Effect of Various Salts on the Synthesis of Ascorbic Acid and Carotene in Cress Seedlings

BY L. W. MAPSON AND E. M. CRUICKSHANK, *Dunn Nutritional Laboratory, University of Cambridge, and Medical Research Council*

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The excessive application of nitrogen to apple trees has been shown to reduce the ascorbic acid content of the fruit (Kessler, 1939). A similar observation on grape-fruit was made by Jones, Van Horn, Finch, Smith & Caldwell (1944) who noted also an inverse relationship between the nitrogen content of the fruit and the ascorbic acid content. Several investigators, however, including Karikka, Dudgeon & Hauck (1944) and Hamner, Bernstein & Maynard (1945), who worked on potatoes and tomatoes respectively, found that the effect of nitrogenous fertilizers on the ascorbic acid content was overshadowed by the effect of environmental factors such as soil and light intensity. Moreover, as has been pointed out by Hamner *et al.* (1945), the effect of nitrogen *per se* is difficult to assess since nitrogenous fertilizers inevitably affect the size of crop, amount of foliage, etc., which have important secondary effects on the synthesis of ascorbic acid.

In the present work we have investigated the influence of nitrogenous and other salts on the synthesis of ascorbic acid, and in doing so have eliminated as far as possible the effect of other factors. We used as experimental material seedlings germinated on filter paper in dishes, since under these conditions factors such as temperature, intensity of illumination and supply of nutrients could more easily be controlled.

Giroud (1938) has pointed out that many fruits which are rich in ascorbic acid are rich also in carotene. Moreover, the work of Neubauer (1939), Moldtmann (1939) and Weber (1940) suggests that the co-existence of ascorbic acid and carotene is not accidental. In view of the possibility that the synthesis of carotene may be related to that of ascorbic acid, we also examined the effect of the different treatments on the synthesis of carotene.

METHODS

Conditions of germination. The seeds were germinated on ashless filter paper in glass crystallizing dishes, provided with glass covers to prevent evaporation. The dishes in any one experiment were subjected to identical conditions of temperature and illumination, the latter being provided by a north light. Since work was carried out at different seasons, illumination and temperature varied slightly as

between individual experiments, the latter fluctuating between 15 and 20°. The seeds were kept moist during germination by the frequent addition of small amounts of the solutions being tested.

Composition of solutions used for germination. In certain of these experiments, a nutrient solution composed as follows was used:

CaCl ₂	0.8 g.
KH ₂ PO ₄	0.1 g.
K ₂ HPO ₄	0.1 g.
MgSO ₄ ·7H ₂ O	0.1 g.
FeCl ₃	Trace

Glass-distilled water to 1 l.

The salts being tested were dissolved either in glass-distilled water (G.D. water), or in the nutrient solution in accordance with the control used, e.g. if nutrient solution was used as control the salts were dissolved in this, though for the sake of brevity, this has not been specifically indicated in the tables.

Reagents. The salts used were of A.R. purity.

Estimation of ascorbic acid. Ascorbic acid was estimated chemically with 2:6-dichlorophenolindophenol, by means of a photoelectric absorptiometer. The procedure adopted was similar to, though not identical with, that used by Bessey (1938). Reference to Table 1 shows that differences between duplicate analyses were so small as to be negligible. Dehydroascorbic acid was estimated after reduction by H₂S.

Since certain of the salts used in our experiments have to some extent affected growth as well as the synthesis of ascorbic acid, we have expressed our results not only in terms of concentration (mg./g. of fresh seedling) but also as the total amount produced by 100 seedlings, which was the number taken for each analysis. In those instances where growth was appreciably affected, the total amount synthesized gives a better indication of the effect of the nutrient supplied.

For the estimation of ascorbic acid, the complete seedling, dissected from the testa, was taken for analysis, after removal of adhering moisture.

Estimation of carotene. As with ascorbic acid, carotene was estimated in the complete seedling from which the testa and adhering moisture had been removed. Seedlings were generally taken from batches which were also used for ascorbic acid estimations. The chromatographic method of Booth (1945) was employed, with alumina-sodium sulphate as adsorbent. The precautions mentioned by him, e.g. extraction in dim light, were taken to avoid destruction of the carotene. Fifty seedlings were taken for analysis, and all analyses were done in duplicate, the figures reported in

the tables being the mean of such duplicates. The standard error of duplicates was ± 0.404 , which amounts to $\pm 3.65\%$ of the mean of the duplicates. The agreement was, therefore, satisfactory. The carotene (in light petroleum solution) was estimated by means of a photoelectric absorptiometer incorporating a Wratten filter no. 47. The instrument was calibrated with solutions of pure β -carotene. Results obtained with this colorimeter agreed well with those obtained in a Pulfrich photometer.

Estimation of pH of cell sap. Seedlings (2 g.) were thoroughly washed with distilled water and ground to a fine mixture with 5 ml. of distilled water in a glass mortar. The pH was determined immediately by means of the glass electrode.

RESULTS

Synthesis of ascorbic acid

Effect of ammonium sulphate on synthesis of ascorbic acid in cress seedlings. When cress was

grown under the conditions described above, in the presence of $(\text{NH}_4)_2\text{SO}_4$ a considerable reduction in the concentration and total amount of ascorbic acid synthesized was observed. This occurred not only when the salt constituted the sole nutrient, but also when it was supplied in conjunction with a nutrient solution containing Ca^{++} , K^+ , Mg^{++} , Fe^{+++} , Cl^- , PO_4^- and SO_4^- (see Table 1). The effect of $(\text{NH}_4)_2\text{SO}_4$ in decreasing the production of ascorbic acid was not a temporary phenomenon, but was observed during all stages of development up to 170 hr.

The extent to which the synthesis of ascorbic acid was reduced depended on the concentration of $(\text{NH}_4)_2\text{SO}_4$ present (Table 2). Growth was affected with the highest concentration (0.1M) tested, but with a concentration of 0.02M there was little or no impairment of growth, though the amount of ascorbic acid formed was greatly reduced. These

Table 1. *Effect of $(\text{NH}_4)_2\text{SO}_4$ on the synthesis of ascorbic acid in cress seedlings*

Exp. no.	Treatment	Duration of exp. (hr.)	Wt. of 100 seedlings (g.)	Ascorbic acid synthesized			
				Concentration		Total produced by 100 seedlings	
				(mg./g.)	(% of control)	(mg.)	(% of control)
1	Control (G.D.* water)	74	1.43	0.52	—	0.74	—
	$(\text{NH}_4)_2\text{SO}_4$, 0.02M	74	1.57	0.095	18.2	0.15	20.0
2	Control (G.D. water)	75	1.62	0.54	—	0.87	—
	$(\text{NH}_4)_2\text{SO}_4$, 0.02M	75	1.72	0.10	18.6	0.17	19.6
3	Control (G.D. water) (1)	90	1.75	0.51	0.50	0.89	0.875
	Control (G.D. water) (2)	90	1.79	0.49		0.86	
	$(\text{NH}_4)_2\text{SO}_4$, 0.02M (1)	90	1.67	0.217	0.218	0.35	0.35
	$(\text{NH}_4)_2\text{SO}_4$, 0.02M (2)	90	1.62	0.22		0.35	
	Control (nutrient solution) (1)	90	1.84	0.42	0.395	0.78	0.775
	Control (nutrient solution) (2)	90	2.1	0.37		0.77	
	Nutrient solution + $(\text{NH}_4)_2\text{SO}_4$, 0.02M (1)	90	1.77	0.147	0.14	0.26	0.27
	Nutrient solution + $(\text{NH}_4)_2\text{SO}_4$, 0.02M (2)	90	1.90	0.14		0.28	
	Control (nutrient solution)	89	2.03	0.33	—	0.67	—
	Nutrient solution + $(\text{NH}_4)_2\text{SO}_4$, 0.02M	89	1.90	0.06	18.0	0.11	16.0

* G.D. = glass distilled.

Table 2. *Effect of concentration of $(\text{NH}_4)_2\text{SO}_4$ on the synthesis of ascorbic acid in cress seedlings*

Treatment	Duration of exp. (hr.)	Wt. of 100 seedlings (g.)	Ascorbic acid synthesized			
			Concentration		Total produced by 100 seedlings	
			(mg./g.)	(% of control)	(mg.)	(% of control)
Control (nutrient solution)	96	2.02	0.31	—	0.62	—
$(\text{NH}_4)_2\text{SO}_4$, 0.002M	96	2.33	0.30	97	0.69	110
$(\text{NH}_4)_2\text{SO}_4$, 0.004M	96	2.25	0.28	90	0.62	100
$(\text{NH}_4)_2\text{SO}_4$, 0.01M	96	2.11	0.25	80	0.53	85
$(\text{NH}_4)_2\text{SO}_4$, 0.02M	96	1.99	0.11	35	0.21	34
$(\text{NH}_4)_2\text{SO}_4$, 0.04M	96	1.90	0.09	29	0.18	29
$(\text{NH}_4)_2\text{SO}_4$, 0.1M	168*	0.76	0.12	39	0.09	15

* Germination delayed.

results are of interest in that they show that a reduction in concentration of ascorbic acid may take place, unaccompanied by any appreciable depression of growth, a finding in accord with the observations of Ray (1934) on germinating pea seedlings.

Dehydroascorbic acid was also determined in some experiments. The amount found was no greater in the seedlings grown in the presence of $(\text{NH}_4)_2\text{SO}_4$ than in those grown in control solutions. The smaller amount of ascorbic acid produced in the former was, therefore, not due to any alteration in the balance between the amounts of reduced and oxidized vitamin present (Table 3).

Since several workers have shown that illumination aids the synthesis of ascorbic acid by the plant, it was thought possible that the inhibiting effect of ammonium sulphate on chlorophyll formation might be the cause of the reduction in the ascorbic acid synthesized. In this event, the depressing effect of $(\text{NH}_4)_2\text{SO}_4$ should not be observed in seedlings in which the formation of chlorophyll was prevented by the complete absence of light.

The results (Table 4) show that, although the total amount of ascorbic acid synthesized was reduced in the dark, the relative amounts synthesized in seeds

Table 3. *Effect of $(\text{NH}_4)_2\text{SO}_4$ on the synthesis of ascorbic acid and dehydroascorbic acid in cress seedlings*

Exp. no.	Treatment	Duration of exp. (hr.)	Ascorbic acid		Dehydroascorbic acid		Total ascorbic acid (D.H.A. + A.A.)	
			Concentration (mg./g.)	Total produced by 100 seedlings (mg.)	Concentration (mg./g.)	Total produced by 100 seedlings (mg.)	Concentration (mg./g.)	Total produced by 100 seedlings (mg.)
1	Control (G.D. water)	90	0.485	0.86	0.08	0.14	0.565	1.00
	$(\text{NH}_4)_2\text{SO}_4$, 0.02M	90	0.215	0.35	0.085	0.13	0.30	0.48
2	Control (nutrient solution)	90	0.365	0.77	0.051	0.105	0.42	0.87
	Nutrient solution + $(\text{NH}_4)_2\text{SO}_4$, 0.02M	90	0.145	0.28	0.06	0.11	0.20	0.39

Table 4. *The production of ascorbic acid in cress seedlings germinated in different salt solutions in the presence and absence of light*

Exp. no.	Treatment	Duration of exp. (hr.)	Wt. of 100 seedlings (g.)	Ascorbic acid synthesized			
				Concentration		Total produced by 100 seedlings	
				(mg./g.)	(% of control)	(mg.)	(% of control)
1	Control (G.D. water)	72, light	1.65	0.45	—	0.74	—
	$(\text{NH}_4)_2\text{SO}_4$, 0.02M	72, light	1.8	0.048	11	0.086	12
	Control (G.D. water)	72, dark	2.3	0.23	—	0.54	—
	$(\text{NH}_4)_2\text{SO}_4$, 0.02M	72, dark	2.04	0.028	12	0.057	11
2	Control (G.D. water)	76, light	—	0.52	—	—	—
	NH_4NO_3 , 0.02M	76, light	—	0.50	96	—	—
	KNO_3 , 0.04M	76, light	—	0.61	117	—	—
	$(\text{NH}_4)_2\text{SO}_4$, 0.02M	76, light	—	0.15	29	—	—
	NH_4Cl , 0.04M	76, light	—	0.16	31	—	—
	Control (G.D. water)	125, light	—	0.59	—	—	—
	NH_4NO_3 , 0.02M	125, light	—	0.48	82	—	—
	KNO_3 , 0.04M	125, light	—	0.66	112	—	—
	$(\text{NH}_4)_2\text{SO}_4$, 0.02M	125, light	—	0.16	27	—	—
	NH_4Cl , 0.04M	125, light	—	0.154	26	—	—
	Control (G.D. water)	54, dark	—	0.37	—	—	—
	NH_4NO_3 , 0.02M	54, dark	—	0.35	95	—	—
	KNO_3 , 0.04M	54, dark	—	0.39	106	—	—
	$(\text{NH}_4)_2\text{SO}_4$, 0.02M	54, dark	—	0.18	49	—	—
	NH_4Cl , 0.04M	54, dark	—	0.18	49	—	—

The effect of ammonium salts in the presence and absence of light. It was noticed that cress seeds germinated in the presence of $(\text{NH}_4)_2\text{SO}_4$ solutions appeared less green and contained less chlorophyll than seeds germinated in water or the nutrient

grown with and without $(\text{NH}_4)_2\text{SO}_4$ remained approximately the same as in seeds grown in the light. It is clear, therefore, that the effect of $(\text{NH}_4)_2\text{SO}_4$ is not due merely to any influence which it has on photosynthetic activity.

Table 5. *Effect of KNO₃ on the synthesis of ascorbic acid in cress seedlings*

Exp. no.	Treatment	Duration of exp. (hr.)	Wt. of 100 seedlings (g.)	Ascorbic acid synthesized			
				Concentration		Total produced by 100 seedlings	
				(mg./g.)	(% of control)	(mg.)	(% of control)
1	Control (nutrient solution)	89	2.03	0.33	—	0.67	—
	KNO ₃ , 0.04M	89	1.95	0.42	127	0.815	122
2	Control (G.D. water)	68	1.42	0.39	—	0.55	—
	KNO ₃ , 0.04M	68	1.31	0.39	100	0.51	93
	Control (G.D. water)	92	2.05	0.33	—	0.68	—
	KNO ₃ , 0.04M	92	2.32	0.35	106	0.81	119
	Control (G.D. water)	140	2.98	0.27	—	0.79	—
	KNO ₃ , 0.04M	140	3.93	0.20	74	0.80	101
3	Control (nutrient solution)	48	—	0.47	—	—	—
	KNO ₃ , 0.04M	48	—	0.45	96	—	—
	Control (nutrient solution)	72	—	0.44	—	—	—
	KNO ₃ , 0.04M	72	—	0.59	134	—	—
4	Control (G.D. water)	76	—	0.52	—	—	—
	KNO ₃ , 0.04M	76	—	0.61	117	—	—

Table 6. *Effect of different ammonium salts on the synthesis of ascorbic acid in seedlings*

Exp. no.	Treatment	Type of seed	Duration of exp. (hr.)	Wt. of 100 seedlings (g.)	Ascorbic acid synthesized			
					Concentration		Total produced by 100 seedlings	
					(mg./g.)	(% of control)	(mg.)	(% of control)
1	Control (G.D. water)	Cress	68	1.42	0.39	—	0.55	—
	NH ₄ Cl, 0.04M	"	68	1.35	0.048	12.0	0.066	12.0
	NH ₄ NO ₃ , 0.02M	"	68	1.53	0.32	82.0	0.49	89.0
	(NH ₄) ₂ SO ₄ , 0.02M	"	68	1.30	0.06	15.0	0.08	15.0
	(NH ₄) ₂ HPO ₄ , 0.02M	"	68	1.17	0.15	39.0	0.17	31.0
	Control (G.D. water)	"	92	2.05	0.33	—	0.68	—
	NH ₄ Cl, 0.04M	"	92	1.59	0.03	9.0	0.05	7.0
	NH ₄ NO ₃ , 0.04M	"	92	1.94	0.23	70.0	0.45	66.0
	(NH ₄) ₂ SO ₄ , 0.02M	"	92	1.64	0.07	21.0	0.12	18.0
	(NH ₄) ₂ HPO ₄ , 0.02M	"	92	1.82	0.17	51.0	0.31	46.0
	Control (G.D. water)	"	140	2.98	0.27	—	0.79	—
	NH ₄ Cl, 0.04M	"	140	2.21	0.05	19.0	0.10	13.0
	NH ₄ NO ₃ , 0.04M	"	140	2.68	0.17	63.0	0.46	58.0
	(NH ₄) ₂ SO ₄ , 0.02M	"	140	2.58	0.05	19.0	0.14	18.0
	(NH ₄) ₂ HPO ₄ , 0.02M	"	140	2.81	0.13	48.0	0.37	47.0
	NH ₄ acetate, 0.04M*	"	140	1.71	0.36	134.0	0.62	79.0
2	Control (G.D. water)	Cress	120	2.35	0.34	—	0.77	—
	NH ₄ HCO ₃ , 0.02M†	"	120	2.23	0.35	103.0	0.78	101.0
3	Control (G.D. water)	Wheat	100	9.5	0.34	—	3.2	—
	NH ₄ acetate, 0.04M	"	100	6.5	0.36	106.0	2.35	74.0
	NH ₄ Cl, 0.04M	"	100	6.3	0.25	74.0	1.56	49.0
	NH ₄ NO ₃ , 0.02M	"	100	9.4	0.32	94.0	3.0	96.0
	(NH ₄) ₂ SO ₄ , 0.02M	"	100	6.6	0.25	74.0	1.68	52.0
	Control (G.D. water)	"	124	10.8	0.28	—	3.1	—
	NH ₄ acetate, 0.04M	"	124	9.8	0.35	125.0	3.35	108.0
	NH ₄ Cl, 0.04M	"	124	9.6	0.24	86.0	2.29	74.0
	NH ₄ NO ₃ , 0.02M	"	124	12.9	0.31	111.0	4.0	128.0
	(NH ₄) ₂ SO ₄ , 0.02M	"	124	8.8	0.25	90.0	2.2	71.0
	Control (G.D. water)	"	148	13.0	0.34	—	4.4	—
	NH ₄ acetate, 0.04M	"	148	12.5	0.38	112.0	4.7	107.0
	NH ₄ Cl, 0.04M	"	148	10.6	0.23	68.0	2.2	50.0
	NH ₄ NO ₃ , 0.02M	"	148	14.6	0.34	100.0	5.0	114.0
	(NH ₄) ₂ SO ₄ , 0.02M	"	148	8.8	0.26	76.0	2.3	52.0

* Estimations at 68 and 92 hr. were not possible since germination was delayed.

† Solution of ammonia, 0.02M, saturated with CO₂.

Effect of potassium nitrate. Seeds were germinated in an aqueous solution of KNO_3 (0.04M), or in a solution of KNO_3 (0.04M) in nutrient solution. The concentration of nitrogen in the medium, therefore, was comparable with that used in the previous experiments. KNO_3 in this concentration did not depress the synthesis of ascorbic acid. In most cases, indeed, rather more ascorbic acid was synthesized than in the controls (Table 5).

Effect of asparagine. In another experiment nitrogen was supplied as asparagine, which had no influence on the synthesis of ascorbic acid. Thus the amount synthesized by seedlings grown in an asparagine solution (0.02M) was 94, 112 and 100% of that formed in seedlings grown in distilled water after periods of germination of 68, 92 and 140 hr., respectively.

Effect of different cations combined with anions utilized to varying extents by the plant

Effect of various cations combined with a 'non-utilizable' anion. From the above results it appeared that NH_4^+ *per se* was not the sole operative factor, but that the extent to which the anion was utilized or disposed of by the plant was of major importance. In agreement with this hypothesis was the fact that the amount of ascorbic acid synthesized by seedlings grown in the nutrient solution (which contained most of the anions in the form of SO_4^{2-} or Cl^-) was always lower than the amount synthesized by seedlings grown in water. The effect of various cations when combined with a 'non-utilizable' anion, viz. chloride, was therefore further investigated. The results of experiments (Table 7) in which cress seed-

Table 7. *The effect of different chlorides on the synthesis of ascorbic acid in cress seedlings*

Exp. no.	Treatment	Duration of exp. (hr.)	Wt. of 100 seedlings (g.)	Ascorbic acid synthesized			
				Concentration		Total produced by 100 seedlings	
				(mg./g.)	(% of control)	(mg.)	(% of control)
1	Control (G.D. water)	66	1.62	0.57	—	0.92	—
	MgCl_2 , 0.02M	66	1.82	0.39	69	0.71	77
	NH_4Cl , 0.04M	66	1.82	0.12	21	0.22	24
	CaCl_2 , 0.02M	66	1.90	0.46	81	0.88	96
	KCl , 0.04M	66	1.91	0.46	81	0.88	96
	NaCl , 0.04M	66	2.20	0.32	56	0.71	77
2	Control (G.D. water)	90	2.28	0.43	—	0.99	—
	MgCl_2 , 0.02M	90	2.72	0.27	63	0.73	74
	NH_4Cl , 0.04M	90	2.18	0.12	28	0.26	26
	CaCl_2 , 0.02M	90	2.72	0.28	65	0.78	79
	KCl , 0.04M	90	2.94	0.30	70	0.88	89
	NaCl , 0.04M	90	2.88	0.28	65	0.81	82

The effect of different ammonium salts. Although the results recorded above suggested that nitrogen in the form of ammonium salts had an effect which differed from that of nitrogen in nitrate or asparagine, in subsequent experiments it became evident that the effect of the ammonium salts varied according to the nature of the anion (Table 6). Thus when NH_4^+ was combined with Cl^- or SO_4^{2-} , anions which are presumably not readily utilized in metabolic processes, the depressing effect was much greater than when it was combined with anions like HCO_3^- , NO_3^- or CH_3COO^- , which can be readily disposed of or transformed into other substances by the plant. The depressing effect of $(\text{NH}_4)_2\text{HPO}_4$ was intermediate, an observation in accord with the fact that PO_4^{3-} is more readily utilized than Cl^- or SO_4^{2-} , but less readily than HCO_3^- , NO_3^- , etc. It is of interest to note that, although $\text{CH}_3\text{COONH}_4$ delayed germination considerably, the amount of ascorbic acid ultimately synthesized was high.

lings were grown in solutions of chlorides of Mg^{++} , Ca^{++} , K^+ and NH_4^+ showed that, whilst the Mg , Ca and K salts all slightly depressed the synthesis of ascorbic acid, the effect of NH_4Cl was much greater.

It seems, therefore, that when the seed is germinated in the presence of a salt containing an anion not readily utilized for metabolic purposes, the synthesis of ascorbic acid is somewhat depressed. When, in addition, such an anion is combined with NH_4^+ , the depressing effect is much more pronounced. These results might be interpreted on the basis of the theory that the factor which favours the synthesis of ascorbic acid by the seedling is the amount of effective base resulting from the utilization of the anion by the plant. With ammonium salts the cation itself is utilized to a greater degree than the cations in other salts, and this still further reduces the amount of base available.

Table 8. *Comparison of the effect of different potassium or sodium salts on the synthesis of ascorbic acid in cress seedlings*

Exp. no.	Treatment	Duration of exp. (hr.)	Wt. of 100 seedlings (g.)	Ascorbic acid synthesized			
				Concentration		Total produced by 100 seedlings	
				(mg./g.)	(% of control)	(mg.)	(% of control)
1	Control (G.D. water)	144	2.15	0.35	—	0.74	—
	K acetate, 0.02M	144	1.85	0.58	166	1.07	144
	KHCO ₃ , 0.02M	144	2.35	0.45	128	1.06	143
	NH ₄ HCO ₃ , 0.02M	144	2.02	0.38	109	0.76	102
2	Control (nutrient solution)	120	2.26	0.25	—	0.57	—
	KHCO ₃ , 0.02M	120	2.19	0.35	140	0.77	136
	NaHCO ₃ , 0.02M	120	2.03	0.38	152	0.77	136
	K acetate, 0.02M	120	1.72	0.46	184	0.79	139
3	Control (G.D. water)	120	1.99	0.35	—	0.69	—
	KHCO ₃ , 0.04M	120	1.47	0.52	149	0.76	110
	KNO ₃ , 0.04M	120	2.3	0.44	126	1.0	145
	KCl, 0.04M	120	2.28	0.24	68	0.55	80
	K ₂ SO ₄ , 0.02M	120	1.82	0.30	86	0.55	80
4	Control (nutrient solution)	144	2.8	0.20	—	0.56	—
	KHCO ₃ , 0.02M	144	2.7	0.27	135	0.73	130
	KHCO ₃ , 0.04M	144	2.26	0.36	180	0.82	147

Table 9. *The effect on ascorbic acid synthesis in cress seedlings of (NH₄)₂SO₄ in the presence of succinic, malic or aspartic acids, or their sodium salts*

Exp. no.	Treatment	Duration of exp. (hr.)	Wt. of 100 seedlings (g.)	Ascorbic acid synthesized			
				Concentration		Total produced by 100 seedlings	
				(mg./g.)	(% of control)	(mg.)	(% of control)
1	Control (G.D. water)	75	1.62	0.54	—	0.87	—
	Succinic acid, 0.02M	75	1.69	0.59	109	1.0	115
	Succinic acid, 0.02M + (NH ₄) ₂ SO ₄ , 0.02M	75	1.88	0.12	22	0.22	25
	(NH ₄) ₂ SO ₄ , 0.02M	75	1.72	0.10	19	0.17	20
2	Control (G.D. water)	120	2.27	0.27	—	0.62	—
	Sodium succinate, 0.02M	120	2.23	0.40	148	0.88	142
	Sodium succinate, 0.02M + (NH ₄) ₂ SO ₄ , 0.02M	120	2.04	0.24	89	0.49	79
	(NH ₄) ₂ SO ₄ , 0.02M	120	1.61	0.11	40	0.18	29
3	Control (G.D. water)	120	2.3	0.375	—	0.87	—
	(NH ₄) ₂ SO ₄ , 0.02M	120	1.68	0.144	39	0.24	28
	Malic acid, 0.02M + (NH ₄) ₂ SO ₄ , 0.02M	120	1.61	0.15	40	0.25	29
4	Control (G.D. water)	144	2.15	0.41	—	0.87	—
	(NH ₄) ₂ SO ₄ , 0.02M	144	1.60	0.14	34	0.23	27
	(NH ₄) ₂ SO ₄ , 0.02M + sodium malate, 0.02M	144	1.46	0.33	81	0.48	55
	Sodium malate, 0.02M	144	1.75	0.58	142	1.01	116
5	Control (G.D. water)	72	1.62	0.54	—	0.88	—
	Aspartic acid, 0.02M	72	1.76	0.54	100	0.95	108
	Aspartic acid, 0.02M + (NH ₄) ₂ SO ₄ , 0.02M	72	1.70	0.11	20	0.19	22
	(NH ₄) ₂ SO ₄ , 0.02M	72	1.72	0.10	19	0.17	19
	Sodium aspartate, 0.02M	72	1.67	0.63	116	1.05	119
	Sodium aspartate, 0.02M + (NH ₄) ₂ SO ₄ , 0.02M	72	1.9	0.22	41	0.42	48

Effect of cations (other than NH_4^+) combined with 'utilizable' anions. On the assumption that the above theory is correct, the supply of salts such as the bicarbonates, nitrates or acetates of Na or K to seedlings should stimulate the synthesis of ascorbic acid, for in these cases the anion can either be disposed of or transferred into other substances, thus providing a potential reserve of K^+ or Na^+ ions. A comparison of the effect of these salts and of KCl and K_2SO_4 is shown in Table 8. Solutions of KHCO_3 , NaHCO_3 , CH_3COOK or KNO_3 all increased the synthesis of ascorbic acid when added alone or in conjunction with the nutrient solution. On the other hand, where the base was combined with a 'non-utilizable' anion such as SO_4^{2-} or Cl^- , the effect of the salt was to reduce the amount of the vitamin synthesized.

Effect of 'utilizable' organic anions and their sodium salts. The view that the amount of base liberated as the result of metabolic change is the determining factor in the synthesis of ascorbic acid is further supported by the data recorded in Table 9. In these experiments, the effect of certain organic acids and their sodium salts in modifying the action of $(\text{NH}_4)_2\text{SO}_4$ was studied. Succinic, malic and

aspartic acids were chosen because of the part they are known to play in intermediary metabolism. In so far as the succinate, malate or aspartate ion is used as an intermediate, a supply of these acids in the form of their sodium salts will serve as a potential reserve of base. It was found that (1) the free acids when supplied alone did not appreciably affect the synthesis of ascorbic acid, (2) the sodium salts caused a distinct increase, and (3) the depressing effect of $(\text{NH}_4)_2\text{SO}_4$ was not modified by the presence of the free acids, but was diminished by the sodium salts. In agreement with these findings is the fact that ammonium succinate (the cation and anion of which are both utilized) had little or no adverse effect on the synthesis of ascorbic acid (Table 12).

Synthesis of carotene

Effect of different ammonium salts. $(\text{NH}_4)_2\text{SO}_4$ and NH_4Cl greatly reduced the synthesis of carotene in germinating cress seedlings (Tables 10 and 11). The amount synthesized when these salts were supplied was on an average only 40% of that formed in controls. This effect was observed also when the salts were supplied in conjunction with the nutrient

Table 10. *Effect of different ammonium salts on the synthesis of carotene in cress seedlings*

Exp. no.	Treatment	Duration of exp. (hr.)	Wt. of 50 seedlings (g.)	Carotene synthesized			
				Concentration		Total produced by 50 seedlings	
				($\mu\text{g./g.}$)	(% of control)	($\mu\text{g.}$)	(% of control)
1	Control (G.D. water)	96	1.02	12.7	—	13.0	—
	$(\text{NH}_4)_2\text{SO}_4$, 0.02M	96	0.76	9.6	76	7.3	56
2	Control (G.D. water)	90	0.99	11.8	—	11.0	—
	$(\text{NH}_4)_2\text{SO}_4$, 0.02M	90	0.78	6.4	54	5.0	45
3	Control (nutrient solution)	90	1.0	12.2	—	12.2	—
	$(\text{NH}_4)_2\text{SO}_4$, 0.02M	90	1.0	4.7	39	4.7	39
4	Control (nutrient solution)	96	1.04	14.4	—	7.5	—
	$(\text{NH}_4)_2\text{SO}_4$, 0.02M	96	1.11	5.4	38	3.0	40
5	Control (G.D. water)	144	1.29	12.7	—	16.4	—
	NH_4HCO_3 , 0.02M	144	1.16	14.3	112	16.3	99
	$(\text{NH}_4)_2\text{HPO}_4$, 0.02M	144	1.20	8.1	64	9.7	59

Table 11. *Effect of different chlorides on the synthesis of carotene in cress seedlings*

Exp. no.	Treatment	Duration of exp. (hr.)	Wt. of 50 seedlings (g.)	Carotene synthesized			
				Concentration		Total produced by 50 seedlings	
				($\mu\text{g./g.}$)	(% of control)	($\mu\text{g.}$)	(% of control)
1	Control (G.D. water)	144	1.12	13.0	—	14.5	—
	NH_4Cl , 0.04M	144	0.83	5.3	41	4.4	30
	MgCl_2 , 0.02M	144	0.89	14.3	110	12.8	88
	CaCl_2 , 0.02M	144	1.1	11.8	91	11.9	82
	KCl, 0.04M	144	1.0	12.9	92	12.9	88
	NaCl, 0.04M	144	1.23	13.0	100	16.0	110
2	Control (G.D. water)	96	0.98	11.3	—	5.6	—
	KCl, 0.04M	96	0.86	10.6	95	4.6	83

solution. When the ammonium salt contained an anion capable of being metabolized, e.g. HCO_3^- , NO_3^- or succinate, there was little or no depressing effect (Table 12). The effect of ammonium salts on the synthesis of carotene was, therefore, in general, similar to their effect on the synthesis of ascorbic acid, though quantitatively the effect was smaller. Thus, for example, in numerous experiments with $(\text{NH}_4)_2\text{SO}_4$ the average reduction in the total amount of carotene synthesized was 58 % (range 70–44 %) as compared with a reduction of ascorbic acid synthesized of 78 % (range 89–60 %). $(\text{NH}_4)_2\text{HPO}_4$, which depressed the synthesis of ascorbic acid to a lesser extent than the chlorides and sulphates of ammonia, had likewise a lesser effect on the synthesis of carotene.

Effect of chlorides. Experiments similar to those recorded in Table 7 were undertaken in which the effect of different chlorides was investigated. The results corresponded with those obtained with ascorbic acid, in that, whilst the carotene synthesized was greatly diminished by the ammonium salt, it was only slightly diminished by the other chlorides tested.

Effect of $(\text{NH}_4)_2\text{SO}_4$ in the presence of succinate or malate. Neither succinic nor malic acids when given in conjunction with $(\text{NH}_4)_2\text{SO}_4$ significantly affected

the amount of carotene synthesized. When, however, the sodium salts of these acids were added to $(\text{NH}_4)_2\text{SO}_4$, the depressing effect of the latter was largely eliminated. The results with succinate are shown in Table 12. In contrast to the findings with ascorbic acid, no increase in the synthesis of carotene was observed when sodium succinate alone was given.

Synthesis of xanthophyll. It may be of interest to note that $(\text{NH}_4)_2\text{SO}_4$ did not decrease the synthesis of xanthophyll, either in the light or in the dark.

Correlation of pH of expressed sap with synthesis of ascorbic acid and carotene

If our assumption is correct that the amount of base liberated as a result of metabolic change is the factor which determines the extent of synthesis of ascorbic acid and carotene in the plant, then one would expect a correlation between the pH of the cell sap and the production of these two constituents. Determinations of pH of the expressed sap of cress seedlings grown in different salt solutions, and of the ascorbic acid and carotene synthesized, were therefore made. The results of some typical experiments are summarized in Table 12.

The data show that the amount of ascorbic acid synthesized was, in fact, correlated with the pH of

Table 12. *Correlation of pH of cell sap with synthesis of ascorbic acid and carotene in cress seedlings*

Exp. no.	Treatment	Duration of exp. (hr.)	Ascorbic acid synthesized				Carotene synthesized				pH of ex-pressed sap
			Concentration		Total produced by 100 seedlings		Concentration		Total produced by 50 seedlings		
			(mg./g.)	(% of control)	(mg.)	(% of control)	(μg./g.)	(% of control)	(μg.)	(% of control)	
1	Control (G.D. water)	120 (light)	0.49	—	0.81	—	14.7	—	13.4	—	4.7
	(NH ₄) ₂ SO ₄ , 0.02M	120 (light)	0.18	37	0.23	29	6.5	44	4.1	31	4.2
	NH ₄ Cl, 0.04M	120 (light)	0.13	27	0.19	24	6.9	47	4.8	36	4.2
	NH ₄ NO ₃ , 0.04M	120 (light)	0.37	76	0.61	76	15.8	107	13.4	100	4.7
	(NH ₄) ₂ HPO ₄ , 0.02M	120 (light)	0.20	41	0.32	40	12.0	82	10	74	4.3
	NH ₄ acetate, 0.04M	120 (light)	0.48	98	0.68	84	12.9	88	8.7	65	4.8
	NH ₄ succinate, 0.02M	120 (light)	0.50	102	0.70	87	14.9	102	13.0	97	4.7
2	Control (G.D. water)	144 (light)	0.50	—	0.84	—	15.0	—	12.8	—	4.7
	NH ₄ NO ₃ , 0.02M	144 (light)	0.39	78	0.63	75	16.9	113	13.1	103	4.8
	KNO ₃ , 0.04M	144 (light)	0.59	118	0.96	114	19.9	133	14.7	115	5.2
	(NH ₄) ₂ HPO ₄ , 0.02M	144 (light)	0.17	34	0.29	35	9.6	64	7.5	59	4.4
3	Control (G.D. water)	120 (light)	0.37	—	0.76	—	13.3	—	9.6	—	4.8
	(NH ₄) ₂ SO ₄ , 0.02M	120 (light)	0.16	43	0.25	33	8.0	60	5.2	54	4.3
	Na succinate, 0.02M	120 (light)	0.60	162	0.94	124	15.0	113	8.9	93	5.4
	(NH ₄) ₂ SO ₄ , 0.02M + Na succinate, 0.02M	120 (light)	0.32	87	0.52	69	12.4	93	8.2	85	4.8
	NH ₄ succinate, 0.02M	120 (light)	0.35	95	0.68	90	13.8	103	10.5	109	4.8
	Succinic acid, 0.02M	120 (light)	0.45	122	0.72	95	13.8	103	8.7	91	4.8
	Succinic acid, 0.02M + (NH ₄) ₂ SO ₄ , 0.02M	120 (light)	0.17	46	0.23	30	6.9	52	3.9	41	4.3
4	Control (G.D. water)	100 (light)	0.37	—	0.79	—	10.3	—	11.5	—	4.8
	NaHCO ₃ , 0.02M	100 (light)	0.46	124	0.95	120	11.8	115	11.8	103	5.25
	KHCO ₃ , 0.02M	100 (light)	0.495	134	1.05	133	11.3	110	10.9	95	5.20
	K acetate, 0.02M	100 (light)	0.73	197	1.21	153	13.4	130	12.1	106	5.18
	NH ₄ HCO ₃ , 0.02M	100 (light)	0.43	116	0.82	103	11.5	112	11.4	99	4.80
5	Control (G.D. water)	96 (dark)	0.26	—	0.58	—	1.93	—	4.37	—	4.70
	(NH ₄) ₂ SO ₄ , 0.02M	96 (dark)	0.12	46	0.20	34	1.96	101	3.28	75	4.30
	KNO ₃ , 0.04M	96 (dark)	0.30	115	0.74	128	2.31	120	5.87	134	4.95

the cell sap, high pH values being associated with increased synthesis, and low pH values with decreased synthesis of ascorbic acid. In seedlings grown in such solutions as KNO_3 , KHCO_3 , NaHCO_3 , sodium succinate or potassium acetate, i.e. where the anion was presumably being preferentially utilized, the pH values of the sap were high. A low value was found when the seeds were grown in solutions of $(\text{NH}_4)_2\text{SO}_4$, NH_4Cl and $(\text{NH}_4)_2\text{HPO}_4$, where the cation was being preferentially utilized. In the former cases the pH of the sap was 0.3–0.5 unit higher, and in the latter cases about 0.3–0.5 unit lower than that of controls.

It will be seen also from Table 12 that when succinic acid was given in conjunction with $(\text{NH}_4)_2\text{SO}_4$, the pH of the cell sap and the production of ascorbic acid corresponded with those of seedlings grown in $(\text{NH}_4)_2\text{SO}_4$ alone. When, however, sodium succinate was supplied in conjunction with $(\text{NH}_4)_2\text{SO}_4$, the pH was raised to 4.8, a value equal to that of the control, and the production of ascorbic acid was increased. It is worth noting that the pH of the sap of seedlings grown in succinic acid alone was not lower than that of seedlings grown in distilled water, which suggests that the acid was being metabolized, or alternatively, was not absorbed by the plant.

Where the amount of ascorbic acid synthesized was only slightly affected, there was little or no detectable change in comparison with controls in the pH of the cell sap. In a few cases, as with seedlings grown in NH_4NO_3 , the production of ascorbic acid was slightly reduced, but no detectable change in the pH of the sap was observed. This failure to establish a more exact correlation may have been due to the imperfections inherent in our method of obtaining the cell sap.

A similar correlation also existed between the pH of the cell sap and the amount of carotene synthesized. It was, however, less close than for ascorbic acid. Whilst a lowering of pH caused a distinct fall in the carotene synthesized in all experiments, a rise in pH slightly increased the production of carotene in some experiments, but not in all (Table 12).

Though it is evident that the production of ascorbic acid and carotene in cress is correlated with the changes in the pH of the cell sap, it is possible that some other factor may have been the primary cause. This point needs emphasis, since in pre-

liminary experiments of a similar nature with wheat, no close correlation was found between the pH of the cell sap and the amount of ascorbic acid synthesized. The decrease in ascorbic acid production was, however, considerably less than that observed with cress. On the other hand with peas, $(\text{NH}_4)_2\text{SO}_4$ reduced the synthesis of ascorbic acid by 40–60% and this was accompanied by a reduction of 0.2–0.3 in the pH of the cell sap. The degree to which different species are affected by nutrient salts evidently varies.

SUMMARY

1. The synthesis of ascorbic acid and of carotene was decreased when cress seedlings were grown (in the presence or absence of light) in solutions of $(\text{NH}_4)_2\text{SO}_4$, NH_4Cl or $(\text{NH}_4)_2\text{HPO}_4$. A similar effect was observed when the salts were supplied in conjunction with a nutrient solution.
2. The synthesis of ascorbic acid and carotene was not affected by NH_4HCO_3 , NH_4NO_3 , ammonium acetate or ammonium succinate.
3. Ascorbic acid (but not carotene) was increased by the sodium and potassium salts of nitric, carbonic, acetic, succinic, malic or aspartic acids, although the free succinic, malic or aspartic acids had little or no influence.
4. The depressing effect of $(\text{NH}_4)_2\text{SO}_4$ was diminished by the addition of the sodium salts of succinic, malic or aspartic acid, but not by the free acids.
5. The synthesis of xanthophyll was not diminished in cress seedlings grown in solutions of $(\text{NH}_4)_2\text{SO}_4$.
6. There was a positive correlation between the pH value of the expressed cell sap and the amounts of ascorbic acid synthesized. A similar correlation was less close in the case of carotene.
7. The observed effect of the salts used may be explained by the hypothesis that the factor determining the extent of the synthesis of ascorbic acid is the amount of base becoming available to the plant after metabolic change.

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