EXPERIMENTAL STUDIES ON PLANT METABOLISM. I. EFFECTS OF AMMONIUM SULPHATE EITHER ALONE OR FORTIFIED WITH PHOSPHORUS AND CARBON SOURCES ON RESPIRATION AND METABOLIC CHANGES IN POTATO SLICES

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SUMMARY

Incubation of potato slices in aerated and non-aerated ammonium sulphate media either alone or fortified with carbon and phosphorus sources induced variable losses in the total carbohydrate content. Whereas the content of reducing sugars was, in general, increased by the different treatments, those of starch and sucrose were decreased. In tissues fed with ammonium sulphate + ATP, the losses of starch were higher and those of sucrose were lower than the comparable losses in tissues fed with ammonium sulphate alone.

Protein breakdown was operative in all ammonium sulphate media with no carbon source but supplemental addition of glucose led to a marked accumulation of protein-N. Amino- and total soluble-N showed different levels in the different media. It is concluded that ammonium sulphate and glucose independently affected the protein content of the tissues and the fraction of proteins synthesized at the expense of glucose was increased in presence of phosphorus sources.

Ammonium sulphate either alone or fortified with ATP caused an initial sharp stimulation followed by marked low rates of CO_2 production by potato slices. When combined with glucose, ammonium sulphate reduced the high rates of CO_2 output observed in its absence. This reduction of glucose respiration was overcome by the presence of phosphorus sources.

The present results are discussed in relation to the action of ammonium sulphate when used either alone or fortified with phosphorus and carbon sources.

1. INTRODUCTION

It is well established that the rate of respiration of plants may be greatly increased during the assimilation of ammonium-nitrogen (FOLKES *et al.* 1952; SYRETT 1958; WILLIS & YEMM 1955; YOUNIS *et al.* 1969b). BECCARI *et al.* (1967, 1969) suggested that the ammonium ion acts as a metabolic activator, particularly on glycolysis, rather than a supplementary nitrogen source. But there are reports indicating that ammonia (NH_3) and/or undissociated ammonium hydroxide may act as respiration inhibitors (ALTSCHUL 1946; VINES & WEDDING 1960). STUART & HADDOCK (1968) found that ammonium sulphate, ammonium carbonate or ammonia gas inhibited water uptake in sugar beet roots whenever

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the pH was sufficiently high to cause the production of ammonia. On removal of ammonia by aeration, inhibition of water uptake by the roots was rapidly reversed. ATP either wholly or partially prevented the ammonia induced inhibition of water uptake by the roots.

Plant injury has been traced to ammonia derived from organic sources such as cotton seed meal (WILLIS & RANKIN 1932) and animal manure (GROGAN & ZINK 1956). Aqua ammonia and ammonium salts were found injurious to plants when placed too close to the root zone (GROGAN & ZINK 1956; LORENZ *et al.* 1955) Ammonia and ammonium compounds have been the inorganic forms of nitrogen most supplied to plants. Therefore, the question of how ammonia is utilized and how it produces the toxic symptoms which frequently result from its use on plants have been a matter of interest.

The object of this paper is to give a preliminary account of the changes in respiration rate and in metabolites of potato tuber slices as influenced by ammonium sulphate treatment.

2. MATERIALS AND METHODS

At the time of each experiment, a stock of potato tubers (*Solanum tuberosum* cv. "Alpha") was purchased from the local market – uniform healthy tubers were selected for experimentation. Cylinders of tissue were removed from the tubers with a No. 10 cork borer and cut into sections roughly 1 mm thick. The discs were cut into water and then rinsed twice, dried on paper towels, and divided into random samples of 20 g each.

The samples were washed in aerated running tap water for 24 hrs in order to wash away the contents fo cut cells and to overcome the period of unstable respiratory activity (LATIES 1963). Then the samples were washed in sterile distilled water several times. Two samples were then extracted for estimation of their initial carbohydrate, nitrogen and phosphorus contents. The remaining samples were transferred, in duplicate, to air-tight respiration chambers, each containing 360 ml of distilled water or sterile culture solution adjusted to pH 7.0; the composition of which is indicated in the tables for each of the 3 experiments conducted. The apparatus was sterilized before use and we found no evidence of microbial contamination fo the washed samples.

The samples, after being introduced into the respective media, were maintained in a current of CO_2-free air at a rate of 4 l/chamber/hr throughout the experimental period. The air currents leaving the respiration chambers were allowed to pass into Pettenkofer tubes containing standard NaOH solutions which were periodically replaced for CO_2 absorption. The respiration chambers were kept at 25 °C for 48 and 24 hrs for experiments 1, and 2 and 3, respectively. The absorbed CO_2 was converted into $BaCO_3$ and the unreacted NaOH was titrated against HCl.

By the end of each experiment, each tissue sample was washed with distilled water, drained, blotted with paper towels and divided into 2 equal portions for carbohydrate and nitrogen and phosphorus determinations.

Carbohydrates: Sugars were extracted from the slices with boiling 80% ethanol. The alcohol was removed by distillation under reduced pressure and the aqueous tissue extract was cleared with basic lead acetate; excess lead being removed by Na_2HPO_4 (BARKER & YOUNIS 1965a).

The direct reducing value (D.R.V.) which was considered to be equivalent to reducing sugars was estimated by the method of SOMOGYI (1937) as described by YOUNIS (1963). The total reducing value (T.R.V.) was estimated by determining the reducing titre after hydrolysis by invertase; sucrose was thus calculated by the difference between T.R.V. and D.R.V.

Starch was extracted with perchloric acid, precipitated with iodine, and recovered as starch which was hydrolysed with acid and determined as glucose (PUCHER *et al.* 1948).

Nitrogen and phosphorus: The potato slices were extracted in 10% trichloroacetic acid; the extract being used for determination of total soluble-N by the conventional Kjeldahl method (PIRIE 1955), amino-N (YEMM & COCKING 1955) and phosphorus fractions (KUTTNER & LICHTENSTEIN 1932). The ground dried residue was used for protein-N determination by the Kjeldahl method.

In the above analyses, remarkably close data were obtained from duplicate samples and so the mean values are presented.

The potato tubers used were obtained from crops of varied origin and this most probably has resulted in the observed differences in the distribution of various metabolites in the initial samples.

3. RESULTS

Experiment 1. Effects of ammonium sulphate either alone or in combination with ATP on the respiratory and metabolic changes in slices.

Carbohydrate content. Incubation of tissue slices in water induced slight, if any, changes in the carbohydrates examined (*table 1*). This is consistent with the low rate of CO_2 output of these samples (*fig. 1*).

Feeding with 0.25 mM ammonium sulphate caused substantial and slight losses in sucrose and starch contents respectively. These losses are far in excess to account for the observed increments in reducing sugars. Thus, the losses in

Table 1. (experiment 1). Analysis of the carbohydrate content of potato tuber slices at the start and after 48 hrs. of incubation in ammonium sulphate at pH 7.0, either alone or fortified with ATP. The values are given as mg glucose equivalent per 100 g original fresh weight of slices.

Culture medium	Reducing sugars	Sucrose	Starch	Total
Initial	258.2	310.2	37635.2	38203.6
Distilled water	257.4	290.2	37608.4	38156.0
0.25 mM $(NH_4)_2SO_4$	345.5	102.5	36759.6	37207.6
0.25 mM $(NH_4)_2$ SO_4 + 0.2 mM ATP	306.0	156.4	32375.8	32838.2

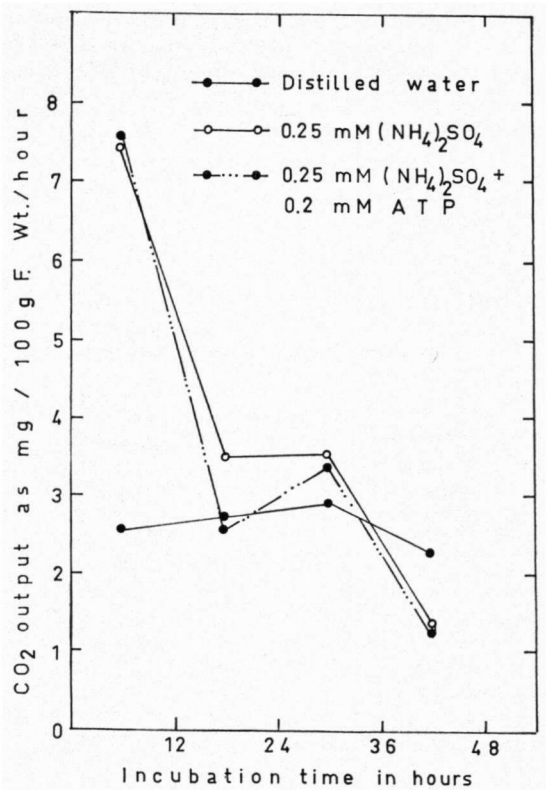

Fig. 1. (experiment 1). Changes in the rate of CO_2 output from potato tuber slices incubated in ammonium sulphate or ammonium sulphate + ATP at pH 7.0, for 48 hrs.

carbohydrates of tissues in ammonium sulphate could be primarily accounted for by the respiratory loss. This seems to be consistent with the inital sharp increase in the respiration of tissues in ammonium sulphate (*fig. 1*).

Similar conclusions could be drawn for the changes in carbohydrate content of tissues fed with 0.25 mM ammonium sulphate + 0.2 mM ATP but the losses of starch were higher and those of sucrose were lower than the comparable losses due to feeding with ammonium sulphate alone.

The behaviour of total sugars followed a similar pattern to that of starch which is the predominant carbohydrate in potato slices (*table 1*).

Nitrogen content. The potato slices starved in water showed no significant change in any nitrogen fraction examined (*table 2*).

Ammonium sulphate (0.25 mM) induced slight protein breakdown concurrently with losses in amino- and total soluble-N. In 0.25 mM ammonium sulphate + 0.2 mM ATP, also slight protein breakdown was operative and this may partly account for the increased amino- and total soluble-N above the levels of the comparable ammonium sulphate samples.

Phosphorus content: Incubation of tissue slices either in water or in 0.25 mM ammonium sulphate induced a slight change in their content of inorganic

Table 2. Analysis of the nitrogen content of potato tuber slices at the start and after 48 hrs. of incubation in ammonium sulphate at pH 7.0, either alone or fortified with ATP. The values are given as mg N per 100 g original fresh weight of slices.

Culture medium	Amino-N	Total soluble-N	Protein-N	Total-N*
Initial	46.6	135.9	61.7	197.6
Distilled water	41.3	125.7	59.1	184.8
0.25 mM $(NH_4)_2$ SO_4	31.1	113.6	53.1	166.7
0.25 mM $(NH_4)_2$ SO_4+ 0.2 mM ATP	41.7	136.8	53.3	190.1

* Total-N is the sum total of total soluble- and protein-N.

Table 3. (experiment 1). Analysis of the phosphorus content of potato tuber slices at the start and after 48 hrs. of incubation in ammonium sulphate at pH 7.0, either alone or fortified with ATP. The values are given as mg P per 100 g original fresh weight of slices.

Culture medium	Total-P	Inorganic-P	Organic-P*
Initial	117.5	17.3	100.2
Distilled water	112.4	16.3	96.1
0.25 mM $(NH_4)_2$ SO_4	108.0	18.3	89.7
0.25 mM $(NH_4)_2$ SO_4+ 0.2 mM ATP	126.6	21.3	105.3

* The organic-P content was calculated from the difference between total-P and inorganic-P.

phosphorus. Combination of ATP with ammonium sulphate appreciably increased the inorganic phosphorus above the water level. In general, organic and total phosphorus showed similar behaviour to that of inorganic phosphorus (*table 3*).

CO_2 output. The tissue samples in water respired at low rate throughout. Ammonium sulphate either alone or fortified with ATP considerably stimulated CO_2 production during the first 12 hrs-period. During the second 12 hrs the rate of CO_2 output was nearly comparable to that of water. Levelling off followed by a marked decrease in CO_2 production was apparent during the third and fourth 12 hrs respectively (*fig. 1*).

Experiment 2. Effects of incubation of potato tuber slices in aerated and non-aerated ammonium sulphate culture media, either alone or fortified with ATP on CO_2 output and metabolic changes.

Carbohydrate content. As compared with initial samples, the content of reducing sugars and sucrose of aerated water samples was not affected whilst starch content was slightly decreased. In non-aerated water, except for a marked decrease in sucrose, the content of reducing sugars and starch of tissue slices remained unaltered (*table 4*).

Table 4. (experiment 2). Analysis of the carbohydrate content of potato tuber slices at the start & after 48 hr of incubation in different media at pH 7.0. The values are given as mg glucose equivalent per 100 g original fresh weight of slices.

Culture medium	Reducing sugars	Sucrose	Starch	Total
Initial	261.0	295.7	37746.9	38303.6
Distilled water, aerated	257.4	289.5	36253.6	36800.5
Distilled water, non-aerated	250.9	196.3	36075.7	36522.9
2.0 mM $(NH_4)_2$ SO_4, aerated	331.1	187.8	35242.8	35761.7
2.0 mM $(NH_4)_2$ SO_4, non-aerated	326.3	160.9	35411.7	35898.9
2.0 mM $(NH_4)_2$ SO_4+ 0.2 mM ATP, aerated	311.2	289.1	33064.4	33664.7
2.0 mM $(NH_4)_2$ SO_4+ 0.2 mM ATP, non-aerated	298.9	214.3	30508.3	31021.5

Aerated and non-aerated 2 mM ammonium sulphate media induced a marked increase in reducing sugars above the control values whereas a marked decrease in sucrose was observed; the magnitude of decrease being greater in non-aerated media. Starch was slightly decreased.

In response to feeding with aerated ammonium sulphate fortified with ATP, the content of reducing sugars increased and that of starch decreased markedly. Sucrose content was comparable to that of tissues in aerated water. Non-aerated comparable tissues showed more consumption of sucrose and starch (*table 4*).

Nitrogen content. All treated samples showed protein breakdown; the magnitude of breakdown being much higher in non-aerated than in aerated samples (*table 5*). Water samples either aerated or non-aerated showed no change in the contents of their amino- and total soluble-N. On the other hand, protein break-

Table 5. (experiment 2). Analysis of the nitrogen content of potato tuber slices at the start & after 48 hr of incubation in different media at pH 7.0. The values are given as mg N per 100 g original fresh weight of slices.

Culture medium	Amino-N	Total soluble-N	Protein-N	Total-N
Initial	59.5	138.5	65.0	203.8
Distilled water, aerated	56.7	140.6	61.0	201.6
Distilled water, non-aerated	58.5	141.6	39.0	180.6
2.0 mM $(NH_4)_2$ SO_4, aerated	78.2	161.3	48.1	209.4
2.0 mM $(NH_4)_2$ SO_4, non-aerated	63.6	128.5	35.1	163.6
2.0 mM $(NH_4)_2$ SO_4+ 0.2 mM ATP, aerated	85.3	185.6	55.4	241.0
2.0 mM $(NH_4)_2$ SO_4+ 0.2 mM ATP, non-aerated	75.6	147.2	34.7	181.9

down in aerated ammonium sulphate either alone or fortified with ATP was accompanied with increments in amino- and total soluble-N. In the comparable non-aerated media the levels of amino- and total soluble-N were lower than those in aerated media (*table 5*).

Phosphorus content. Essentially similar results to those of experiment 1 were obtained irrespective of aerating or non-aerating the culture media.

CO_2 output. In aerated culture media the response of the CO_2 production by the slices to ammonium sulphate, either alone or fortified with ATP, was similar to that of experiment 1, regardless of the higher concentration of ammonium sulphate used (*fig. 2*).

Experiment 3. Effects of ammonium sulphate and glucose either alone or in combination with different phosphorus sources on CO_2 production and metabolic changes in potato tuber slices.

Carbohydrate content. In glucose-fed samples, there was a marked increase of reducing sugars, a slight decrease of sucrose and a marked breakdown of starch (*table 6*). This observed loss in carbohydrates could be accounted for partly by the respiratory loss and partly by the utilization for some kinds of metabolism. The changes in carbohydrate fractions in 5 mM ammonium sulphate (*table 6*) were, in general, similar to those induced by 0.25 and 2 mM ammonium sulphate (*tables 1* and *4*).

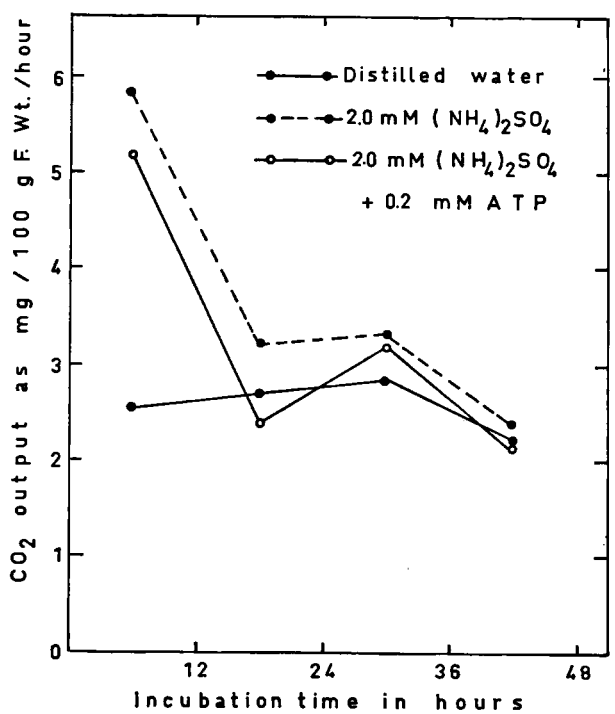

Fig. 2. (experiment 2). Changes in the rate of CO_2 output from potato tuber slices incubated in aerated ammonium sulphate or ammonium sulphate + ATP culture media at pH 7.0, for 48 hrs.

Table 6. (experiment 3). Analysis of the carbohydrate content of potato tuber slices at the start and after 24 hrs of incubation in different media at pH 7.0. The values are given as mg glucose equivalent per 100 g original fresh weight of slices.

Culture medium	Reducing sugars	Sucrose	Starch	Total
Initial	261.0	301.1	37726.4	38288.5
Distilled water	258.0	297.8	36351.2	36907.0
0.056 M glucose	343.0	270.0	30492.8	31106.4
5 mM $(NH_4)_2$ SO_4	334.9	193.3	36009.6	36537.8
0.056 M glucose + 5 mM $(NH_4)_2$ SO_4	340.6	214.6	33634.3	34189.5
0.056 M glucose + 5 mM $(NH_4)_2$ SO_4 + 66 mM KH_2PO_4	241.2	219.9	31371.5	31832.6
0.056 M glucose + 5 mM $(NH_4)_2$ SO_4 + 0.2 mM ADP	249.9	245.9	30148.4	30644.2
0.056 M glucose + 5 mM $(NH_4)_2$ SO_4 + 0.2 mM ATP	260.0	295.8	25805.7	26361.5

An interaction between ammonium sulphate and glucose seems to have occurred and thus the levels of carbohydrates in the respective tissues, in general, lay in between those in tissues incubated in glucose or ammonium sulphate. When ammonium sulphate + glucose media were fortified with phosphorus, marked losses in total carbohydrates occurred concurrently with the sharp increase in CO_2 production (*fig. 3*). In particular, phosphorus sources seem to have favoured the utilization of starch (*table 6*).

Nitrogen content. Feeding with glucose induced marked accumulation of amino- and protein-N whereas the total soluble-N content was comparable to that of water samples. This may indicate the utilization of glucose *via* the glycolytic and the tricarboxylic acid cycle pathways and thus leading to carbon skeletons which were aminated and ultimately led to formation of proteins (*table 7*).

In ammonium sulphate the reduction in amino- and protein-N was associated with an increase in the total soluble-N. Protein- and amino-N levels in tissues in ammonium sulphate + glucose lay in between those in tissues in ammonium sulphate and in glucose media.

Addition of different phosphorus sources to the glucose + ammonium sulphate media slightly increased the amino-N content of the differently treated samples above that of water samples. Induction of synthesis of appreciable amounts of proteins was also apparent. Furthermore, total soluble-N increased above the water level; the magnitude of increase being highest in case of ATP (*table 7*).

Phosphorus content. In general, similar results to those of experiments 1 and 2 were obtained. In culture media without phosphorus, the phosphorus content of the respective tissues showed slight, if any, changes. On the other hand, incu-

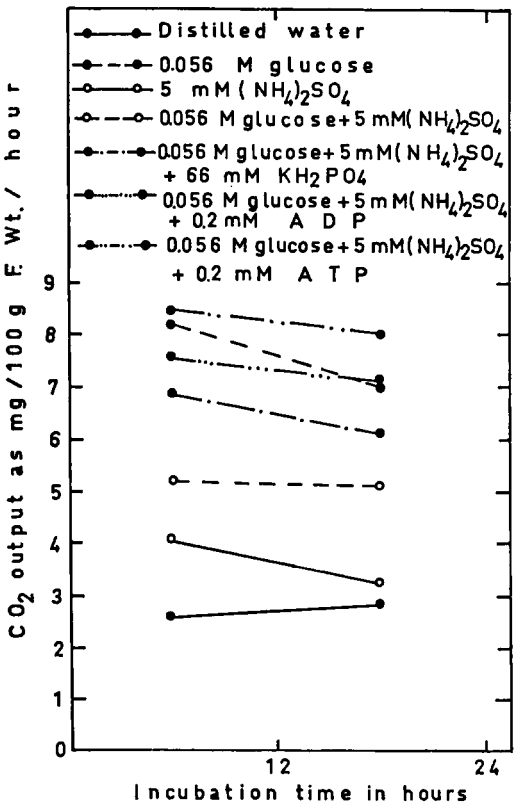

Fig. 3 (experiment 3). Changes in the rate of CO_2 output from potato tuber slices incubated in different culture media at pH 7.0, for 24 hrs.

Table 7. (experiment 3). Analysis of the nitrogen content of potato tuber slices at the start and after 24 hrs of incubation in different media at pH 7.0. The values are given as mg N per 100 g original fresh weight of slices.

Culture medium	Amino-N	Total soluble-N	Protein-N	Total-N
Initial	52.7	147.2	60.3	207.5
Distilled water	55.2	135.2	589.	194.1
0.056 M glucose	84.6	132.2	74.6	206.8
5 mM $(NH_4)_2$ SO_4	43.3	173.5	42.0	215.5
0.056 M glucose + 5 mM $(NH_4)_2$ SO_4	50.0	160.4	59.9	220.3
0.056 M glucose + 5 mM $(NH_4)_2$ SO_4 + 66 mM KH_2 PO_4	59.3	165.1	65.1	230.2
0.056 M glucose + 5 mM $(NH_4)_2$ SO_4 + 0.2 mM ADP	62.8	170.6	67.8	238.4
0.056 M glucose + 5 mM $(NH_4)_2$ SO_4 + 0.2 mM ATP	65.2	231.5	70.3	301.8

bation ot tissue slices in glucose + ammonium sulphate media fortified with different phosphorus sources led to a marked increase in inorganic and organic phosphorus contents of the tissues.

CO_2 output. Feeding with glucose induced an initial sharp increase followed by a slight decrease in the rate of CO_2 production. 5 mM ammonium sulphate appreciably increased the CO_2 output during the first 12 hrs but during the second 12 hrs the rate of CO_2 production was only slightly higher than that of the control. The rate of respiration of slices incubated in ammonium sulphate + glucose lay in between those of ammonium sulphate and of glucose (*fig. 3*).

The rate of respiration of tissue slices in ammonium sulphate + glucose media fortified with phosphorus was much higher than that of samples in water. Of the three phosphorus sources, ATP had the highest effect, with less effect in case of ADP and least in case of KH_2PO_4.

4. DISCUSSION

Changes in carbohydrate content. All the concentrations of ammonium sulphate used in the present work were, in general, similar in their effects on the changes in the carbohydrate fractions examined. Thus, whereas the content of reducing sugars was increased, those of starch and sucrose were decreased, the magnitude of decrease in the latter being greater than in the former carbohydrate fraction. That the utilization of internal as well as sugars taken up by the slices proceeded at a faster rate in presence of phosphorus sources than in their absence is consistent with the prior phosphorylation of sugars before entry into the metabolic pathways (BARKER & YOUNIS 1965b; BEEVERS 1961) and with the possibility that NH^+_4 mops up the ATP content of plant tissues (SYRETT 1958). The observed loss in carbohydrates could be accounted for partly by the respiratory loss and partly by the utilization for some kinds of metabolism (*viz.* protein synthesis).

In non-aerated water and ammonium sulphate the lower levels of sucrose than in the comparable aerated media (*table 4*) might have been due to shortage of ATP. Thus, it is stated that the conversion of starch to sugar in potatoes might be inhibited by anoxia through a lack of adenosine and uridine triphosphates (TURNER 1960; BARKER 1968). This is also supported by the fact that, the losses in starch of tissues in non-aerated ammonium sulphate + ATP were higher than those of tissues in the comparable aerated media (*table 4*), this more consumption of starch being due to the presence of ATP in the non-aerated media.

The breakdown of sucrose and starch in tissues treated with ammonium sulphate is not known whether to be hydrolytic involving invertase and amylase or phosphorylytic involving sucrose and starch transglucosylases. It seems probable from the present results that both mechanisms are operative in media containing no phosphorus source. But in the presence of a phosphorus source the phosphorylytic breakdown of starch most probably has proceeded at a much faster rate than that of the amylolytic breakdown. In support of this explanation ARREGUIN-LOZANO & BONNER (1949) pointed out that the starch transglu-

cosylase reaction is accelerated if inorganic phosphate is increased. For sucrose the situation might have been different, since the equilibrium between breakdown and synthesis is towards synthesis; if levels of sucrose in tissues in ammonium sulphate are to be compared with those in ammonium sulphate + phosphorus. However, this problem of breakdown of starch and sucrose remains for further investigations.

Changes in nitrogen content. The total-N in tissues in ammonium sulphate fortified with ATP was higher than in tissues in ammonium sulphate alone. Thus it seems that ATP has partly increased the uptake and utilization of ammonia-N, two processes requiring the expenditure of energy (SYRETT 1958; YOUNIS *et al.* 1969b) and partly reduced the probable leakage of nitrogen fractions from tissues into the media. Thus ATP may be involved in maintaining the permeability control mechanism and the structure of the cell. In this connection the observation of STUART & HADDOCK (1968) that ATP either wholly or partially prevented the ammonia-induced inhibition of water uptake by roots is of interest. They also suggested that ATP may be involved in maintaining the structure of water pathways through the root.

In non-aerated media, the greater reduction in total-N content of tissues compared to that in aerated media could be due in part to less uptake and utilization of ammonium sulphate and in part to leakage of nitrogen fractions into the media. In non-aerated media the expected shortage of ATP could account for less uptake of ammonium sulphate. Also it is expected, in tissues maintained under anaerobic conditions, that excessive exudation would probably occur since the metabolism of plant tissues is severely disturbed under these conditions (BARKER & EL-SAIFI 1952; KRAMER 1956).

The loss in nitrogen fractions from tissues most probably have been due to leakage into the media. We have no evidence in the present study to support this conclusion and to our knowledge, ammonium sulphate has not been reported to induce leakage from sliced storage organs. However, outward diffusion of various metabolites, including nitrogen compounds, into the ambient culture media containing respiratory inhibitors and/or nitrogenous compounds has been demonstrated (SAID & YOUNIS 1952; STENLID 1949; YOUNIS 1960; YOUNIS 1962, YOUNIS *et al.* 1969a; YOUNIS *et al.* 1969b; YOUNIS 1969).

From *table 7* it is clear that glucose induced the formation of 15.7 mg protein-N/100 g original fresh weight of slices as compared with the water control whereas 5 mM ammonium sulphate alone induced the disappearance of 16.9 mg protein-N/100 g original fresh weight of slices. The level of protein-N in glucose + ammonium sulphate was also higher than that in ammonium sulphate alone by 17.9 mg protein-N. It is thus apparent that ammonium sulphate and glucose acted independently on proteins in potato tuber slices.

But the inclusion of a phosphorus source in the glucose + ammonium sulphate media induced protein-N levels higher than those of ammonium sulphate + glucose and lower than those of glucose alone. It seems then that the protein fraction synthesized at the expense of glucose was further increased in presence of a phosphorus source and ATP proved to be the best.

Thus, it is now known that the *in vivo* synthesis of amides, peptides and proteins is achieved at the expense of an energy source, usually ATP (WEBSTER 1959). That amino acids act as precursors of protein synthesis is apparent from the increased levels of amino acids concurrent with the increased levels of proteins.

CO_2 output. The initial sharp stimulation followed by inhibition of CO_2 production (*figs. 1* and *2*) may be related to the internal effective concentrations of ammonium sulphate. At the start of the experiment the concentration of NH^+_4 might have been stimulatory and with progress of time the NH_4^+ concentration might have been increased enough to inhibit the CO_2 output. Previous work has indicated the use of ammonia and ammonium salts as stimulants and inhibitors of plant respiration (ALTSCHUL *et al.* 1946; BECCARI *et al.* 1969, VINES & WEDDING 1960; YOUNIS *et al* 1969b). The degree of inhibition was found to be related to the pH which controls the amount of ammonia in solutions containing NH_4^+ (VINES & WEDDING 1960).

Feeding slices with 0.056 M glucose considerably increased the CO_2 output and thus the respiratory enzymes seem to have sustained such an increased supply of glucose as a respiratory substrate. It is known that the assimilatory reactions of glucose utilize ATP (SYRETT 1958; YOUNIS *et al.* 1969b) and thus, in glucose media, the more rapid ATP consumption seems to have been balanced by a greater synthesis of ATP connected with an increased respiration rate.

The fact that glucose + ammonium sulphate induced a lower production of CO_2 (*fig. 3*) is in accord with the results of VINES & WEDDING (1960). They found that the exogenous respiration of glucose, citrate and malate, by barley roots and other plant tissues, was inhibited by ammonia in the same way as the endogenous respiration. Our results (*fig. 3*) clearly show that the inhibition, by ammonium sulphate, of the exogenous respiration of glucose by tissue slices was partially and completely overcome by the presence of KH_2PO_4 and ADP and ATP in the culture media respectively. This is also in accord with the suggestion that NH_4^+ diminishes the ATP content of plant tissues. But in the present work only the total concentration of organic phosphorus which is incorporated in many different nucleotides in the tissues was measured, and changes in this total fraction may not reflect very closely changes in the ATP concentration at the sites where phosphorylations are taking place. However, a tendency towards a decrease in organic phosphorus (*table 3*) associated with an increase in inorganic phosphorus is apparent.

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