its proper place in a range of salt treatments which passes from strong potassium salts on the one hand to strong calcium salts on the other.

What then measures the respiration of tissue not undergoing salt absorption according to LUNDEGÅRDH? LUNDEGÅRDH makes the assumption that there can be no anion absorption in bicarbonate solutions. Although this anticipates the results of another investigation similar to this one, it is clear that the bicarbonate ion exerts its own specific effects on metabolism. These—like the ones already described—are related to absorption. Other work by HOAGLAND, privately communicated to the authors, also shows that potassium cations are absorbed from bicarbonate solutions. This latter criticism would perhaps not be of such great importance were it not that LUNDEGÅRDH uses the respiration of roots at zero anion absorption to calibrate the respiratory efficiency of different batches of roots and, by the use of calculated factors, attains the semblance of close agreement between what are in reality a number of separate and obviously variable experiments. That the methods used by LUNDEGÅRDH still fail to standardize the variables in the material is evident from even a casual perusal of the absolute data; thus comparisons, except between the behavior of strictly comparable samples drawn from the same large batch of seedlings, could be justified only by a statistical analysis which is lacking. For all these reasons the theory of "Anionenatmung" is still to be regarded as unproved, relative to roots; with respect to potato discs, however, it is inconsistent with the established facts.

The recently published work of VAN EIJK (**22**) contributes the interesting result that the respiration of salt marsh plants (*Aster tripolium*) is much affected by salts (NaCl). He discusses the results from the standpoint of LUNDEGÅRDH but his calculated values for the constant anion respiration fluctuate over a wide range. His data do show that salts affect the respiration of halophytes; they do not establish the anion respiration as a special component of that respiration which is produced by salt absorption.

Relation between nitrogen metabolism and respiration

The case of barley plants and potato discs compared.—Gregory and Sen (3) recognized the possibility of a close connection between carbon dioxide output and nitrogen metabolism. In barley leaves the respiration was controlled by nutritional factors during development (potassium and nitrogen supply) and under these treatments high respiration was not associated with a high sugar content but rather with a high concentration of amino acids. The relatively low respiration of high-carbohydrate Lemna plants when grown under conditions of low nitrogen supply was similarly observed by White and Templeman (23). Discussing later experiments concerned with the effect of phosphorus nutrition on the composition and respiration of barley leaves, Richards (15) stressed mainly a supposed reciprocal relationship between carbon dioxide output and protein content. This relationship is regarded from a novel standpoint: not from the more familiar and old idea that protein is a measure of the total active substance capable of respiration, but from the converse standpoint that a certain fixed and continuous production of carbon dioxide is required to maintain a given amount of protein.

The results of the experiments on the effect of salts and oxygen on potato discs agree with the recent work on barley plants, in so far as respiration is not determined by sugar concentration. The behavior of potato discs suggests, however, that *neither* the concentration of amino acids nor of protein regulates the respiration rate and that the portion of the total carbon dioxide output which is linked with nitrogen metabolism is determined by the *conversion of amino acids to protein.* There is as yet no evidence for potato discs which indicates that the *concentration* of amino acids regulates protein synthesis (13); on the contrary, it appears to be regulated mainly by other factors (oxygen tension and the presence of inorganic cations) which determine the activity of systems in the cells that catalyze oxidation and also deaminate amino acids. Pearsall and Billimoria (14) rightly emphasize the diversity of factors which govern protein synthesis in different species and the vital properties such as age in the case of leaves.

Both the system investigated and the method of approach of Gregory and his associates differ in important respects from the case of aerated potato discs in salt solutions and, therefore, too close a parallel should not be drawn. Gregory and his co-workers modified the composition of their plants by nutritional means and, after steady states were established, the respiration rates were determined during periods so short that the composition of the leaves did not change appreciably. The rates observed were, therefore, regarded as regulated by the initial composition. Actual changes in the nitrogenous fractions associated with a given amount of carbon dioxide respired were apparently not investigated. In fact, it is implicit in

the scheme suggested that for the steady state net changes in the nitrogenous fractions would not be observed but that nevertheless the carbon dioxide output would be regulated by the continuous cycle of protein synthesis, breakdown, and resynthesis. The rate of the postulated cyclical process was conceived to be regulated apparently by the steady concentrations of the nitrogenous compounds of the cells caused by the nutritional treatments applied.

During development and storage the potato tuber does attain a steady level of soluble nitrogen compounds—a level which in GREGORY'S scheme might appear as the resultant of balanced tendencies to synthesis and breakdown. The conditions which obtain in the aerated cut discs, however, so encourage synthesis that any hypothetical tendency to simultaneous breakdown is masked and evidence of a continuous cycle of nitrogen metabolism is lacking. Under such conditions it is the new external conditions imposed which determine the response of the cut discs and their initial uniform composition, with reference either to salts or metabolites is *not* a determining factor. In fact, in so far as the metabolism is regulated by salts, it seems that *during entry these have access to more potent spheres of influence than the ions already stored in the cells* since their effects are disproportionately large relative to the amounts actually absorbed. This is in harmony with the idea—inescapable from these investigations—that salt uptake and protein synthesis of potato discs are processes which are linked together.

Protein breakdown is a conspicuous feature of mature leaves (**14**), especially of monocotyledons which are incapable of further growth; in the aerated potato discs, however, synthesis may be long maintained. In short, the nitrogen metabolism of the rapidly metabolizing cells of the potato discs does not appear as a cyclical process in which the rate is regulated by the steady concentrations of the components as envisaged by GREGORY and SEN (**3**), but rather as a linear one in which there is a progressive gain of protein at the expense of a reserve supply of amino acids and a steady state is not established. Other data show that this condition may be long maintained—in fact until most of the soluble nitrogen is converted to protein.[16] Potato cells are able to grow quite effectively in contrast with isolated or mature leaves of grasses. For this reason, considering the relation of respiration to nitrogen metabolism, greater emphasis must be placed upon the one-way process of synthesis than upon a hypothetical cycle of balanced synthesis and breakdown since the former can be demonstrated. It may be assumed with confidence that similar considerations will apply to other actively growing cells. The chief charm of the cyclical explanation is the ease with which, at some point, it can be adapted to demands made upon it;

[16] Thin potato discs in air will increase in protein content until this equals over 60 per cent. of the total nitrogen.

but this should not obscure the fact that it stands or falls by the evidence for the continuous cycle of protein synthesis and breakdown. It is precisely in those cases where cells are no longer able to grow[17] that the evidence for continuous breakdown must appear strongest. Also in the case here described nitrogen metabolism and respiration alike are regulated not by the concentrations of any nitrogenous or carbohydrate components of the tissue but by those external variables (oxygen and salt supply) which limit the extent to which the cells exercise their evident capacity for synthesis. This capacity is determined ultimately by the vital properties of the system concerned and not by the concentrations of any of the nitrogenous components. With these reservations, the views of GREGORY and SEN go far toward a general explanation of the data herein presented.

Accordingly, the interpretation of these results must rest upon a direct connection between protein synthesis and respiration. This connection becomes credible when it is appreciated that the stored amino acids may not be directly used in protein synthesis but first liberate ammonia which, with products derived from sugar, generates protein. Such views impinge upon old, and still open, controversies regarding the true nature of the relationship of both respiration and amino acids to protein synthesis and breakdown. Further reference should be made to the recent book by CHIBNALL (**2**) as extended reference to them is not possible here. The main conclusions, however, can rest upon the demonstrable facts that carbon dioxide output and the utilization of amino nitrogen in protein synthesis are inseparably linked in potato discs. Furthermore, both processes are regulated by variables (oxygen and the presence of salts) which determine the activity in the tissue of an enzyme system which can liberate ammonia from amino acids in vitro. That the corresponding α-keto-acids thus liberated contribute to the carbon substrates from which the increased carbon dioxide output is derived is an hypothesis which can be justified at present only by its probability.

The evident connection between protein synthesis and accumulation, which is the logical sequel to the parallelism between accumulation and growth previously noted, invites speculation on the mechanism of salt uptake. The obvious mode of approach is to postulate loose anion and cation combinations with the positive and negative ions of amphoteric amino acids. In this form the ions might be conducted across the protoplast only to be released into the vacuole when many of the acid and basic groups disappear

[17] CHIBNALL (**2**) in his recent book concludes that some factor from the root, probably hormonic, regulates the protein level of attached leaves. This is another way of saying that the response of attached leaves as part of the integrated system of shoot and root is different from those detached—a difference which seems determined by their capacity to grow whether this in turn is regulated by hormones or other properties of the organized system of which they form a part.

with synthesis. Until the nature of such postulated compounds of salts with amino acids is exactly specified, until the milieu in which synthesis occurs can be designated, and until the reason why the same mechanism does not equally conduct salts outward is clear, any attempt to build a theory of salt uptake on such premises is unwarranted.

Without the implication of the importance of synthesis BROOKS (**1**) has postulated anion and cation combinations with the proteins of the protoplasm, in which phase he believes the primary accumulation occurs. LUNDEGÅRDH (**9**) envisages the entry of anions (A) in combination with unspecified organic bases (R) and the essential stage of accumulation to occur at the inner surface when the compound (RA) activates the breakdown of glucose. While it may seem that there is a common element in these three views it must be clearly recognized however, that all such facile speculation is premature. Despite the evident effects of salt concentration on total uptake, protein synthesis, and respiration direct quantitative relations between them are elusive. In fact, it appears from a consideration of the results that such cannot be expected. The essential point is that *accumulation in potato discs occurs only if protein synthesis takes place* but mere passive absorption to equality of concentration can occur irrespective of protein synthesis. It remains for future work to specify more closely the contact between salt accumulation and the aerobic synthesis of protein from amino acids and to distinguish between effects which are due to the energy value of the extra respiration thus stimulated, effects which could be produced by any other substrate of aerobic respiration; and those which are peculiarly dependent on protein synthesis because of some essential reactions which this entails.

Summary

1. The effect of a range of salt concentrations on respiration and metabolism of potato discs is described. The experiments were carried out under controlled conditions conducive to salt accumulation. The salts used were bromides, chlorides, and nitrates of potassium; and calcium and potassium sulphates. The carbon dioxide output of the discs and the effect of the treatment on carbohydrate, soluble nitrogen fractions, amino and amide nitrogen, ammonia, and protein nitrogen were determined.

2. The effects of salt concentration on starch hydrolysis are slight. The tendency is for increased concentration of potassium salts to decrease and calcium salts to increase starch hydrolysis. This is the converse of the more important effect of salts on all the processes which involve oxidation.

3. Increased external concentration of potassium salts increases respiration and all other reactions which are favored by oxygen, whereas corresponding concentrations of the calcium salts with a common anion depress these processes.

4. The salt concentrations which induce high respiration do not produce high sugar content. High rates of respiration and lower sugar content obtain in the tissue exposed to strong potassium salts and the converse is true of the discs treated with calcium salts. Sugar concentration does not determine respiration.

5. The effective ions of the salts are the cations. The specific effects of the cations are accentuated by the anions and these, like the contrast between the effect of potassium and calcium salts at the same equivalent concentration, are influenced by the anions in the order $NO_3 > Cl > Br > SO_4$ which is also the order in which they influence absorption of a common cation.

6. The effects of salts on respiration are closely connected with their effect on protein synthesis from stored amino acids. Potassium salts stimulate, calcium salts depress both processes.

7. In potassium nitrate solutions the bulk of the nitrate absorbed passes via amino acid to protein. In calcium nitrate solutions less nitrate is absorbed, much less of it appears as protein and the nitrogen metabolism is diverted from amino acids to amide and ammonia.

8. Only the protein synthesis which proceeds from amino acids is linked to respiration. The contact between protein synthesis and respiration seems to be the oxidative deamination of amino acid. Amino acids do not yield protein directly but are first deaminated; they then release ammonia and a carbon residue (probably keto-acid) which contributes to the substrates of aerobic respiration. The activity of the oxidase system of potato which produces oxidizing agents which deaminate amino acids is stimulated by the salts that also increase protein synthesis.

9. Amides, easily hydrolyzed at pH 6.5 in hot solutions, the stability of which is similar to that of glutamine, accumulate in the actively respiring tissue and appear to be the products derived from sugar and ammonia from which protein is formed.

10. Protein synthesis and the aerobic respiration with which it is associated are the phases of the metabolism of potato discs most closely concerned with salt accumulation. Accumulation occurs even in strong (0.075 equivalents per liter) solutions of potassium salts but in calcium salts of this strength, in which a net gain of protein is not produced, the absorption of anions does not exceed equality with the external solution.

11. The salt effects are exerted in centers accessible to salts being absorbed; these centers, however, are inaccessible to salt merely present in the external solution or accumulated in the sap.

12. The bearing of the results upon the present status of the problem of salt accumulation is discussed. The necessity for further work to elaborate the connection between salt absorption, protein synthesis, deamination of

amino acids, and the aerobic respiration with which it is associated, is emphasized.

12. Comparison is made between the relation of respiration to nitrogen metabolism as revealed by work on mature barley leaves and potato discs.

13. The data are discussed relative to LUNDEGÅRDH's theory, to which exception is taken.

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LITERATURE CITED

1. BROOKS, S. C. Selective accumulation with reference to ion exchange by the protoplasm. Trans. Faraday Soc. **33**: 1002–1006. 1937.
2. CHIBNALL, A. C. Protein metabolism in the plant. Yale University Press, New Haven. 1939.
3. GREGORY, F. G., and SEN, P. K. Physiological studies in plant nutrition. VI. The relation of respiration rate to the carbohydrate and nitrogen metabolism of the barley leaf as determined by nitrogen and potassium deficiency. Ann. Bot. N.S. **1**: 521–561. 1937.
4. HOAGLAND, D. R. The absorption of ions by plants. Soil Sci. **16**: 225–246. 1923.
5. ————, and BROYER, T. C. General nature of the process of salt accumulation by roots with description of experimental methods. Plant Physiol. **11**: 471–507. 1936.
6. ————, and STEWARD, F. C. Metabolism and salt absorption by plants. Nature **143**: 1031. 1939.
7. ————, and ————. Salt absorption of plants. Nature **145**: 116. 1940.
8. LUNDEGÅRDH, H., and BURSTRÖM, H. Untersuchungen über die Atmungsvorgänge in Pflanzenwurzeln. Biochem. Zeitsch. **277**: 223–249. 1935.
9. ————. Untersuchungen über die Anionenatmung. Biochem. Zeitschr. **290**: 104–124. 1937.
10. LUNDEGÅRDH, H. An electrochemical theory of salt absorption and respiration. Nature **143**: 203–204. 1939.
11. ————. Salt absorption of plants. Nature **145**: 114. 1940.
12. ————, and BURSTRÖM, H. Untersuchungen über die Salzaufnahme der Pflanzen. III. Quantitative Beziehungen zwischen Atmung und Anionenaufnahme. Biochem. Zeitschr. **261**: 235–251. 1933.
13. PETRIE, A. H. K., and WOOD, J. G. Studies on the nitrogen metabolism

of plants. I. The relation between the content of proteins, amino acids, and water in the leaves. Ann. Bot. N.S. **2**: 33–60. 1938.

14. PEARSALL, W. H., and BILLIMORIA, M. C. Effects of age and season on protein synthesis in detached leaves. Ann. Bot. N.S. **2**: 317–333. 1938.
15. RICHARDS, F. J. Further studies of the effect of potash deficiency on the rate of respiration in leaves of barley. Ann. Bot. **46**: 367–388. 1932.
16. STEWARD, F. C. The absorption and accumulation of solutes by living plant cells. II. A technique for the study of respiration and salt absorption in storage tissue under controlled environmental conditions. Protoplasma **15**: 497–516. 1932.
17. ————. The absorption and accumulation of solutes by living plant cells. IV. Surface effects with storage tissue. A quantitative interpretation with respect to respiration and salt absorption. Protoplasma **17**: 436–453. 1932.
18. ————. Salt accumulation by plants—The rôle of growth and metabolism. Trans. Faraday Soc. **33**: 1006–1016. 1937.
19. ————, and HARRISON, J. A. The absorption and accumulation of salts by living plant cells. IX. The absorption of rubidium bromide by potato discs. Ann. Bot. N.S. **3**: 427–454. 1939.
20. ————, and PRESTON, C. Metabolic processes of potato discs under conditions conducive to salt accumulation. Plant Physiol. **15**: 23–61. 1940.
21. ————, STOUT, P. R., and PRESTON, C. The balance sheet of metabolites for potato discs showing the effect of salts and dissolved oxygen on metabolism at 23° C. Plant Physiol. **15**: 409–447. 1940.
22. VAN EIJK, M. Analyse der Wirkung des NaCl auf die Entwicklung, Sukkulenz und Transpiration bei *Salicornia herbaceae,* sowie Untersuchungen über den Einfluss der Salzaufnahme auf die Wurzelatmung bei *Aster tripolium.* Rec. d. trav. bot. Néerl. **36**: 559–657. 1939.
23. WHITE, H. L., and TEMPLEMAN, W. G. The interaction of factors in the growth of Lemna. X. The interaction of nitrogen and light intensity in relation to respiration. Ann. Bot. N.S. **1**: 191–204. 1937.