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BIOCHEMICAL STUDIES OF MICROELEMENTS IN GREEN PLANTS

II. THE CHEMICAL COMPOSITION OF RICE PLANT AND BARLEY AFFECTED BY IRON DEFICIENCY

By

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Due to the shortage of iron supply, a chlorosis is usually caused in plants. The concentration of mineral or other constituents of such chlorotic plants must be altered as well as the chlorophyll content. Many studies concerning this status have been made by several investigators (2) (3) (7) (8) (13) (14) (15) (16).

Now such alteration in the chemical composition of iron deficient tissues may be regarded as the overall consequence of their metabolic processes impaired, directly or indirectly, by the lack of iron which is considered to play diverse roles in plant metabolism. On this standpoint, rice plant and barley have been cultured on a solution containing all the nutrient elements at normal levels but lacking in iron exclusively. Then at first, their growth and chemical composition have been pursued during the development of deficiency symptoms and their restoration brought about by the addition of iron to the deficient plants. Moreover, it seems to be one of the characteristic features of iron deficiency symptoms that the higher the position of a leaf in a deficient plant is, the more severe is the yellowing of it, while the lowest leaf is maintained almost completely in a green state throughout the long period of growth, in contrast with the case of nitrogen, potassium or magnesium deficiencies in which the yellowing of leaves is more severe with the lower leaves. Thus the concentration gradient of chemical components within the chlorotic barley plants has been studied to find the characteristic of iron deficiency.

Materials and Methods

The culture experiments were undertaken with rice plants in summer and with barley in autumn. Their seeds were germinated in distilled water added with 0.5 ppm of Fe. This addition of iron was necessary to start the culture with the normal green seedlings, because, concerning the nutrient supply from the endosperm to the embryo, iron alone seemed to be so insufficient even at a very early stage of the seedling development that the seedlings became somewhat chlorotic without the external supply of iron in small amounts. The ten-day old seedlings thus brought up were transplanted on the solution in 2-liter beakers as previously described (4), then grown in a green house. The solution for the iron deficient culture had the following composition; $(NH_4)_2SO_4$, 0.0012M; $NH_4H_2PO_4$, 0.0006M; KNO_3, 0.0015M; $Ca(NO_3)_2$, 0.00075M; $MgSO_4$, 0.001M; B, 0.5 ppm; Mn, 0.5 ppm; Zn, 0.05 ppm; Cu, 0.02 ppm; Mo, 0.01 ppm: In addition 1 ppm of Fe was given to the solution for the culture of complete nutrition.

In the first experiment to follow the changes of chemical composition with the progress of growth, the samples of the whole shoot were taken three times at weekly intervals. Immediately after the third sampling, by this time chlorotic symptoms had fairly well developed in the deficient plants, 1 ppm of Fe was added to them, then they were sampled on the third and tenth days after the addition of iron in order to examine the recovering process. This design of sampling was done for both species of plants.

Next, another series of barley were cultured under the complete and the deficient conditions for 30 days. Then their shoots were sampled and separated into three portions, the upper, the middle and the lower leaves. These portions corresponded respectively to the considerably chlorotic, the mild chlorotic and the green leaves of the deficient plants. This cultural experiment was made for a comparative study of the concentration gradient of the chemical components in the deficient plants to that in the normal plants.

All the samples were oven-dried at 70° C, then analysed. In the first experiment, analyses were made for total-nitrogen, protein-nitrogen, total-sugar, ash, phosphorus, potassium and iron, whereas in the second expriment, the soluble-nitrogen fraction was further fractionated into amino-, amide-, ammonium-, nitrate- and undetermined residual nitrogen, and the concentrations of calcium, magnesium, manganese, zinc and copper were also estimated. Total-nitrogen was estimated by the modified Gunning method. Soluble-nitrogen was fractionated after extraction by the sodium-tungustate solution according to Schlenker's method (12) and the nitrogen in the insoluble matter was determined by the Kjeldahl method and asigned as protein-nitrogen. For the estimation of mineral elements the following methods were applied to the solution of ash: P, Allen's method (1) of colorimetry; K, flamephotometry; Ca, titration by permanganate after separation as oxalate; Mg, Fe, Mn, colorimetry by titan-yellow, o-phenanthroline, periodate respectively; Zn, Cu, colorimetry by dithizone.

All the results of analyses were expressed on dry weight basis.

Results

symptom and growth

Both in the rice plants and barley of iron deficient culture, the chlorotic new leaf came out about ten days after transplanting and it grew up to show intervenal striped yellowing. The next leaf was more chlorotic and yellow almost all over its leafblade. However, the lowest leaf maintained its healthy green state until the end of the culture. By the addition of iron to the chlorotic plants, a healthy green new leaf appeared. However, the chlorotic leaves which had already developed could not recover completely from chlorosis, though they became more or less green by the end of the cultural period.

The modes of plant growth in the first experiment are shown in Fig. 1 for rice and Fig. 2 for barley. The retardation of dry matter syntheses in the deficient rice plants appeared after two weeks from the transplanting, whereas in the barley it appeared after about three weeks. In the barley plants, however, it was noteworthy that the length of the deficient shoot was taller throughout the cultural period than that of normal and that the produc-

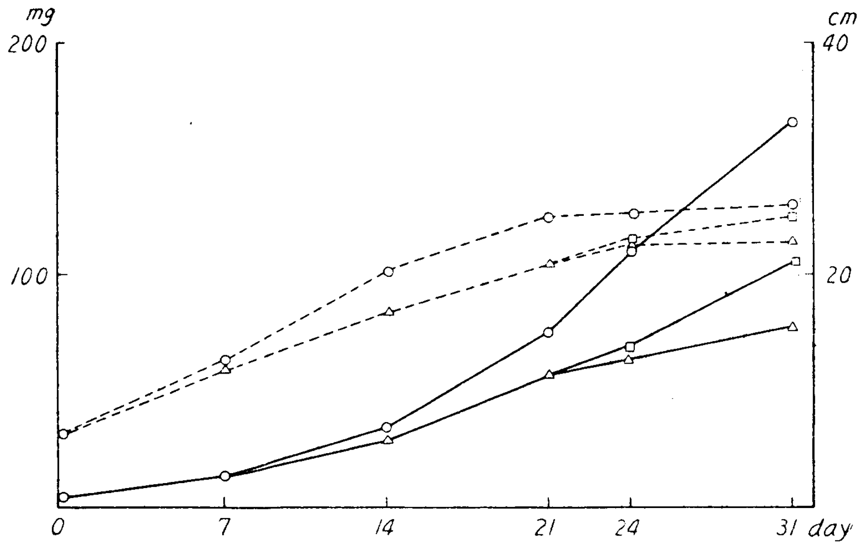

Fig. 1. The growth of rice plant
○·····○ : Length of normal shoot
△·····△ : Length of deficient shoot
□·····□ : Length of recovering shoot
○——○ : Dry matter of normal shoot
△-- △ : Dry matter of deficient shoot
□——□ : Dry matter of recovering shoot

tion of dry matter in the chlorotic plants distinctly exceeded that of the normal after the addition of iron. Such status was not seen in the rice plants.

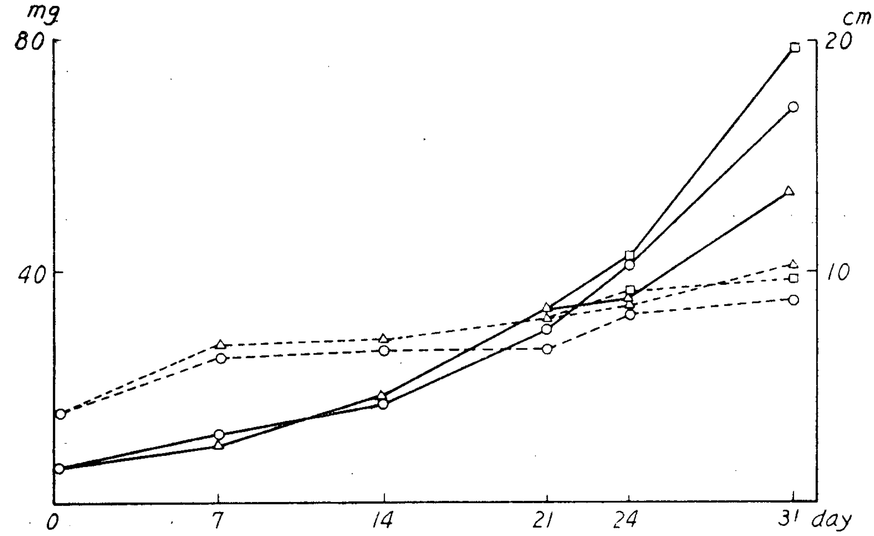

Fig. 2. The growth of barley plant.
○……○ : Length of normal shoot
△……△ : Length of deficient shoot
□……□ : Length of recovering shoot
○——○ : Dry weight of normal shoot
△——△ : Dry weight of deficient shoot
□——□ : Dry weight of recovering shoot

Changes of chemical composition

The changes of the concentrations of total- and protein-nitrogen are presented in Figs. 3 and 4. It can be seen from these results that the concentration of total-nitrogen in the deficient barley was higher than that in

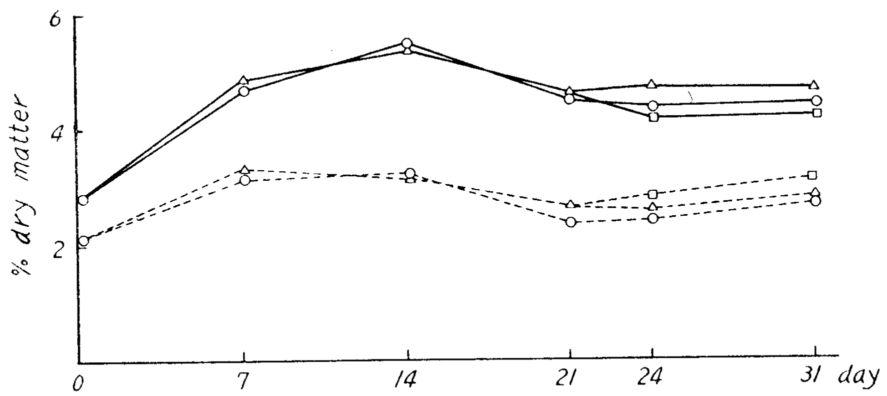

Fig. 3. The changes of total- and protein-nitrogen in rice plant.
○……○ : Total-nitrogen concentration in normal plant
△……△ : Total-nitrogen concentration in deficient plant
□……□ : Total-nitrogen concentration in recovering plant
○——○ : Protein-nitrogen concentration in normal plant
△——△ : Protein-nitrogen concentration in deficient plant
□——□ : Protein-nitrogen concentration in recovering plant

the normal from the very early stage of growth, the latent period of chlorosis, and increased gradually with the progress of deficiency. Similar trend appeared

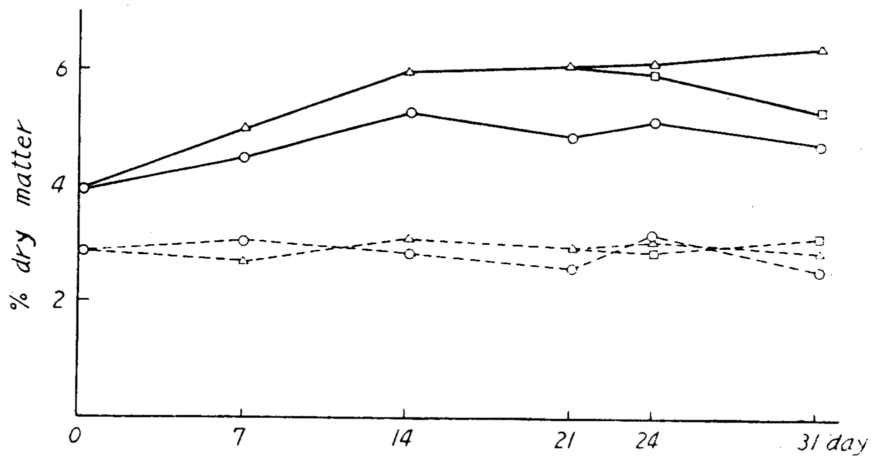

Fig. 4. The changes of total- and protein-nitrogen in barley plant.
○—○ : Total-nitrogen concentration in normal plant
△—△ : Total-nitrogen concentration in deficient plant
□—□ : Total-nitrogen concentration in recovering plant
○······○ : Protein-nitrogen concentration in normal plant
△······△ : Protein-nitrogen concentration in deficient plant
□······□ : Protein-nitrogen concentration in recovering plant

in the rice plants about two weeks later. The levels of protein-nitrogen in the deficient plants showed little difference from those of the normal plants throughout the growing period in both species, although it might be said that the former was slightly higher.

It may be noted that the results concerning the protein level appeared to conflict with those obtained by some workers (2) (13) (17) in which the protein levels of chlorotic leaves have been found to be lower. Due to the supply of iron to the chlorotic plants the concentration of total-nitrogen decreased rapidly approaching to the normal levels in both species.

The results obtained for total-sugar are given in Table 1. In general, the concentration in the deficient plants was lower than that in the normal plants throughout the growing period, though a few exceptions existed.

Table 1. The changes of concentration of total-sugar (% dry matter)

Sample \ Date of Sampling	0 (Seedling)	7	14	21	24	31
Normal rice	23.0	22.4	17.7	27.4	27.7	25.5
Deficient ,,		19.3	18.1	24.7	24.1	29.9
Recovering ,,					25.8	29.1
Normal barley	25.8	29.1	19.8	23.0	21.4	26.4
Deficient ,,		25.5	18.7	17.5	20.0	19.0
Recovering ,,					18.3	23.6

The changes of the concentrations of ash, phosphorus, potassium and iron are shown in Tables 2, 3, 4 and 5. As regards ash, phosphorus and

Table 2. The changes of concentration of ash (% dry matter)

Sample \ Date of Sampling	0 (Seedling)	7	14	21	24	31
Normal rice	6.8	7.9	9.4	7.0	8.4	7.9
Deficient "		7.6	10.4	10.8	12.7	13.1
Recovering "					10.6	8.8
Normal barley	4.6	6.6	10.1	7.3	8.5	8.0
Deficient "		10.6	10.6	11.0	13.0	13.1
Recovering "					10.7	8.8

Table 3. The changes of phosphorus concentration (% dry matter)

Sample \ Date of Sampling	0 (Seedling)	7	14	21	24	31
Normal rice	0.64	0.82	0.97	0.75	0.77	0.67
Deficient "		0.90	1.05	0.87	0.95	0.97
Recovering "					0.89	0.81
Normal barley	0.51	0.71	0.74	0.52	0.61	0.46
Deficient "		0.71	0.70	0.69	0.84	0.73
Recovering "					0.68	0.49

Table 4. The changes of potassium concentration (% dry matter)

Sample \ Date of Sampling	0 (Seedling)	7	14	21	24	31
Normal rice	1.35	2.19	2.60	2.54	2.27	2.17
Deficient "		2.44	3.23	3.15	2.99	3.08
Recovering "					2.96	2.72
Normal barley	1.75	2.25	3.05	3.14	3.40	2.70
Deficient "		2.68	4.41	4.20	4.39	4.34
Recovering "					4.18	2.73

Table 5. The changes of iron concentration (ppm dry matter)

Sample \ Date of Sampling	0 (Seedling)	7	14	21	24	31
Normal rice	174	220	189	157	160	176
Deficient "		128	85	66	55	47
Recovering "					141	364
Normal barley	152	190	144	145	155	182
Deficient "		98	73	48	41	32
Recovering "					83	145

potassium, similar tendencies could be observed in the deficient plants of both species. Their concentration increased within one or two weeks after transplanting when the visual symptoms were not apparent, then increased progressively with the development of chlorosis. In the recovery process, they tended to decrease towards the normal levels.

The concentration of iron in the deficient plants decreased with the progress of chlorosis. But the deficient plants absorbed iron rapidly after the addition of it, especially in the deficient rice plants the rate of the absorption was strikingly high resulting in a higher level of iron about two times of the normal one within ten days. This result seems to agree with the observation of Jacobson and Oeltri (9) upon iron deficient sunflower.

Concentration gradient of constituents within the plants

The concentration gradients of total- and protein-nitrogen are shown in Fig. 5, and the concentrations of fractionated soluble-nitrogenous compounds are given in Table 6.

Table 6. The concentrations of soluble-nitrogen fractions in barley (% dry matter)

N-fraction	Plant	Lower-leaves	Middle-leaves	Upper-leaves
Soluble-N	Normal	0.98	1.17	1.61
	Deficient	1.30	2.71	2.86
α-Amino-N	Normal	0.381	0.394	0.326
	Deficient	0.304	0.362	0.265
Amide-N ($\times 2$)	Normal	0.049	0.090	0.118
	Deficient	0.071	0.172	0.440
NH_4-N	Normal	0.021	0.015	0.009
	Deficient	0.008	0.018	0.020
NO_3-N	Normal	0.132	0.140	0.104
	Deficient	0.190	0.344	0.165
Undetermined residual-N	Normal	0.35	0.44	0.94
	Deficient	0.65	1.04	1.54

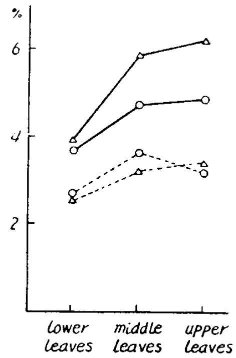

Fig. 5. Concentration of total- and proten-N in barley
○—○ : Total-N in normal plant
△—△ : Total-N in deficient plant
○······○ : Protein-N in normal plant
△······△ : Protein-N in deficient plant

The concentration gradients of total- and protein-nitrogen within both normal and deficient barley were in such status as the abscissa of Fig. 4 was substituted by the upward order of leaf instead of the date. Here it may be again stressed that the protein levels in the chlorotic leaves were maintained as high as those of the normal.

The concentration gradient of soluble-nitrogen is represented as the difference between the curves of total- and protein-nitrogen. It can be seen from this figure that the concentration of this fraction in the deficient leaves was higher than that in the normal increasing progressively with the leaf order. But the ratio of concentration of soluble-nitrogen within the deficient plant of that within the normal was found to reach the maximum at the

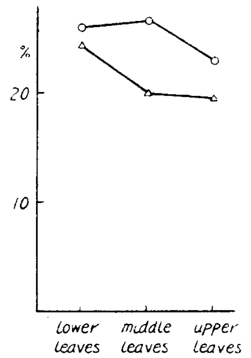

Fig. 6. Concentration of total sugar in barley.
○—○ : Normal plant
△—△ : Deficient plant

middle leaves.

The concentration of each fraction of soluble-nitrogen was also increased in the deficient plants as well as that of soluble-nitrogen itself, though the concentration of amino-nitrogen was lowered exceptionally. The concentration gradient of each fraction in the deficient plants appeared to have similar tendency as in the normal. But in the upper leaves of the deficient plant, the most chlorotic leaves, the concentration of amide-nitrogen was so high that the ratio of it to amino-nitrogen was remarkably reversed.

The total-sugar concentration of the deficient leaves was considerably lowered with the advance of chlorosis. (Fig. 6.)

The concentration gradients of mineral elements are shown Fig. 7. In order to comprehend the relative concentration of mineral elements in the

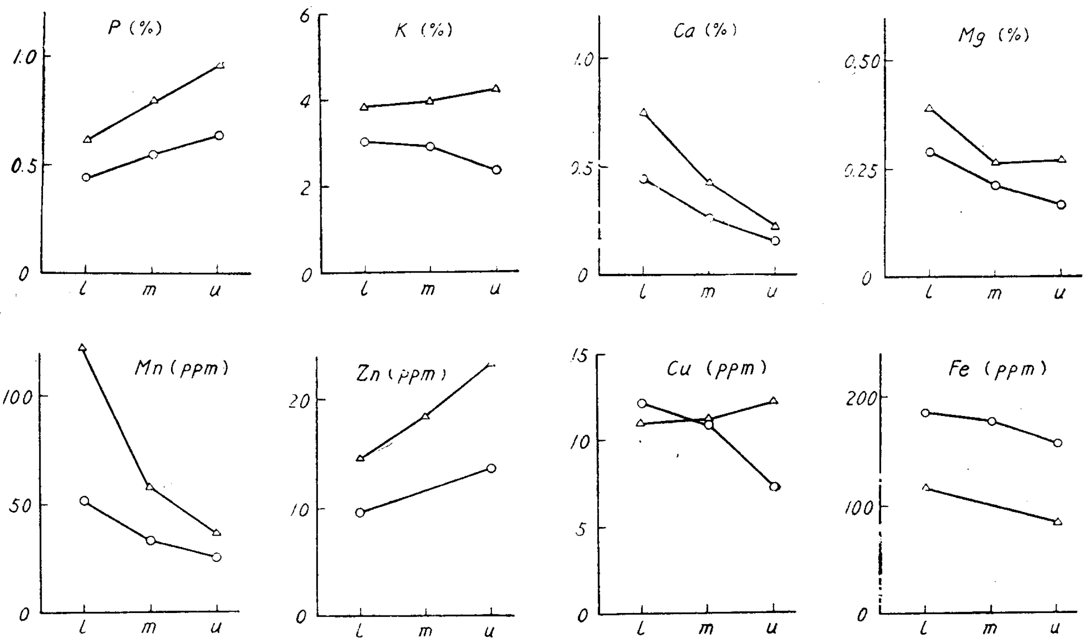

Fig. 7. Concentration of mineral elements in barley.
○—○ : Normal plant   *l* : Lower leaves
△—△: Deficient ,,   *m* : Middle leaves
u : Upper leaves

deficient leaves the proportions of their concentration to those in the corresponding protion of the normal leaves are summarized in Fig. 8.

The concentrations of all the mineral elements examined except iron were higher in the deficient leaves but the patterns of their concentration gradient and the relative concentrations were various.

The concentration gradient of phosphorus within the deficient plants had approximately the same tendency as that of the normal, rising progressively with the leaf order. However, the relative concentration tended to increase towards the upper leaves. Similar status could be seen with zinc. On the other hand, the concentration gradient of both potassium and copper within the deficient plants showed an inverse pattern to that in the normal. In the deficient plants their concentration gradients rose towards the upper leaves, i.e. more chlorotic leaves, whereas in the normal plants they fell. Therefore, the relative concentration of potassium and copper increased with the leaf order in a much greater rate than the case of phosphorus and zinc.

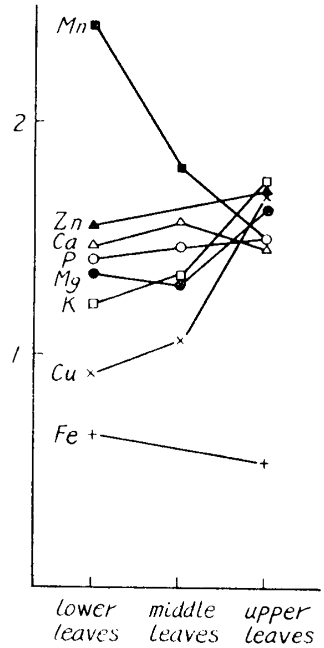

Fig. 8. Relative concentrations of mineral elements in deficient barley.

The concentration gradients of magnesium, calcium and manganese in the deficient plants had similar tendencies as in the normal, and in general dropped in the progressively upper leaves. However, the relative concentrations of these elements in the deficient plants showed patterns different from one another. Concerning calcium and magnesium, the relative concentration showed respectively the maximum and minimum at the middle leaves, while the relative concentration of manganese in the lower leaves was highest of all the results of mineral elements and it decreased rapidly with the leaf order. This status seems to distinguish manganese from the other elements.

The concentration of iron in the deficient plants was, of course, lowered and the concentration gradient was downward, just against the severity of chlorosis.

Discussion

In this experiment the protein level in the iron deficient chlorotic leaves is found to be substantially unchanged compared with that of the normal leaves. This finding differs from the results obtained by some workers (2) (13) (17) who have found it to be lowered in the chlorotic leaves. The cause of this discrepancy is difficult to understand, but it may be due to the differences in the species of plant and the form of nitrogen source of the medium in this experiment. In this experiment rice and barley have been used and the nitrogen source consisted of an equivalent concentration of ammonium and nitrate nitrogen, while those workers have used another species of plant and as the nitrogen source nitrate only. Whatever may be the case, as far as the results obtained in this experiment is considered, it may be regarded as the characteristic of iron deficiency that the protein level within the leaf-tissue remains unchanged. While one of the characteristic of the copper deficiency is considered to increase the protein level in the leaf-tissue (5) (10) and that of the zinc deficiency to decrease it (5) (6). The amount of protein synthesized in the considerably chlorotic plant is less since the dry matter production itself is decreased. Therefore it may be thought that iron governs the protein synthesis in the plant though indirectly. On the other hand, though the concentration gradient of each fraction of soluble-nitrogen within the deficient plants fluctuates with approximately similar tendency to that in the normal, the concentration of amino-nitrogen is lowered in the deficient plants while the other fractions increase and especially the intensive amide formation can be seen in most chlorotic leaves. Thus, to make clear the nitrogen metabolism in plants with relation to iron, the problem about the quality of protein and amino acid must be further studied.

As to the status of mineral elements it may be regarded that the characteristic of iron deficiency in the barley is as summarized in Fig. 8.

Concerning the interrelation between iron and potassium in the metabolism of plants, Bolle-Jones (3) concluded that potassium enhances the efficiency of iron utilization. Thus it may be probable that the particular tendency of potassium in the chlorotic plants already pointed out is attributed to its accumulation by iron deficient tissues in order to enhance the efficiency of iron utilization. Copper also shows a tendency similar to potassium. Hence, if such a reasoning is applied for it, it may be presumed that copper has the nature to complement the shortage of iron. According to Nason *et al.* (11) the activity of polyphenoloxidase, a copper enzyme, is greatly enhanced in the leaf homogenate of iron deficient plants. Therefore, it may be possible that in the oxidase-system of the iron deficient leaves the iron enzymes are partially replaced by the copper enzyme, and accordingly the iron deficient leaf accumulates a greater concentration of copper.

On the other hand, it has been well known that manganese has an antagonistic nature against iron in the plant metabolism and in this experiment the concentration gradient of manganese in the deficient is found to fall more rapidly than that in the normal. Hence, the presumption may be made that this result is caused by the diminished absorption of manganese by the iron deficient tissue to avoid its antagonistic action upon iron. However, the concentration of manganese itself is much higher in the deficient leaves, then such an interpretation of the result is complicated. Thus for the teleological interpretation of the results, this sort of analysis should be made more extensively on the deficient plant of each nutrient element other than iron, together with the study of their function in the metabolism of plants.

The difficulty of redistribution of iron within the plant is simplly concluded from visual observation of deficiency symptoms and the results of iron analysis. But if iron is supplied to the deficient plants externally it can readily be absorbed. On the other hand, the status of the soluble nitrogenous compounds, most of mineral elements and the total-sugar in the deficient plants is found to be affected generally far less in the lower leaves than in the upper, except manganese. Connecting these facts, it may be considered that once iron is assimilated into the matured tissue it turns into a fairly stable state from which iron is not released easily and the metabolism in that tissue does not suffer substantially from the iron deficiency.

Summary

Rice plant and barley were grown on solutions in which iron was exclu sively eliminated. Then the changes in chemical composition affected by the lack of iron were pursued with the development of deficiency symptoms and the recovery from it caused by the iron addition. Moreover the concentration gradient of chemical constituents within the deficient plants were examined. The results obtained were as follows:

1. As the chlorotic symptom advances the concentration of total-nitrogen, phosphorus and potassium increased and in the recoverring process their concentrations tended to decrease and iron was rapidly absorbed.
2. The protein level in the deficient leaves did not change significantly even if the chlorosis was considerably severe. It may be presumed that this status of protein level is the characteristic of iron deficiency.
3. The concentrations of chemical constituents examined generally increased, while total-sugar, amino-nitrogen and iron decreased.
4. The relative concentrations of the constituents in the deficient plants to those of the normal plants were in general close to unity at any rate in the lower leaves and diverged from the normal levels in the upper leaves.
5. Such a tendency appeared most remarkably with potassium and copper. But manganese only showed an entirely inverse tendency.
6. The amide formation was very intensive in the chlorotic leaves.

References

1) Allen, R.J. (1940). Biochem. Jour., **34**, 858.
2) Benett, J.P. (1945). Soil Sci., **90**, 91.
3) Bolle-Jones, E.W. (1955). Plant and Soil, **6**, 129.
4) Fujiwara, A. and Tsutsumi, M. (1954). Tohoku Jour. of Agri. Res., **5**, 47
5) Fujiwara, A. and Tsutsumi, M. Unpublished Data.

6) Hoagland, D.R. (1948). Lectures on the Inorganic Nutrition of Plants p. 40, Chronica Botanica Co. Waltham, Mass.
7) Iljin, W.S. (1952). Plant and Soil, **3**, 239.
8) Iljin, W.S. (1953). Plant and Soil, **4**, 11.
9) Jacobson, L. and Oertli, J.J. (1956). Plant Physiol., **31**, 199.
10) Lucas, R.E. (1948). Soil Sci., **65**, 461.
11) Nason, A. Oldewurtel, H.A. and Propst, L.M. (1952). Arch. Biochem. Biophys., **38**, 1.
12) Schlenker, F.S. (1934). Plant Physiol., **18**, 141.
13) Sideris, C.P. and Young, H.Y. (1956). Plant Physiol., **31**, 211.
14) Smith, P.E., Reuther, W., and Specht, A. W. (1950). Plant Physiol., **25**, 496.
15) Steinberg, R.A., Specht. A.W. and Roller, E.M. (1955). Plant Physiol., **30**, 123
16) Wadleigh, C.H. and Brown, J.W. (1952). Bot. Gaz., **113**, 373.
17) Weinstein, L.H. and Robbins, W.R. (1955). Plant Physiol., **30**, 27.