

STUDIES ON THE EFFECTS OF SOME DICARBOXYLIC ACIDS ON THE UPTAKE AND UTILIZATION OF INORGANIC NITROGEN

II - Effects of some Dicarboxylic Acids on the Uptake and Utilization of Ammonium and Nitrate Nitrogen by Sweet Potato Tuber Disks

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INTRODUCTION

It has been recently shown by El-Shishiny and Nosseir (unpublished work) that certain dicarboxylic acids figured in the krebs cycle, when supplied together with ammonium salts to carrot root disks, exert a marked influence upon the course of the metabolic reactions of the tissues. In control samples cultured in ammonium salt solutions, a considerable part of the ammonia absorbed is recovered as amide—N, mainly glutamine. However, if oxalacetate, fumarate, malate, succinate, or α —ketoglutarate is acquired from the culture solution, the formation of glutamine was greatly stimulated. Reasons have been advanced by Chibnall (1), and Vickery & Pucher (14) for the assumption that the specific precursors of asparagine and glutamine are respectively oxalacetic and α —ketoglutaric acids which arise as intermediary products in carbohydrate metabolism. Carrot root disks obviously contain enzyme systems that provide for rapid and extensive metabolic transformation of the organic acids present or introduced into the cells.

A further series of experiments is described in the present paper. These were carried out in order to extend the observations to include aspartic acid and nitrate—N. Disks of sweet potato tubers were employed, and tests were accordingly made to see whether the effect of added acids was similar to that observed earlier. Potato tuber is selected because of its availability in the garden of the department and also because it represents a species with a moderately intense glutamine metabolism, El-Shishiny (2).

MATERIAL AND METHODS

The technique of the disk culture experiments was the same as in the earlier work described by El-Shishiny (2), and El-Shishiny & Nosseir (3). A stock of disks sufficient for each experiment was prepared from sweet potato tubers variety « Balady » grown in the botanic garden of the Faculty. About twenty grams of disks taken at random (40 disks) were used for each treatment. The samples after being washed for 48 hours in aerated distilled water, El-Shishiny (2), were transferred into 350 ml. culture solution kept at 25° C. ($\pm 0.1^\circ$ C.) in a constant-temperature water bath. A current of CO₂—free air was passed through each culture solution at a constant rate of 4 liters per hour to serve for aeration and for the determination of CO₂ output. After a culturing period of 24 hours, the disks were drained, washed several times with distilled water, and analysed for the final distribution of the various nitrogenous fractions. The medium, together with the washings were made to a convenient volume and analysed for organic and inorganic nitrogenous fractions. The analytical methods for the determination of the various nitrogenous fractions were those long in use in this laboratory and described by El-Shishiny (2), and El-Shishiny & Nosseir (3).

Two experiments were carried out following the same technique. The results were fully consistent with those summarized in tables I A and I B. In each experiment, twelve samples after being washed for 48 hours were transferred into culture vessels each containing 350 ml. of sterilized distilled water or culture solution according to the following scheme : samples 1 & 2 distilled water; 3, ammonium chloride; 4, potassium nitrate; 5, NH₄ Cl + fumarate; 6, KNO₃ + fumarate; 7, NH₄ Cl + malate; 8, KNO₃ + malate; 9, NH₄ Cl + succinate; 10, KNO₃ + succinate; 11, NH₄ Cl + aspartate; 12, KNO₃ + aspartate. The concentration of ammonium chloride and potassium nitrate was 0.005 M, while that of organic acid salts was 0.0025 M.

Chemicals used : Aspartic acid of highest purity was purchased from L. Light & Co. Ltd. The other chemicals were purchased from the B.D.H. The organic acids were neutralized with KOH to pH 7 before they were supplied to the tissues.

RESULTS AND DISCUSSION

Effects of dicarboxylic acids figured in the krebs cycle and of aspartic acid upon the uptake and utilization of ammonium and nitrate-N :

The data depicted in table I B show the uptake and utilization of ammonium - and nitrate - N, in mg. nitrogen, by 100 g. of sweet potato tuber

disks cultured in 0.005 M ammonium chloride and in 0.005 M potassium nitrate alone and with aspartate, fumarate, malate or succinate. The results reported in this table show that 68.03 mg. ammonium—N and 74.69 mg. nitrate—N were removed in 24 hours from the single salt solutions of 0.005 M ammonium chloride and potassium nitrate respectively. When ammonium chloride and potassium nitrate were the only sources of nitrogen, the rate of ammonium assimilation exceeded very much the rate of nitrate assimilation since 98.7 per cent of the absorbed ammonium—N and only 61.6 per cent of the absorbed nitrate—N were changed into organic nitrogen. Thus the ammonium nitrogen was very rapidly metabolised and did not accumulate in the cells. On the other hand, nitrate—N utilization is slower and nitrate accumulates and reaches high levels in the cells of sweet potato disks.

Culturing sweet potato tuber disks on ammonium chloride with fumarate, malate or succinate brought about increases in the rates of ammonium—N uptake and assimilation. There results only 7.0%, 9.7%, and 7.4% increase in ammonium—N uptake and the rate of ammonium assimilation was similarly increased by 6.5%, 9.6%, and 6.6% respectively over the control in ammonium chloride. This might be due to the large amounts of ammonia—N removed from the culture solution of ammonium chloride alone. The increase in the rate of ammonium assimilation and the disturbance of the metabolic activity when organic acids were present in the external medium indicate that these acids were acquired from the external media by the cells and there underwent metabolic transformations. Pucher & Vickery (10) showed that all of the individual members of the krebs tricarboxylic acid cycle that were tested entered the cells of tobacco leaves during the culture in their potassium salt solutions and exerted a marked influence on the course of metabolic reactions of the tissues.

On the other hand, aspartate expressed a depressing effect on the uptake and on the assimilation of ammonium—N by sweet potato tuber disks. When aspartate is given in the culture solution with ammonium chloride, the uptake of ammonium—N was decreased by 38.9% and the ammonium—N assimilation was also decreased by 44.9% as compared by uptake and assimilation of the control disks cultured in ammonium chloride alone.

Similarly, aspartate and fumarate exerted depressing effects on the uptake of nitrate—N from potassium nitrate culture media by 39.4% and 10.4% respectively, while malate and succinate were without remarkable effects. Nitrate—N assimilation was also depressed since decreases of 33.2%, 23.0%, 6.7, and 9.1 % were shown consequent to addition of aspartate, fumarate, malate, and succinate respectively to KNO_3 in the external media. The differential effect of the different acids on ammonium and

nitrate — N uptake by sweet potato tuber disks might be due to their specific effects on the permeability as well as on the metabolism of the tissues.

Effects of ammonium and nitrate salts with some possible asparagine precursors of the krebs cycle on the distribution of the various nitrogenous fractions :

The changes in the distribution of the various nitrogenous fractions in the disks of sweet potato roots treated with ammonium chloride or potassium nitrate with aspartate, fumarate, malate, or succinate were depicted in table I A. The first three columns show the composition of the samples cultured in water, NH_4Cl , or KNO_3 to serve as controls to assist in judgements of the relative magnitude of the changes that occurred as a result of the presence of the organic acids used. The subsequent columns show the changes in composition brought about by the different treatments. The units being mg. N computed for a sample that weighed 100 g. at the start.

Table I A shows that amides are formed and accumulated in tissues cultured in ammonium chloride in response to the increase of the level of ammonium — N in the cells. The presence of fumarate, malate, and succinate stimulated ammonium assimilation and increased the level of amides and amino acids in the tissues. The increase in the amides is mainly due to glutamine. Even, when the equilibrium of amino acids was one sidedly disturbed by feeding sweet potato root tissues with aspartate and ammonia, glutamine considerably increases as also shown for carrot root tissues by El-Shishiny and Nosseir (4). The primary formation of glutamine even when aspartic acid is supplied to the tissues proves that there must be, in the plant cells, either a very active deaminase system or the amino group of aspartic acid must first be transferred to α -ketoglutaric acid and the formed glutamic acid is then amidated. Rautanen (11) showed that glutamic acid and glutamine appreciably increase during the uptake of aspartic acid by pea seedlings. He suggested the central importance of α -ketoglutaric acid as a primary acceptor of ammonia. Evidence of a primary synthesis of glutamine is supported by the investigations with N^{15} on barley roots by Yemm Willis (17). Moreover, El-Shishiny (2) has shown that the amide metabolism in sweet potato roots is mainly glutamine metabolism.

It has been recently shown by El-Shishiny & Nosseir (unpublished work) that oxalacetate, fumarate, malate, or succinate, when acquired together with ammonium — N by carrot root disks from the culture solutions, stimulated greatly glutamine formation. The difference in response of various species to impressed conditions that provide the increased concentration of ammonia in plant cells have been interpreted by Vickery & Pucher (14) in terms of relative availability of the non-nitrogenous precursors required

TABLE I (A)

Effect of culturing disks of sweet potato tubers for 24 hours in 0.005 M solutions of ammonium chloride and potassium nitrate with and without 0.0025 M dicarboxylic acids. (M gm. N per 100 g. fresh weight of tissue)

Nitrogenous fractions	Controls in			Changes in the nitrogenous fractions as compared with controls in KNO_3 or NH_4Cl							
	Water	KNO_3	NH_4Cl	KNO_3			NH_4Cl				
				Aspartate	Fumarate	Malate	Succinate	Aspartate	Fumarate	Malate	Succinate
Ammonium - N	0.98	1.13	1.86	+ 0.56	+ 0.06	+ 0.62	- 0.48	+ 3.65	+ 0.38	+ 0.13	+ 0.69
Nitrate - N	—	23.05	—	-14.03	+ 2.83	+ 4.58	+ 5.70	—	—	—	—
Glutamine - N	4.82	4.52	12.33	+ 2.30	+ 0.74	+ 2.92	+ 1.93	+ 7.54	+ 5.46	+ 7.00	+ 2.46
Asparagine - N	2.18	2.24	8.84	0.00	- 0.22	- 0.04	+ 0.80	+ 0.46	+ 1.38	+ 1.06	+ 0.16
Total - N of amides	7.00	6.76	21.22	+ 2.30	+ 0.52	+ 2.88	+ 2.78	+ 8.00	+ 6.84	+ 8.08	+ 2.62
Amino acid - N	31.72	47.49	28.38	+11.73	+10.47	+ 3.66	+ 6.07	+30.55	+16.29	+ 5.44	+ 8.10
Rest - N	29.26	51.22	66.12	-14.67	-15.43	- 4.65	- 3.02	-26.78	-10.52	+ 0.24	- 0.16
Protein - N	81.96	88.57	59.79	+ 5.00	- 2.40	- 2.68	- 1.68	- 1.80	- 5.28	- 5.33	- 4.35

TABLE I (B)

Effect of some dicarboxylic acids on the uptake and assimilation of ammonium and nitrate nitrogen

Absorbed-ammonium-N	—	—	68.03	—	—	—	—	41.55	72.77	74.60	73.05
Absorbed nitrate-N	—	74.69	—	45.31	66.95	76.20	76.19	—	—	—	—
Absorbed amino-N	—	—	—	18.15	—	—	—	36.84	—	—	—
Assimilated ammonium-N	—	—	67.14	—	—	—	—	37.01	71.50	63.56	71.56
Assimilated nitrate-N	—	46.04	—	30.74	35.47	52.97	41.84	—	—	—	—
Assimilated amino-N	—	—	—	- 9.33	—	—	—	9.63	—	—	—

for the synthesis of the respective amides which arise in the transformation of organic acids during respiration. The results obtained for sweet potato roots in the present investigation as those recently obtained for carrot roots afford no conclusive evidence in favour of this postulate.

The maintenance of positive amino acid, amide and protein balance consequent to feeding sweet potato tuber disks with ammonium—N together with fumarate, malate, or succinate suggest the effective absorption and utilization of these compounds in anabolic reactions leading to glutamine accumulation. It seems therefore, that if such a cycle as that derived by Chibnall (1) from krebs and Johnson's cycle (7) could be operative in sweet potato tuber disks, then under conditions such that ammonia is accumulating, α -ketoglutaric acid might be withdrawn from the respiratory cycle for the synthesis of glutamine, while a small fraction of oxalacetic acid produced via interconversions of the acids used (Green, 5) or produced as a result of metabolic changes in the tissues, might be withdrawn for the synthesis of asparagine. It seems in this case that the mechanism is such that little oxalacetic acid becomes available at any time to react with ammonia even when the level of succinic, fumaric or malic acid is artificially increased. These acids seem to undergo conversion in the process of respiration which apparently made α -ketoglutaric acid available to react with ammonia directly or undergo transamination with aspartic acid metabolically formed to give glutamine as suggested by Leonard & Burris (8). The amination and amidation of α -ketoglutaric acid to give glutamine have been reported by Vickery et al (15 & 16), Chibnall (1), Sideris & co-workers (12), Mac Vicar & Burris (9), Yemm & Willis (17), and Kretovich & Yakovleva (6).

On the other hand, feeding sweet potato tuber disks with KNO_3 alone or in combination with aspartate, fumarate, malate, or succinate results in a considerable increase in the amino acid—N fraction and negligible changes in the amides. But the rate of synthesis of amino acids in presence of the organic acid salt was much higher than that of the control tissues supplied with KNO_3 alone while that of glutamine and asparagine was almost unchanged. This result together with the fact that nitrate assimilation did not lead to any significant increase in ammonia—N level in the tissues might indicate that nitrate was not reduced in the cells into ammonia or that the rate of ammonium transformation into organic—N other than amide—N is as fast as its production from the nitrate.

The energy required for the formation of amides and amino acids might have been derived partly through the oxidation of the original substrate in the cells and partly through the oxidation of the supplied organic acids,

since respiration of sweet potato tuber disks increased with NH_4Cl or KNO_3 and showed further increase with organic acids supplied to the external medium. Speck (13) suggested that the oxidation of succinate, malate, and oxalacetate supplied to pigeon liver dispersion supply the energy required for the utilization of ammonia and for amino acid and glutamine synthesis.

SUMMARY

The effect of aspartate, fumarate, malate, and succinate on the uptake and utilization of ammonium- and nitrate — N by sweet potato tuber disks was studied.

The rate of nitrate utilization was lower than absorption, leading to accumulation of nitrate — N in the cells. Ammonium — N, on the other hand, was utilized as soon as it entered the cells and there was a very small increase in its level inside the tissues.

Fumarate, malate, and succinate stimulated the uptake and utilization of ammonium — N and there was no accumulation of this fraction in the cells. This is probably due to the rapid synthesis of amino acid- and glutamine — N.

KNO_3 alone or supplied in combination with aspartate, fumarate, malate or succinate caused a considerable increase in the amino acid — N content and negligible changes in the amide — N. The rate of synthesis of amino acids in presence of the organic acid salt was higher than that of the control tissues cultured in KNO_3 alone while that of amides was almost unchanged. No significant increase in ammonia — N level in KNO_3 — treated tissues was observed.

BIBLIOGRAPHY

1. Chibnall, A. C. Protein metabolism in the plant. Yale University Press, New Haven, 1939.
2. El-Shishiny, E. D. H. Absorption and assimilation of inorganic nitrogen from different sources by storage root tissues. *J. Exp. Bot.* 6 : 6 - 16, 1955.
3. El-Shishiny, E. D. H. & Nosseir, M. A. The relation of optical form to the utilization of amino acids. I. Utilization of stereoisomeric forms of glutamic acid by carrot root disks. *Plant Physiol.* 32 : 360 - 364, 1957.
4. The relation of optical form to the utilization of amino acids. II. Utilization of stereoisomeric varieties of aspartic acid and asparagine by carrot root disks. *Plant Physiol.* 32 : 639 - 643, 1957.

5. Green, D. E. The malic dehydrogenase of animal tissues. *Biochem. J.* 30 : 2095 - 2110, 1936.
6. Kretovich, V. L. & Yakovleva, V. I. Biosynthesis of glutamic acid and glutamine in pea and wheat seedlings. *Plant Physiol. U. S. S. R.*, 6 : 174 - 178, 1959.
7. Krebs, H. A., & Johnson, W. A. The role of citric acid in intermediate metabolism in animal tissues. *Enzymologia*, 4 : 148 - 156, 1937.
8. Leonard, M. J. K. & Burris, R. H. A survey of transaminases in plants. *J. Biol. Chem.*, 170 : 701 - 709, 1947.
9. Mac Vicar, R. & Burris, R. H. Studies on nitrogen metabolism in tomato with use of isotopically labelled ammonium sulphate. *J. Biol. Chem.*, 176 : 511 - 516, 1948.
10. Pucher, G. W. & Vickery, H. B. The metabolism of organic acids of tobacco leaves I. Effect of culture of excised leaves in solutions of organic acid salts. *J. Biol. Chem.*, 178 : 557 - 575, 1949.
11. Rautanen, N. On the synthesis of the first amino acids in green plants. *Annales Acad. Sci. Fennicae, Ser. A, II*, No. 33, 1948.
12. Sideris, C. P., Young, H. Y. & Chun, H. H. Q. Effect of nitrogen on the nitrogenous fractions of *Ananas comosus* (L.) Merr. *Plant Physiol.*, 22 : 127 - 148, 1947.
13. Speck, J. F. The synthesis of glutamine in pigeon liver dispersions. *J. Biol. Chem.*, 179 : 1387 - 1403, 1949.
14. Vickery, H. B. & Pucher, G. W. The metabolism of amides in green plants. III. The mechanism of amide synthesis. *J. Biol. Chem.*, 128 : 703 - 713, 1939.
15. Vickery, H. B., Pucher, G. W., Leavenworth, C. S. & Wakeman, A. J. The metabolism of amides in green plants. II. The amides of rhubarb leaf. *J. Biol. Chem.*, 125 : 527 - 538, 1938.
16. Wakeman, A. J. & Leavenworth, C. S. Chemical investigations of the rhubarb plant. *Bull. Conn. Agric. Exp. Sta.*, No. 424, 1939.
17. Yemm, E. W. & Willis, A. J. Respiration of barley plants. IX. The metabolism of roots during the assimilation of nitrogen. *New Phytol.*, 55 : 229 - 252, 1956.