STUDIES ON THE EFFECT OF CERTAIN ENZYMIC POISONS ON THE METABOLISM OF STORAGE ORGANS. VII. THE EFFECT OF IODOACETATE ON THE METABOLISM OF NITROGEN COMPOUNDS BY RADISH ROOT SLICES

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SUMMARY

Feeding with various nitrogen compounds led to marked changes in the total amount and in the relative composition of the nitrogen pool in the radish slices. Possible modes of utilization of these compounds are given. Iodoacetate greatly retarded the uptake and utilization of NH_4Cl and aspartic acid. Whereas the uptake of KNO_3 was inhibited by iodoacetate, its utilization was markedly enhanced. The losses of nitrogen fractions from the differently treated tissues were almost recovered in the culture media.

The marked losses in carbohydrates would almost correspond in amount with the increased CO_2 output and with the synthesis of nitrogenous constituents. However, some of the lost carbohydrates from tissues treated with iodoacetate + either NH_4Cl or aspartic acid could be markedly accounted for in the culture media.

The rise in CO_2 output in iodoacetate is mainly attributed to increased accessibility, in the metabolic region, of substrates to enzymes. The differential rates of CO_2 production by tissues incubated in nitrogen media, either alone or supplemented with iodoacetate are interpreted on the bases that different rates of uptake of nitrogen compounds and of utilization of ATP are possible. Also the relation between nitrogen assimilation and the breakdown of carbohydrates is discussed.

1. INTRODUCTION

It is now well authenticated that iodoacetate, in low concentrations, increases the respiration of plant tissues mainly through increasing the accessibility, in the metabolic region, of substrates. Thus, it was found that low concentrations of iodoacetate induced characteristic changes in metabolites and considerably increased the CO_2 output and the permeability of the cell membranes as was evident from the increased leakage of certain metabolites from the tissues (BARKER & MAPSON 1964; BARKER & YOUNIS 1965a, b; YOUNIS 1969a, b; YOUNIS *et al.* 1969a, b).

In previous communications (YOUNIS *et al.* 1969b, e) iodoacetate (4×10^{-4}M) greatly retarded the uptake of sugars, substantially decreased the carbohydrate content and caused exudation of metabolites from the radish root slices into the culture media. Protein synthesis *via* amino acids was operative in iodoace-

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tate treated tissues and the fraction of protein synthesized, in sugar media, *via* sugar + nitrate was inhibited by iodoacetate. Furthermore, sugars and iodoacetate when joined together were found te mask the accelerating effect of each other on the CO_2 output by radish slices.

Thus it was thought of interest, in this work, to investigate the effects of a stimulatory concentration of iodoacetate (4×10^{-4} M) on the uptake and utilization of different nitrogen sources coupled with carbohydrate metabolism in radish root slices.

2. MATERIALS AND METHODS

Uniform healthy roots of *Raphanus sativus* var. *aegyptiacus* (about 50 days old) were selected for experimentation. Slices were prepared, sampled and pretreated (washed in running aerated tap water for 48 hours) as described by YOUNIS *et al.* (1969b).

After the pretreatment of slices two samples were analysed to determine the initial carbohydrate and nitrogen contents as in YOUNIS *et al.* (1969a). The remaining samples were transferred to air-tight chambers each containing 400 ml of culture solution according to the following scheme: Samples 1 & 2, distilled water; 3 & 4, 7.14 mM NH_4Cl; 5 & 6, 7.14 mM KNO_3; 7 & 8, 7.14 mM aspartic acid; 9 & 10, 4×10^{-4}M iodoacetate; 11 & 12, 4×10^{-4}M iodoacetate + 7.14 mM NH_4Cl; 13 & 14, 4×10^{-4}M iodoacetate + 7.14 mM KNO_3; 15 & 16, 4×10^{-4}M iodoacetate + 7.14 mM aspartic acid. The apparatus and the solutions were sterilized before use and we found no evidence of microbial contamination of the washed slices.

After transferring the samples into respective media, 50 ml of each were taken for analysis. 50 ml aliquots were also taken after 24 and 48 hours for determining the uptake of nitrogen and the leakage of different metabolites from tissues into the media. The samples were kept at 25 °C and aerated for 48 hours by passing CO_2-free air through each culture solution at a constant rate of 4 litres per hour. The air currents, after leaving the culture chambers, were passed through standard solutions of NaOH to determine the rates of CO_2 production by the different treated tissues.

After 48 hours the tissue samples were drained, washed with distilled water, dried on paper towels and divided into 2 equal portions for carbohydrate and nitrogen determinations. The maximum deviation between carbohydrate and nitrogen contents of duplicate samples was about 5% and thus the mean values are presented.

3. RESULTS AND DISCUSSION

3.1. Uptake of nitrogen

The present results indicate that aged radish root slices had a marked ability to absorb (*table 1*) and utilize nitrogen (*tables 2* and *3*). In the first 24 hours nitrogen uptake was much higher than in the second 24 hours. The rate of up-

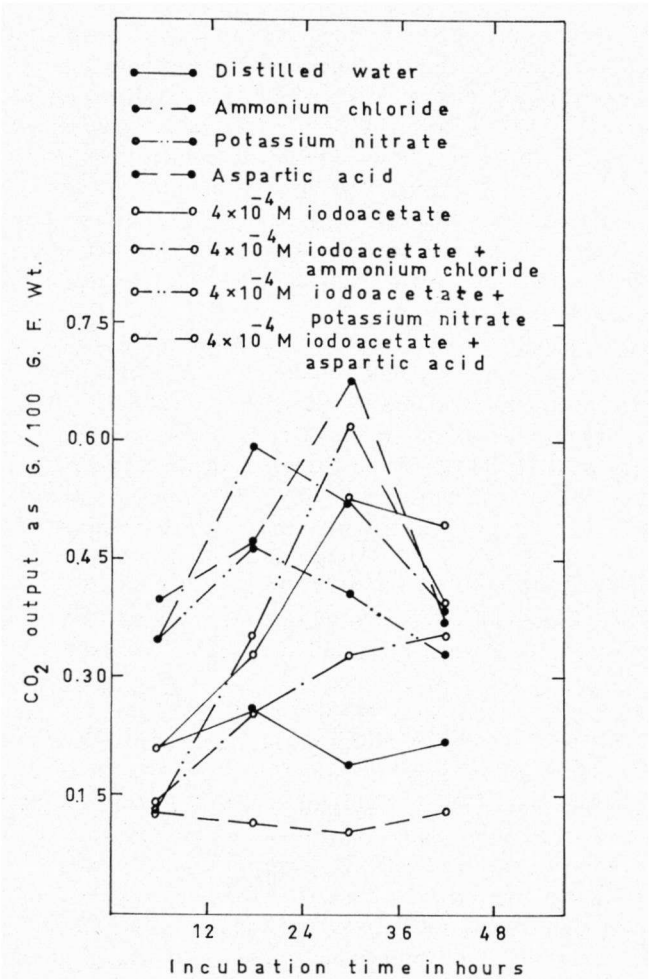

Fig. 1. Changes in the rate of CO_2 output from aged radish root slices incubated in different culture media, for 48 hours.

take was highest from aspartic acid, less from KNO_3 and least from NH_4Cl. The differences in the degree of absorption of these compound may be the result of the differences in their rates of penetration into the cells. It may be suggested, as adopted earlier for glutamic acid (EL-SHISHINY & NOSSEIR 1957) and for sugars (GLASZIOU 1960; YOUNIS *et al.* 1969d), that the nitrogen compound first combines with some cellular constituent at the surface and then this complex is transported into the cell; differences in penetrability of these complexes might have been possible.

The nitrogen uptake was accompanied with higher respiration rates (*fig. 1*). This seems to indicate the dependence of nitrogen uptake upon respiratory energy as suggested by WEBSTER (1954), also by BIRT & HIRD (1956) and YOUNIS *et al.* (1969c) who reported inhibition of nitrogen uptake by KCN.

In support of this conclusion, iodoacetate, which is known to inhibit phosphorylation (JAMES 1953), retarded the rate of nitrogen uptake to nearly half that rate in its absence (*table 1*). This retardation was operative from the beginning since the uptake of nitrogen was much more retarded in the first then in the second 24 hours. An induction of leakage of nitrate-N in the first 24 hours was also apparent.

The retarding action of iodoacetate was more pronounced on the uptake of aspartic acid than on the uptake of the other two nitrogen sources (*table 1*). However, this might be related to the pH of the medium; that of aspartic acid being much more acidic than those of HN_4Cl and KNO_3 and thus the inhibitory action of iodoacetate was accentuated. In this connection it may be mentioned that BOSWELL (1950) and LATIES (1949) reported decrease in inhibition of respiration of plant tissues by iodoacetate on decreasing the acidity.

3.2. Changes in nitrogen fractions

Incubation of slices in water or in iodoacetate induced changes in the nitrogen fractions (*table 2*), in general, similar to those observed by YOUNIS *et al.* (1969a).

Feeding with nitrogen sources slightly increased the protein- as well as the total soluble-N of the tissues except in the case of aspartic acid where the total soluble-N markedly decreased inspite of the highest amount of nitrogen absorbed by the respective tissues. Variable changes in the soluble-N fractions as compared with those of water controls were apparent (*table 2*). The inclusion of iodoacetate in the nitrogen media substantially decreased the soluble-N fractions whereas the protein-N levels in the tissues were slightly changed.

Analysis of the media revealed no nitrogen in water (*table 3*). In iodoacetate nitrogen leached out in appreciable amounts. Nitrogen administration with or without iodoacetate induced leakage of soluble-N into the media.

Careful examination of *tables 2* and *3* revealed certain metabolic changes of

Table 1. The effects of iodoacetate on the uptake of nitrogen by radish root slices. The values are given as mg N per 100 g original fresh weight of slices.

Culture medium	1st 24 hr	2 nd 24 hr	Total
7.14 mM NH_4Cl	28.5	7.0	35.5
7.14 mM KNO_3	30.2	9.0	39.2
7.14 mM aspartic acid	29.2	16.8	46.0
4×10^{-4} M iodoacetate + 7.14 mM NH_4Cl	3.6	8.3	11.9
4×10^{-4} M iodoacetate + 7.14 mM KNO_3	-6.9*	24.4	17.5
4×10^{-4} M iodoacetate + 7.14 mM aspartic acid	0.0	16.7	16.7

* Refers to leakage from tissue slices.

Table 2. Analysis of the nitrogen content of radish root slices at the start and after 48 hours of incubation in different media. The values are given as mg N per 100 g original fresh weight of slices.

Culture medium	Ammonia -N	Amide -N	Amino -N	Nitrate -N	"Peptide -N"	Total soluble -N	Protein -N	Total -N
Initials	2.6	6.2	8.4	20.9	8.4	46.5	44.1	90.6
Distilled water	1.6	5.0	8.4	19.2	8.9	43.1	46.8	89.9
7.14 mM NH_4Cl	16.8	10.3	9.0	8.4	4.8	49.3	50.8	100.1
7.14 mM KNO_3	4.0	2.0	4.2	35.4	4.9	50.5	51.2	101.7
7.14 mM aspartic acid	8.1	3.6	11.0	6.0	0.6	29.3	49.8	79.1
4×10^{-4} M iodoacetate	2.3	0.5	0.0	1.2	7.3	11.3	52.4	63.7
4×10^{-4} M iodoacetate + 7.14 mM NH_4Cl	8.6	1.5	0.0	3.5	3.0	16.6	49.7	66.3
4×10^{-4} M iodoacetate + 7.14 mM KNO_3	2.1	3.5	2.8	4.8	4.4	17.6	50.4	68.0
4×10^{-4} M iodoacetate + 7.14 mM aspartic acid	1.4	0.5	8.3	0.7	2.7	13.6	46.1	59.7

interest. Thus, the presence of large amounts of ammonia-N in the aspartic acid media and in the respective tissues seems to indicate that a good deal of aspartic acid has been oxidatively deaminated both at the cytoplasmic surfaces and inside the radish cells. The rest of the utilized amino-N was transformed into amides, peptides and proteins. Ammonia production associated with amino acid utilization was earlier reported (STEPHENSON & GALE 1937; YOUNIS 1960) and similar modes of utilization of alanine (SAID & YOUNIS 1953) and aspartic acid (YOUNIS *et al.* 1969c) were described. Apparently iodoacetate strongly inhibited the oxidative deamination of aspartic acid and its incorporation into proteins. Hence, the complete absence of ammonia-N in the respective media, its low level in the tissues and the slight suppression of protein formation could be explained.

The radish slices fed with KNO_3 showed higher levels of amino- and ammonia-N both in the tissues and in the media than in those of water controls and thus it is possible that nitrate, through the classical reduction pathway (cf. STEWARD & STREET 1947), might have contributed to ammonia formation with simultaneous appearance of peptides and proteins. Iodoacetate seems to have enhanced these transformations.

NH_4Cl alone or with iodoacetate induced the accumulation of a good deal of the absorbed ammonia-N; the rest being assimilated into peptides and proteins, a part of the former fraction was excreted into the culture media.

Moreover, the losses of nitrogen fractions from the differently treated tissues were almost recovered in the culture media. Iodoacetate had an accelerating

Table 3. Average values of the various nitrogen fractions leached from radish root slices into the culture media during 48 hours of incubation. The values are given as mg N per 100 g original fresh weight of slices.

Culture medium	Ammonia -N	Amide -N	Amino -N	Nitrate -N	"Peptide -N"	Total soluble -N
Distilled water	0.0	0.0	0.0	0.0	0.0	0.0
7.14 mM NH_4Cl	–	2.3	4.4	10.4	9.1	26.2
7.14 mM KNO_3	4.8	1.2	8.3	–	13.2	27.5
7.14 mM aspartic acid	27.4	11.8	–	11.1	7.3	57.6
4×10^{-4} M iodoacetate	4.1	7.2	4.2	8.4	2.4	26.3
4×10^{-4} M iodoacetate + 7.14 mM NH_4Cl	–	6.5	0.0	17.1	18.3	41.9
4×10^{-4} M iodoacetate + 7.14 mM KNO_3	7.7	3.5	12.3	–	21.8	45.3
4×10^{-4} M iodoacetate 7.14 mM aspartic acid	0.0	6.6	–	20.4	25.9	52.9

Table 4. Analysis of the carbohydrate content of radish root slices at the start and after 48 hours of incubation in different media. The values are given as mg glucose equivalent per 100 g original fresh weight of slices.

Culture medium	Glucose	Fructose	Sucrose	Polysaccharides	Total sugars
Initials	235.7	266.8	57.3	286.8	846.6
Distilled water	89.3	122.2	12.5	188.4	412.4
7.14 mM NH_4Cl	3.1	10.8	2.9	162.5	179.3
7.14 mM KNO_3	12.1	60.3	4.0	178.6	255.0
7.14 mM aspartic acid	23.2	30.8	6.1	213.3	273.4
4×10^{-4} M iodoacetate	9.8	6.2	0.0	144.3	160.3
4×10^{-4} M iodoacetate + 7.14 mM NH_4Cl	2.6	4.2	2.9	176.1	185.8
4×10^{-4} M iodoacetate + 7.14 mM KNO_3	0.0	5.7	0.0	162.8	168.5
4×10^{-4} M iodoacetate + 7.14 mM aspartic acid	3.7	12.2	8.3	283.9	308.1

effect on the leakage of nitrogen fractions which is consistent with increased permeability of cell membranes (BARKER &YOUNIS 1965b; YOUNIS 1969a, b; YOUNIS *et al.* 1969a, b).

It is also apparent that protein synthesis is not favoured in highly acidic media. Thus the low levels of proteins in aspartic acid media either alone or in combination with iodoacetate might be explained.

3.3. Changes in carbohydrates

Starvation in water or incubation in iodoacetate induced substantial losses in all the carbohydrate fractions examined (*table 4*). Compared with water samples, NH_4Cl, KNO_3 and aspartic acid induced further losses in the glucose, fructose and sucrose contents whereas the polysaccharides content appeared either to decrease slightly in NH_4Cl and in KNO_3 or to show a higher content in aspartic acid media. The presence of iodoacetate in the nitrogen media, in general, induced more losses in the soluble sugars than those observed in its absence. Polysaccharides, on the other hand, showed slight changes in NH_4Cl and KNO_3 and in aspartic acid, a value comparable to that of initial samples was apparent (*table 4*).

In water, nitrate and iodoacetete media, reducing sugars diffused out of the tissues in small amounts while in NH_4Cl and aspartic acid media no sugars were detected (*table 5*). The inclusion of iodoacetate in the nitrate media doubled the rate of leakage of sugars but in the NH_4Cl and aspartic acid media great amounts of sugars leached out with a higher magnitude in the latter than in the former medium (*table 5*). Thus, in the two latter media some of the lost sugars from tissues could be accounted for in the culture media.

The above variable losses in the carbohydrate fractions accompanied the variably increased rates of CO_2 output (*fig. 1*) during the assimilation of nitrogen by the radish root cells. As no external supplies of carbohydrate were provided, these losses in carbohydrates can be related to respiration and the synthesis of nitrogenous constituents. In this connection it is interesting to mention that YEMM (1954) indicated that in barly roots and yeast the breakdown of carbohydrates was adequate to meet the needs for both the synthesis of nitrogenous constituents and the production of CO_2. Feeding different nitrogen sources either alone or in presence of a stimulatory concentration (10^{-3}M) of KCN to radish slices, similar observations were recorded by YOUNIS *et al.* (1969c).

Table 5. Average values of the reducing sugars leached from radish root slices into the culture media during 48 hours of incubation. The values are given as mg glucose equivalent per 100 g original fresh weight of slices.

Culture medium	Reducing sugars
Distilled water	4.2
7.14 mM NH_4Cl	0.0
7.14 mM KNO_3	8.1
7.14 mM aspartic acid	0.0
4×10^{-4} M iodoacetate	8.1
4×10^{-4} M iodoacetate + 7.14 mM NH_4Cl	101.9
4×10^{-4} M iodoacetate + 7.14 mM KNO_3	16.1
4×10^{-4} M iodoacetate + 7.14 mM aspartic acid	270.7

The marked leakage of sugars from the tissues to the different media containing iodoacetate is in accord with the previous suggestion that iodoacetate increases the permeability of cell membranes (see above references). This effect of iodoacetate appeared to be more pronounced in acidic (aspartic acid) than in neutral (KNO_3 and NH_4Cl) media.

3.4. CO_2 output

In water an initial increase was followed by a lower rate of CO_2 production. 4×10^{-4}M iodoacetate induced a sharp increase followed by a slight decrease in CO_2 output (*fig. 1*). This high rate of respiration is mainly due to an increase in accessibility of substrates to enzymes in the metabolic region (BARKER & YOUNIS 1965; YOUNIS 1969a, b; YOUNIS *et al.* 1969a).

Feeding slices with nitrogen sources led to rapid increase in the rate of CO_2 production. The high rates were maintained for a longer time in the case of aspartic acid. Afterwards, a slow and a quick fall in CO_2 production were apparent in NH_4Cl and KNO_3 and in aspartic acid media respectively. These effects of nitrogen sources on the rate of respiration seem to be associated with their uptake and assimilation that are closely coupled with a rapid breakdown of carbohydrates of the slices. It is very probable that the depletion of the limited carbohydrate reserves in the tissues is an important factor causing the secondary decline in the rate of CO_2 production. Thus, an essential condition for rapid assimilation of nitrogen is a high level of readily available carbohydrates in the cells (YEMM 1954). In support of the present results, nitrogenous compounds have been found to increase the rate of respiration of various tissues (YEMM 1954; SYRETT 1958; SAID & YOUNIS 1952, 1953; YOUNIS 1960; YOUNIS *et al.* 1969c; YOUNIS & SULEIMAN 1970).

Supplemental addition of iodoacetate to the nitrogen media induced a rate of CO_2 production lower than that of water controls during the first 12 hours. This low rate was maintained throughout the experiment in iodoacetate + aspartic acid media whereas in iodoacetate + NH_4Cl a continuous increase in CO_2 production was apparent. A sharp continuous increase followed by a late sharp fall in CO_2 production of samples in iodoacetate + KNO_3 was observed (*fig. 1*).

The immediate reduction in CO_2 output due to inclusion of iodoacetate in the nitrogen media might have resulted from an initial fall in ATP concentration which could be due in part to rapid initial utilization of ATP in assimilatory reactions (SYRETT 1958; YEMM & FOLKES 1958) and in part to inhibition of phosphorylation by iodoacetate (JAMES 1953). The fact that iodoacetate + KNO_3 did not decrease the rate of respiration below that induced by KNO_3 whereas iodoacetate + NH_4Cl elicited a lower rate than that of NH_4Cl alone could be attributed to the relative rates of utilization of ATP in their assimilation. The steps involved in nitrate utilization in radish slices may not require an ample supply of ATP as do those involved in NH_4Cl utilization.

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