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Aconitase Levels in the Leaves of Iron-Deficient Mustard Plants (*Sinapis alba*)

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There have been a number of reports that aconitase in animal tissues is affected by disturbances of iron or ascorbic acid metabolism. Some studies have been made with guinea pigs (Takeda & Hara, 1955; Banerjee & Singh, 1960) but with discordant results. Beutler (1959) has reported that the aconitase level in kidney homogenates from rats fed on an iron-deficient diet was only about half that of controls receiving iron supplements; the activity of the homogenates could not be raised to the normal level by incubation with ascorbate and ferrous sulphate.

We have reported a similar depression of aconitase activity in the leaves of mustard plants (Bacon, DeKock & Palmer, 1959). The present paper describes these experiments in detail and provides further evidence for a specific effect of iron-deficiency on aconitase in this species.

METHODS AND MATERIALS

Growth of plants. A description of most aspects of the growing of the mustard plants has already been given (see DeKock, Hall & MacDonald, 1960*b*). The seeds were germinated in sand and transferred after 1 week to solution culture, and the seedlings in each crock thinned to 15. The first samples for analysis were taken when flower buds were visible, usually 3 weeks after sowing. The plants were grown in a greenhouse, and during the winter (September to April) received extra illumination (about 400 foot-candles) from fluorescent tubes suspended about 30 cm. above the crocks. These tubes were switched on from 5 a.m. to 9 p.m. (G.M.T.) each day and provided local heating as well as light. The ambient temperature of the greenhouse was maintained between 5° and 20° during cold weather, and the plants were shielded from direct sunlight during sunny weather.

Enzyme assays. Aconitase and fumarase were measured as described by Bacon, Palmer & DeKock (1961), and are expressed respectively as μmoles of *cis*-aconitate or fumarate formed/g. fresh wt./hr.

Malic dehydrogenase was measured in the same homogenate by a modification of the method applied by Price & Thimann (1954) to particulate fractions from disintegrated leaf tissue. Before use the homogenate was titrated with 0·8 mM-2:5-dichlorophenol-indophenol until a slight blue colour persisted; in this way the interference of ascorbic acid and other reducing substances was eliminated. The equivalent of 0·05 ml. of original homogenate was added to two test tubes each containing 0·5 ml. of freshly prepared 0·2 M-KCN, 0·5 ml. containing 1 mg. of diphosphopyridine nucleotide (DPN), 0·25 ml. of 0·8 mM-2:5-dichlorophenol-indophenol and 0·1 M-2-amino-2-hydroxymethylpropane-1:3-diol(tris)–HCl buffer (pH 7·5) to a final volume of 4 ml. Each tube was read against water in an EEL portable colorimeter, with a no. 625 (green) filter; 0·25 ml. of 0·3 M-sodium L-malate was added to one and buffer to the other and readings were repeated every 2 min. for 12 min. No change in absorption occurred in the control; decolorization of the test solutions was approximately linear with time and the fall in absorption between 2 min. and 10 min. was used to calculate the enzyme activity, which is expressed as μmoles of malic acid oxidized/g. fresh wt. of leaf/hr.

Other estimations. Chlorophyll was extracted with acetone from fresh leaf samples and measured spectrophotometrically (cf. Arnon, 1949).

Ascorbic acid was measured in metaphosphoric acid extracts by titration with 2:5-dichlorophenol-indophenol (Roe, 1954).

Dry matter was measured by drying to constant weight at 80°.

Kjeldahl nitrogen was measured in fresh material by a micromethod.

Materials. The substrates described by Bacon *et al.* (1961) were used. Potassium dihydrogen (+)-*iso*citrate, prepared from the leaves of *Sedum prealtum* by the method of Vickery & Wilson (1958), was used in some aconitase assays in place of sodium (±)-*iso*citrate prepared from a commercial sample of the lactone, but with no effect upon the values obtained (cf. Pierpoint, 1960).

RESULTS

Plants were grown in the presence of 0·2, 1·0 and 5·0 p.p.m. of iron, in most cases supplied as the ferric salt of ethylenediaminetetra-acetic acid (ferric EDTA). Those receiving only 0·2 p.p.m. were always chlorotic and often considerably stunted; no obvious differences were seen between plants receiving 1·0 and 5·0 p.p.m. of iron, but measurements of height and fresh weight, and scoring for chlorosis have shown that there is some response to the higher concentration (cf. DeKock *et al.* 1960*b*). When iron was supplied as the ferric salt of *NN'*-ethylenebis-[2-(*o*-hydroxyphenyl)glycine] (Chel 138; Geigy Chemical Co. Ltd., Manchester) growth was good and chlorosis slight at the 0·2 p.p.m. concentration. DeKock *et al.* (1960*b*) found no significant effect of iron concentration on

Table 1. *Aconitase activities of leaves from plants receiving various concentrations of iron, potassium, calcium, phosphate and nitrate*

Seeds were sown on 18 July 1958 and transferred to nutrient solution in 5 l. glazed crocks on 23 July 1958. The various treatments were arranged in a $4 \times 3 \times 2$ factorial design, each treatment being repeated twice and the treatments randomized in two blocks of 24. The concentrations of the various nutrients were as follows: Fe 1, 2, 3,: 0·2, 1·0, and 5·0 p.p.m. of iron as ferric EDTA; K/Ca 1, 2, 3, 4, represent the following ratios of potassium to calcium in a total of 13 m-equiv./l.: 1/12, 3/10, 6/7, 10/3; P/N 1, 2, represent respectively ratios of phosphate to nitrate of 1/11 and 2/10 in a total of 12 m-equiv./l. Samples (0·5 g.; 8–10 leaves) were taken from each crock for measurement of aconitase activity. Three analyses were made on 12–13 August and the remainder between 22 August and 1 September.

	Fe 1	Fe 2	Fe 3	P/N 1	P/N 2	Mean	
K/Ca 1	7	19	45	26	22	24	S.E.M. ±2·2
K/Ca 2	10	21	45	33	18	25	
K/Ca 3	9	18	43	23	24	23	
K/Ca 4	10	19	44	20	28	24	
P/N 1	10	17	49			25	S.E.M. ±1·5
P/N 2	8	22	40			23	
Mean	9	19	44				

S.E.M. ±1·9

S.E.M. of 4 in K/Ca × Fe table ±3·8
S.E.M. of 6 in K/Ca × P/N table ±3·1
S.E.M. of 8 in P/N × Fe table ±2·7

Table 2. *Repetition of experiment of Table* 1

Seeds were sown on 17 March 1959, transferred to crocks on 25 March 1959 and sampled from 9 to 17 April 1959. All leaf samples were taken from position 1, the first true leaf.

	Fe 1	Fe 2	Fe 3	P/N 1	P/N 2	Mean	
K/Ca 1	10	22	5	11	14	13	S.E.M. ±1·6
K/Ca 2	8	22	6	10	14	12	
K/Ca 3	9	18	2	11	9	10	
K/Ca 4	8	16	18	13	14	14	
P/N 1	9	19	6			11	S.E.M. ±1·1
P/N 2	8	20	10			13	
Mean	9	20	8				

S.E.M. ±1·3

S.E.M. of 4 in K/Ca × Fe table ±2·7
S.E.M. of 6 in K/Ca × P/N table ±2·2
S.E.M. of 8 in P/N × Fe table ±1·9

the height or fresh weight of such plants. The concentrations of iron used were incorrectly stated (as 0·1, 0·5, 2·5 p.p.m.) in a preliminary communication (Bacon *et al.* 1959) and also by DeKock, Commisiong, Farmer & Inkson (1960*a*). Some of the plants used in the present experiments were in fact grown at these lower concentrations of iron (see Tables 4 and 5), and these received part of their nitrogen in the form of ammonium salts, but the majority of plants used are directly comparable with those analysed for mineral constituents by DeKock *et al.* (1960*b*), and for peroxidase, catalase, haematin pigments and chlorophyll by DeKock *et al.* (1960*a*).

Effect of concentration of iron on actonitase activity

Table 1 shows the results of growing mustard plants in solutions in which the concentrations of phosphorus, potassium and calcium, as well as of iron, were varied. A factorial arrangement with duplication of treatments provided 48 crocks for analysis. The majority of the measurements were made during a period of 12 days at the end of August 1958. Table 2 summarizes the results of a similar experiment carried out in April 1959. The 12 possible combinations of iron concentrations and potassium/calcium (K/Ca) ratios are represented by the figures (each the mean of four crocks) in the top left-hand corner of each table; the six combinations of iron concentration with phosphate/nitrate (P/N) ratios are given below them, and the eight combinations of K/Ca ratio with P/N ratio on their right. In the margins are the mean values for all crocks receiving the same concentration of a particular nutrient: thus there were 12 crocks

Table 3. *Effect of leaf position on aconitase and malic dehydrogenase activities*

Seeds were sown on 29 May 1959 and transferred to 24 crocks on 4 June 1959. Iron concentration was the same as in Tables 1 and 2; in one block of 12 crocks it was given as ferric EDTA, in the other as ferric Chel 138. In all crocks K/Ca was 3/10 and P/N 2/10. Samples (0·5 g.; 8–10 leaves) from position 2 were analysed on 19–24th June and similar samples from position 5 of the same plants on 24–29th June. Aconitase and malic-dehydrogenase activities were measured on the same homogenate and are expressed respectively as μmoles of *cis*-aconitate formed or malate oxidized/g. fresh wt./hr.

	Chelate	Leaf position	Concn. of iron (p.p.m.) 0·2	1·0	5·0
Aconitase	EDTA	2	14	21	8
		5	17	39	36
	Chel 138	2	18	30	33
		5	18	44	52
Malic dehydrogenase	EDTA	2	35	38	40
		5	47	56	56
	Chel 138	2	41	46	45
		5	62	56	67

receiving each K/Ca ratio, 24 each P/N ratio, and 16 each iron concentration.

In each case iron had a marked effect on the aconitase activity, 1·0 p.p.m. of iron giving approximately twice the activity with 0·2 p.p.m. This effect was confirmed in numerous later experiments. The effects of alterations in the other constituents were slight or inconsistent, and for many subsequent experiments plants were grown with variations of the iron concentration only, the K/Ca ratio being fixed at 3/10 and the P/N ratio at 2/10.

With 5·0 p.p.m. of iron there was a striking difference between the two experiments, the aconitase activity being higher than at 1·0 p.p.m. in one (Table 1) and lower in the other (Table 2). Two differences in experimental conditions were considered: (*a*) in the first experiment only natural illumination was used, while in the second intense artificial illumination supplemented the rather weak natural light; (*b*) a different leaf was chosen for analysis in the second experiment. Since the difference in illumination could not easily be reproduced attention was paid to leaf position.

The mustard plants grown with an adequate supply of iron under our conditions have an unbranched stem with eight or nine leaves before the terminal inflorescence. In the first experiment the third leaf down from the inflorescence, i.e. leaf number 6 or 7, was taken from the plants receiving 1·0 and 5·0 p.p.m. of iron, but, because they failed to develop more than two or three leaves, leaf 1 or 2 had to be used from the 0·2 p.p.m. plants. For the second experiment leaf 1 was taken from all plants. Table 3 shows results of an experiment in which enzyme activities of leaves 2 and 5 were compared.

With iron supplied as ferric EDTA the aconitase activity of leaf 2 was much less with 5·0 p.p.m. of iron than with 1·0 p.p.m. (cf. Table 2), but that of leaf 5 was only slightly less. In both leaves the activity with 1 p.p.m. was greater than with 0·2 p.p.m. Table 3 also gives the results of an experiment conducted simultaneously on an adjoining bench, in which iron was given as ferric Chel 138. Because the crocks were not randomized with respect to chelate this difference may not be wholly responsible for the large differences in absolute activities which were found, particularly at the higher concentrations of iron. The general pattern of response to iron was not significantly

Table 4. *Simultaneous measurements of aconitase action on* iso*citrate,* cis*-aconitate and citrate*

Seeds were sown on 7 April 1960 and transferred to 12 crocks on 15 April. In the nutrient solution some nitrogen was supplied as ammonium salts (5 m-equiv./l.), the remainder as nitrate (10 m-equiv./l.); phosphate, potassium, and calcium were present at 2, 3, and 5 m-equiv./l. respectively. The crocks were randomized as one block, each concentration of iron (ferric EDTA: 0·1, 0·5, 2·5, p.p.m.) being repeated four times. Samples (0·5 g.; 8–10 leaves; position 2) were taken on 27–29 April. Each was homogenized and tested in the standard assay system with 0·05 M-(±)-*iso*citrate, 0·25 mM-*cis*-aconitate or 0·1 M-citrate as substrate; the assays were carried out simultaneously at room temperature (26–27°). All activities are expressed as μmoles of *cis*-aconitate formed or disappearing/g. fresh wt./hr.

Substrate	Concn. of iron (p.p.m.)	Aconitase activity: Individual measurements	Mean
*iso*Citrate	0·1	8, 6, 7, 5	6
	0·5	17, 16, 13, 23	17
	2·5	29, 37, 27, 43	34
cis-Aconitate	0·1	8, 5, 4, 4	5
	0·5	13, 13, 11, 15	13
	2·5	18, 24, 21, 24	22
Citrate	0·1	2, 5, 6, 4	4
	0·5	6, 13, 10, 11	10
	2·5	18, 16, 13, 22	17

Table 5. *Effect of iron concentration on dry-matter content of leaves*

In experiment (*a*) sufficient leaves from position 3 were taken from each crock to give a sample weight of 1·0–1·2 g.; each figure represents one crock. In the other two experiments all the leaves from position 1 were taken from each crock, the sample weight varying from 0·5 to 1·1 g.; each figure is the average of the results from two crocks. In (*a*) the K/Ca ratios were as in Table 1, and P/N was 1/11; in (*b*) and (*c*) the nutrient solution was that described in Table 4. In each experiment the dry matter is expressed as per cent fresh weight.

	0·2 p.p.m. of iron			1·0 p.p.m. of iron		
	K/Ca 1	K/Ca 2	K/Ca 3	K/Ca 1	K/Ca 2	K/Ca 3
(*a*)	11·7	10·3	10·3	6·5	9·6	8·3
		Mean 10·5			Mean 8·1	
		Concn. of iron (p.p.m.)	0·1	0·5	2·5	
(*b*)			8·3	8·5	9·1	
(*c*)			9·7	8·9	10·2	

affected by the difference in chelates, but when Chel 138 was used the aconitase activity in both leaves was greater with 5·0 p.p.m. of iron than with 1·0 p.p.m., suggesting that the form in which iron is supplied may also have an influence on the response to the higher concentration.

There was a variable response to 5·0 p.p.m. of iron in subsequent experiments in which leaf 1 or 2 was used (cf. Table 6). Low values were usually characterized by extreme variability: thus the individual values for the assays (at Fe 3) in Table 2 were: 20,* 5, 2, 12, 2, 18,* 0, 0, 9, 0, 10,* 10, 0, 0, 14, 22,* and for Fe 3-EDTA, leaf 2, in Table 3: 0, 0, 4, 27. The effect of the highest K/Ca ratio in Table 2 (values marked with *) in supporting a relatively high aconitase activity is not at all apparent in Table 1. In Table 6 (see below) the opposite effect is seen, the activity with 5·0 p.p.m. of iron decreasing with increasing K/Ca ratio.

The effect of iron concentration was similar when aconitase was measured with *cis*-aconitate or citrate instead of *iso*citrate as substrate. For example, Table 4 shows the results of simultaneous spectrophotometric measurements with all three substrates. A statistical examination was made of the differences between the logarithms of these measurements; it showed that the ratios of activity at 0·1 p.p.m. to that at 0·5 p.p.m. were indistinguishable for the three substrates, and so also were the ratios between activities at 0·5 and 2·5 p.p.m. These results support the view that essentially the same enzyme system is being measured in iron-deficient and normal plants. (In this experiment, carried out under winter conditions in April 1960, with leaf 2, the concentrations of iron were halved, and some nitrogen was supplied in the form of ammonium ions. It will be noticed that the enzyme activity at 2·5 p.p.m. was twice that at 0·5 p.p.m.)

Other changes with concentration of iron

The effect of iron concentration was further investigated to see whether it could be explained by changes in the gross composition of the leaf, or by a general depression of enzymic activity. Measurements on several batches of plants showed only small differences in dry matter (as % fresh weights) between leaves taken from plants receiving different concentrations of iron (Table 5). In one case the Kjeldahl nitrogen was also measured, giving values of 0·98, 0·90 and 0·83 g. of nitrogen/100 g. fresh wt. for 0·1, 0·5 and 2·5 p.p.m. of iron respectively. There was no consistent effect of iron on the ascorbic acid content of the leaves.

Malic-dehydrogenase and fumarase activities were measured simultaneously with aconitase in a few experiments. Table 3 includes measurements of malic dehydrogenase, which show a small but significant increase with iron concentration. As with aconitase, the activity of leaf 5 was throughout greater than that of leaf 2. The results of some simultaneous estimations of fumarase and aconitase are given in Table 6. Fumarase was significantly less at 1·0 p.p.m. than at 0·2 or 5·0 p.p.m.; a similar tendency was found in other experiments. Table 6 also shows an effect of K/Ca ratio on both enzyme activities at the 5·0 p.p.m. level.

Changes during recovery from iron deficiency

If the chlorotic leaves of iron-deficient plants are painted with solutions containing iron they slowly turn green, and their aconitase activity increases. However, this type of curative treatment is not a very satisfactory subject for study, because the leaves often show areas of 'scorching'. Two other types of experiment were therefore carried out.

Feeding of iron to detached leaves. Leaves from iron-deficient plants (0·1 p.p.m.) were detached from the plants and their petioles immersed in a nutrient solution identical with that used to grow the plant except for the omission of iron. These leaves served as controls. Others were placed in nutrient solution containing iron salts or chelates. If the leaves were kept under the same conditions of temperature and illumination as the parent

Table 6. *Effect of various concentrations of iron, potassium and calcium on the fumarase and aconitase activities of leaves*

Seeds were sown on 23 April 1960 and transferred to 24 crocks on 29 April. Levels of iron, potassium and calcium were as in Table 1; phosphate to nitrate 2/10 in all. The treatments were arranged in a 4 × 3 factorial design, each treatment being repeated twice, and the treatments were randomized in one block of 24. Samples (0·5 g.; 8–10 leaves; position 1) were analysed from 16 to 19 May 1960.

Aconitase	K/Ca 1	K/Ca 2	K/Ca 3	K/Ca 4	Mean	
Fe 1	4	6	8	12	8	S.E.M. ± 2·6
Fe 2	18	12	13	26	17	
Fe 3	24	25	16	12	20	
Mean	15	14	12	17		

S.E.M. ± 3·0
S.E.M. of 2 in body of table ± 5·2

Fumarase	K/Ca 1	K/Ca 2	K/Ca 3	K/Ca 4	Mean	
Fe 1	28	42	29	30	32	S.E.M. ± 2·5
Fe 2	24	19	12	26	20	
Fe 3	33	42	22	16	28	
Mean	28	34	21	24		

S.E.M. ± 2·9
S.E.M. of 2 in body of table ± 5·0

Table 7. *Effects of feeding iron to leaves detached from iron-deficient plants*

Plants were grown in solution containing 0·1 p.p.m. of iron as ferric EDTA and other nutrients as described in Table 4. Leaves were detached and a sample was assayed for aconitase. The petioles of the remainder were placed in culture solution, with or without iron supplements. The controls were assayed at the end of the experiment; the others at intervals. In some experiments chlorophyll was also estimated; the results are given as aconitase activities and (in parentheses) as μmoles of chlorophyll/g. fresh wt. For experiment (*a*) leaves from positions 1 and 2 were used; for the others from position 1 only. — Signifies that no measurement was made.

Expt.	Form and concn. of iron	Time of treatment (days) 0	1	2	3	4
(*a*)	None	10	—	—	9	—
	Chel 138; 10 p.p.m.	—	17	23	42	—
(*b*)	None	7 (0·63)	9 (0·73)	—	7	—
	Chel 138; 10 p.p.m.	8 (0·64)	20 (0·89)	25 (1·07)	23 (1·17)	—
(*c*)	None	9 (1·1)	—	—	—	9 (1·08)
	EDTA; 10 p.p.m.	—	—	14 (0·98)	21 (1·11)	23 (0·89)
(*d*)	None	5 (—)	—	—	9 (0·32)	—
	$FeSO_4$; 100 p.p.m.	—	11 (—)	16 (0·67)	14 (0·40)	—
	$FeCl_3$; 10 p.p.m.	—	5 (—)	6 (0·39)	6 (0·39)	—

Table 8. *Changes in aconitase and chlorophyll content during recovery from iron deficiency*

Plants were grown in 24 crocks of nutrient solution containing 0·1 p.p.m. of iron as ferric EDTA and other nutrients as described in Table 4. From the two-leaf stage the plants in 12 crocks were given extra iron as ferric Chel 138 to a concentration of 2·5 p.p.m. Leaves 1 and 2 from one plant in each crock were pooled from each set of 12 crocks, and analysed for aconitase activity (μmoles of *cis*-aconitate formed/g. fresh wt./hr.) and chlorophyll content (μmoles/g. fresh wt.).

Expt. (*a*)					Expt. (*b*)				
	Aconitase		Chlorophyll			Aconitase		Chlorophyll	
Day of treatment	0·1 p.p.m. of iron	2·5 p.p.m. of iron	0·1 p.p.m. of iron	2·5 p.p.m. of iron	Day of treatment	0·1 p.p.m. of iron	2·5 p.p.m. of iron	0·1 p.p.m. of iron	2·5 p.p.m. of iron
0	8	13	0·61	0·58	0	14	11	1·59	1·61
2	9	17	0·58	0·77	3	11	24	1·46	1·78
5	10	26	0·54	0·94	6	10	24	1·44	1·91
7	8	28	0·60	1·19	10	18	47	1·54	2·23
9	12	26	0·55	1·27	13	14	45	1·13	2·20
16	16	22	0·72	1·41					

plants a rise in aconitase activity in the treated leaves was usually apparent after 24 hr. (Table 7). The leaves used, although chlorotic, were by no means devoid of chlorophyll. The increase in aconitase did not seem to be dependent upon an increase in the chlorophyll content [cf. Expt. (*c*), Table 7].

Feeding of iron to deficient plants. If the concentration of iron in the nutrient solution was increased from 0·1 to 2·5 p.p.m. the chlorotic leaves gradually turned green, but, as with detached leaves, this increase in aconitase activity did not require a proportionate increase in the chlorophyll content and the two processes, although simultaneous, had no obvious connexion (Table 8). It will be noticed that the response of aconitase was seen in experiments (*a*) and (*b*) at quite different chlorophyll levels.

Attempts to activate aconitase in homogenates

If extracts were preincubated at 0° with 0·5 mM-ferrous ammonium sulphate and 0·01 M-cysteine for 1 hr. there was no increase in the aconitase activity. This was true of all the extracts tested, whether from iron-deficient or normal plants. In such experiments the concentrations of iron and cysteine in the subsequent assay were respectively 8 μM and 0·2 mM. When higher concentrations of iron (50 μM) and cysteine (1 mM) were present in the assay with *iso*citrate there was a stimulation of activity but this proved to be due to a non-enzymic reaction. When the aconitase activity was measured by the decrease in extinction with *cis*-aconitate as substrate the addition of iron and cysteine diminished the values obtained.

A similar effect was found with ascorbate. If ascorbate at concentrations equivalent to 500–1000 mg./100 g. of original leaf tissue was added to the extraction medium the aconitase activity measured with *iso*citrate appeared to be enhanced, but with *cis*-aconitate it was diminished. The presence of ascorbate equivalent to 100 mg./100 g. of leaf sample taken added about 0·06 to the change in extinction measured in 30 min. at 25°. This is equivalent to an aconitase activity of 12 μmoles/g. fresh wt./hr. If the endogenous ascorbic acid of the leaf (50–100 mg./100 g.) is assumed to contribute in this way to the assay it would introduce an error of about 0·5–2·0 μmoles/g. fresh wt./hr. under standard conditions.

Tests were also made of the results of mixing extracts prepared from plants of 0·2 and 5 p.p.m. iron concentrations. Extracts were mixed in various proportions, at least 30 min. at 0° being allowed before any mixture was tested. In five such experiments the following activities of mixtures were measured (in each case the value calculated from the activities of the separate extracts is given in parentheses) 13(11), 14(15), 22(29), 23(27), 37(40). It will be noted that, if anything, the activity of the mixture was less than that calculated.

DISCUSSION

The results reported here show that when the iron supply to mustard plants in solution culture is so low that growth and chloroplast formation are affected, the aconitase activity extractable from each gram of leaf is markedly and consistently diminished. If the iron supply to the leaf is increased, the aconitase activity rises to the normal level.

That aconitase is specifically affected by iron deficiency is shown by the effects upon two other enzymes of the citric acid cycle: malic dehydrogenase is only slightly affected, and fumarase is, if anything, affected in the opposite way.

The variable results obtained at the highest concentration of iron used cannot yet be explained. The low average values obtained in certain experiments were partly due to the inclusion of extracts with no measurable aconitase activity. In seeking an explanation for these zero values, found especially in the experiments of Tables 2 and 3, it must be remembered that each extract tested was representative of leaves taken from nine to ten plants. On the other hand it may be significant that zero values were never obtained with plants of the 1 p.p.m. concentration, and only once (experiment of Table 6) with plants of the lowest concentration, despite the generally lower trend of these values. It is possible that as the leaves age they accumulate some inhibitory substance, but if this is so the inhibitor must have a specific action, because the malic-dehydrogenase activities (Table 3) were not affected.

With the evidence so far available it would seem that the lower aconitase activity in iron-deficient plants is due to a lack of the enzyme system as a whole and not merely due to a lack of iron. The activity of extracts from iron-deficient leaves was not restored by treatment with ferrous iron and cysteine, which has been shown repeatedly to activate aconitase from animal tissues (cf. Morrison 1954). It is conceivable that a particular complex of iron is required as an activator, but, if so, this substance is evidently not present in excess in normal leaves, because mixtures of extracts from normal and deficient plants did not show an activity greater than the sum of the activities measured separately. (Therefore there is still no direct evidence that iron is required for the action of aconitase in plants.)

The present experiments were undertaken to

test a hypothesis put forward by DeKock (1954–55) that in iron-deficient plants there is a deficiency of ferrous iron and that consequently the aconitase activity is diminished. A possible connexion between the effects upon aconitase and upon the concentrations of citric acid and malic acid and hence upon the phosphorus/iron and potassium/calcium ratios is further discussed by DeKock *et al.* (1960*b*). According to these arguments the concentration of citric acid should be raised and that of malic acid depressed, or at least the ratio of citric acid to malic acid should be greater in the leaves of iron-deficient plants. Experiments in progress (see also DeKock & Morrison, 1958) show this to be the case, but proof is still lacking that the lower level of aconitase is directly responsible. As we have already remarked (Bacon *et al.* 1961), it is difficult to decide, simply from a knowledge of its level of activity in extracts, whether an enzyme is a limiting factor in a sequence of metabolic reactions. What is required is a direct examination of the interconversion of organic acids in normal and deficient leaves.

SUMMARY

1. Aconitase activity has been measured in extracts of the leaves of mustard plants growing in water culture and receiving three concentrations of iron (0·2, 1 and 5 p.p.m.).

2. The aconitase activity in plants receiving 0·2 p.p.m., which always show signs of iron-deficiency, was about half that in those receiving 1 p.p.m. At 5 p.p.m. the activity was variable, but usually greater than at 1 p.p.m.

3. Simultaneous measurements of malic-dehydrogenase activity showed that it was only slightly depressed by iron-deficiency; fumarase activity was slightly raised.

4. When iron-deficient plants or leaves were fed with adequate iron the aconitase activity was restored to the normal level.

5. The aconitase activity of extracts from deficient plants could not be increased by treatment with ferrous iron and cysteine, nor by any other means tested.

6. The significance of these results is discussed in relation to the possible connexion of iron with aconitase action, and to possible effects of aconitase deficiency on the metabolism of the leaf.

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Some Enzymic Reactions Concerned in the Metabolism of Acetoacetyl-Coenzyme A in Athiorhodaceae

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Photosynthetic bacteria of the Athiorhodaceae family grow either aerobically in the dark or anaerobically in the light. Carotenoids and bacteriochlorophyll are formed only during growth in the light or under low conditions of aeration in the dark (van Niel, 1944; Cohen-Bazire, Sistrom & Stanier, 1957; Lascelles, 1959).

Organisms grown anaerobically in the light (pigmented) are considerably more active in catalysing the enzymic reactions concerned in the early stages of bacteriochlorophyll synthesis than are those cultivated aerobically in the dark (non-pigmented). The increase in activity, which is due to enhanced ability to synthesize the enzymes,