of these findings, there are no R's in the insulin particle and the specification of the subunit may be written to the form $\frac{1}{3}\ P = Q_1 + Q_2 \ldots Q_m$, where m need not be unity, with, say, about 102 residues to be distributed among all the Q's. To this complement correspond two of Sanger's A and B components (*5*). It will thus be necessary, ultimately, to prove, for any proposed structure of insulin, that these 4 peptide chains could be obtained from it by Sanger's procedures.

Meanwhile, in the light of early general studies of the language of the vector space (*21*), it was becoming clear that the vector map for the wet insulin crystal calculated from Crowfoot's intensities could be interpreted, in the neighborhood of the origin, in terms of cage structures of about the tetrahedral dimensions of the C_1 structures (*22*), which had already been shown to maintain their coherence with as few as 48 skeletal residues. On this basis, it was suggested (*22*) that each subunit of insulin may perhaps correspond to two sub-sub-units of this type, either as separate entities or (since at that time dissociation below thirds had not been observed) as an "intergrowth." Naturally nothing could be presumed as to whether the two cages are identical in the nature and placing of every residue or as to whether the actual residue numbers, or even the skeletal residue numbers, $48 + k$, $48 + k'$, are the same, or as to which of the residues of the original 72-residue skeleton are deleted.

The two latest developments are therefore of great interest. On the basis of a physicochemical investigation, Fredericq and Neurath (*23*) claim that the insulin particle is dissociable into structures of mol wt *ca* 6000, a result which was strongly suggested by the picture in terms of two cages for each subunit, though the possibility that these form an intergrowth was mentioned, in default at that time, of just this evidence. It seems questionable whether we may infer, even from the finding of a solubility curve indicative of a single component (*23*), that these two "6000" members corresponding to a subunit are identical in the nature and placing of every residue. That this may not be so is, in fact, suggested by the still more recent work of Harfenist and Craig (*24*).

It is not feasible at this time to draw final conclusions from the experimental data on insulin, which are of varied types, with margins of error not precisely specifiable and certain discrepancies still unresolved. In such a case, it is often of value to proceed on a different tack and study, as in this communication, how far the data can be fitted into a model suggested on structural grounds. Actually, the cumulative evidence seems to fit as well as it is reasonable to expect with the picture given above, which analyzes each of the 3 subunits of the insulin structure into two structures, a Q_1 and a Q_2, separate or as an intergrowth, and corresponding together to two of Sanger's A components and two of his B components. Taking the number of residues to be, say, 102, we have for each Q the necessary minimum of 48 skeletal residues.

References

1. Wrinch, D. *Science,* **107**, 445 (1948).
2. ———. *Biol. Bull.,* **95**, 247 (1948).
3. ———. *Wallerstein Lab. Communs.,* **11**, 175 (1948).
4. ———. *Nature,* **139**, 972 (1937).
5. Sanger, F. *Advances in Protein Chem.,* **7**, 2 (1952).
6. Porter, R. R., and Sanger, F. *Biochem. J.,* **42**, 287 (1948).
7. Porter, R. R. *Biochem. et Biophys. Acta,* **2**, 105 (1948).
8. Chibnall, A. C. *Proc. Roy. Soc.* (London), **B131**, 136 (1942).
9. Porter, R. R. Cited by F. Sanger in *Biochemical Symp. No. 3.* New York: Cambridge Univ. Press, 29 (1950).
10. Bailey, K. *Biochem. J.,* **49**, 23 (1951).
11. Fromageot, C. *Biochem. et Biophys. Acta,* **6**, 283 (1950).
12. Ellenbogen, E. Ph.D. thesis, Harvard Univ. (1949).
13. Pedersen, K. O. *Cold Spring Harbor Symposia Quant. Biol.,* **14**, 148 (1949).
14. Fromageot, C. *Ibid.,* 49.
15. Crowfoot, D. *Ibid.,* 72.
16. Oncley, J. L. Private communication (1948).
17. Oncley, J. L., and Ellenbogen, E. Paper presented at 116th mtg. Am. Chem. Soc. (1949).
18. Low, B. *Cold Spring Harbor Symposia Quant. Biol.,* **14**, 77 (1949).
19. Chibnall, A. C. *J. Intern. Soc. Leather Trades' Chemists,* **30**, 1 (1946).
20. Brand, E. *Ann. N. Y. Acad. Sci.,* **47**, 187 (1946).
21. Wrinch, D. *Phil. Mag.,* **30**, 98, 490 (1939).
22. ———. *J. Chem. Phys.,* **16**, 1007 (1948).
23. Fredericq, E., and Neurath, H. *J. Am. Chem. Soc.,* **72**, 2684 (1950).
24. Harfenist, E., and Craig, L. C. *Ibid.,* **73**, 877 (1951).

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Chelates as Sources of Iron for Plants Growing in the Field[1]

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Iron deficiency is widely recognized as one of the most important problems in plant nutrition. It is known to occur in most of the major fruit-producing areas of the world. The symptom of iron deficiency is similar in most plants in that the interveinal areas of the leaves become chlorotic while the veins remain green (Fig. 1). In citrus the leaves often turn an ivory color and usually drop, many of the branches die, and production of the trees is greatly reduced.

Iron deficiency is found under two widely different sets of conditions. In the first place, it occurs extensively in crops growing on calcareous soils, where it is referred to as lime-induced chlorosis. Second, in certain regions, as in Florida, iron deficiency occurs most commonly in crops growing on acid soils. The presence of iron chlorosis does not always mean, however, that there is a shortage of iron in the soil. Even in the very light sandy soils of Florida there is usually sufficient iron for plant growth, provided this element can be utilized effectively.

It has been reported by various workers (*1–3*) that iron chlorosis can be induced by an excessive accumulation in the soil of heavy metals such as copper, manganese, zinc, nickel, or cobalt. Hewitt (*1*) has listed these elements according to their increasing ability to induce iron chlorosis in plants. The same worker makes

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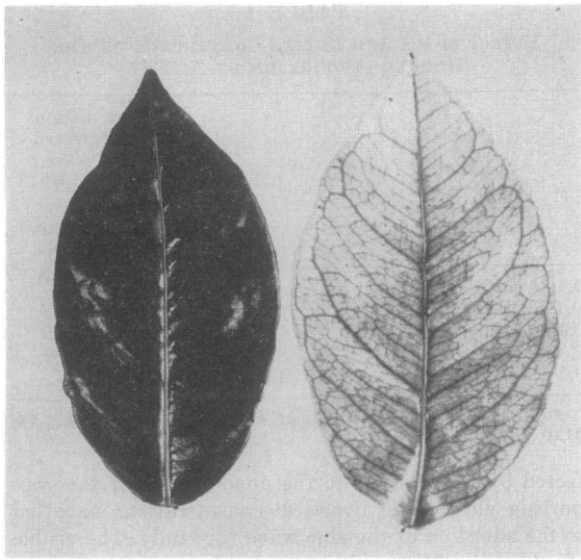

FIG. 1. Grapefruit leaves. Leaf on left is from a tree treated with 30 g chelated iron. Leaf on right shows typical iron chlorosis and is from an untreated tree.

a distinction between the toxic effects of an excess of these metals and their ability to induce iron chlorosis.

Wallace (*4*) and Chapman *et al.* (*5*) found that iron chlorosis was associated with plants growing in potassium-deficient cultures. Hoffer (*6*) has shown that when potassium is deficient iron is precipitated in large amounts at the nodes of cornstalks.

Iron deficiency is the most difficult of all mineral deficiencies to correct. Application of inorganic iron salts to the soil or as a foliage spray has not been successful in correcting this deficiency in citrus. Injection methods of putting dry iron compounds in holes bored in trees have been used, along with forcing iron solution into trees. Although these methods have been successful in supplying iron to trees, they are not sufficiently practical for large-scale use.

In solution and sand culture work it is difficult to keep iron in solution when applied as an inorganic salt, because it precipitates as iron phosphate. Hopkins and Wann (*7*) found that iron could be kept in solution, even under fairly alkaline conditions, when it was supplied as iron citrate. Studies at this station showed that when roots of iron-deficient grapefruit trees growing in the field were placed in flasks containing dilute solutions of ferrous sulfate it was not possible to detect any increase in uptake of iron in the trees, as compared with untreated trees. However, when citric acid was added to the iron sulfate, so that the ratio was 1 : 10 by weight, considerable iron was taken up by the tree, as determined by chemical analysis and greening of the chlorotic leaves. When similar solutions were put on the soil around chlorotic trees there was no apparent increase in the uptake of iron, as compared with the untreated trees.

Studies were then made with chelating agents that form more stable iron complexes than those formed with the citrate ion. Jacobson (*8*) found that ferric potassium ethylenediamine tetraacetate was a good source of iron for plants growing in solution cultures. During the past year we have applied various combinations of iron chelated with sodium ethylenediamine tetraacetate to the soil in solution and also in dry mixtures around iron-deficient grapefruit and orange trees. The pH of the soil on which these trees were growing ranged from 4.5 to 6.0.

Single applications of chelated iron during the dormant season brought about complete greening of the chlorotic leaves on these trees within 6 weeks. The new leaves that came out in the spring on treated chlorotic trees were green (Fig. 1) and appeared 2–3 weeks earlier than those on untreated trees. Considerable response was obtained when as little as 6 g of chelated iron was applied to the soil around iron-deficient trees. Chemical analysis of new leaves sampled in March, 2 months after application of chelated iron to the soil, shows that considerable iron was taken up by the trees (Table 1). Leaves from treated trees contained more than twice as much iron as those from untreated ones. There was only slightly more iron in the leaves from trees treated with 2500 g Fe applied as ferrous sulfate than in untreated trees. This small difference was not enough to produce any noticeable greening of chlorotic leaves.

TABLE 1

IRON CONTENT OF LEAVES FROM ORANGE TREES TREATED WITH CHELATED IRON AND IRON SULFATE

Fe applied/ tree (g)	Form	Total Fe ppm (Dry wt basis)
Untreated		40
10	Chelated	100
20	"	85
30	"	86
40	"	85
50	"	90
2500	Ferrous sulfate	50

The reason for the much greater uptake of iron from the chelate than from ferrous sulfate is not known. It is believed that shortly after ferrous sulfate is applied to the soil the iron is precipitated as hydrated ferric oxide, and this form is much less soluble than chelated iron.

It is not known whether iron ethylenediamine tetraacetate is absorbed by plants. Heck and Bailey (*9*) expressed belief that plants do not absorb certain chelates. Hutner *et al.* (*10*) pointed out that ethylenediamine tetraacetic acid was not metabolized by microorganisms. From these studies it seems probable that the complex is not absorbed by the plant. Jones and Long (*11*), using radioactive iron, found that exchange took place between the iron complexed by ethylenediamine tetraacetic acid and that in the ionized state. Since the soluble chelate makes intimate contact with the roots it may be postulated that the roots are able to take up the iron from the complex by ion exchange.

It is believed that chelates in addition to those of iron may be effective sources of plant nutrients. In many instances zinc, manganese, and molybdenum cannot be applied effectively to the soil. Foliage sprays are often undesirable because the residue causes an increase in insect infestation. Studies are now in progress at this station to determine the usefulness of various metal complexes for plants.

References

1. HEWITT, E. J. *Ann. Rept. Long Ashton Research Sta.* (1948).
2. MILLIKAN, C. R. *Proc. Roy. Soc. Victoria,* **61**, 25 (1948).
3. CHAPMAN, H. D., LIEBIG, G. F., JR., and VANSELOW, A. D. *Soil Sci. Soc. Am. Proc.,* **4**, 196 (1939).
4. WALLACE, T. *J. Pomol. Hort. Sci.,* **7**, 184 (1928).
5. CHAPMAN, H. D., BROWN, S. M., and RAYNER, D. S. *Citrus Leaves,* **25**, (3), 17 (1945).
6. HOFFER, G. N. *Purdue Univ. Agr. Expt. Sta. Bull., 298* (1930).
7. HOPKINS, E. F., and WANN, F. B. *Botan. Gaz.,* **84**, 407 (1927).
8. JACOBSON, L. *Plant Physiol.,* **26**, 411 (1951).
9. HECK, W. W., and BAILEY, L. F. *Ibid.,* **25**, 573 (1950).
10. HUTNER, S. H., *et al. Proc. Am. Phil. Soc.,* **94**, 152 (1950).
11. JONES, S. S., and LONG, F. A. *J. Phys. Chem.,* **56**, 25 (1952).

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The Binding of Metal Ions by ACTH: A Property Correlated with Biological Activity

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A survey of the polarographic behavior of a number of metal ions in solutions containing ACTH led to the discovery that the hormone binds zinc and copper (II) ions in acid solution, and that a relationship exists between the extent of binding under certain fixed conditions and the adrenocorticotropic activity as judged by rat assay. Manganese (II), cobalt (II), and nickel (II) were also observed to bind in acid solution, but without any definite relationship to bioactivity; no binding occurred with antimony (III), bismuth (III), lead (II), and tin (II). The formation of complexes between certain metal ions and amino acids has been the subject of numerous publications, and has been shown to involve the carboxyl group—i.e., to be favored by high pH. The unique characteristic of the binding of zinc ion by ACTH is that it is favored by *low* pH, but is substantially unaffected by buffer composition (Table 1). It is not practicable to carry out polarographic zinc ion measurements in buffers of pH much less than 4.5, because of interference by the hydrogen discharge wave; on the other hand, the copper (II) wave is usually affected by its nearness to the anodic wave of the supporting electrolyte. Practical considerations have led to the adoption of the zinc wave for study. The visible effect of ACTH on the zinc depolarization wave involves (1) the suppression of the zinc diffusion current; (2) distortion of the wave, which increases progressively with the extent of binding to the point where, above 50% diminution, the diffusion current loses all definition; and (3) a progressive shift of the zinc half-wave potential to more negative values.

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TABLE 1

EFFECT OF pH AND BUFFER COMPOSITION ON THE BINDING OF ZINC ION BY ACTH*

Buffer composition	pH	Diminution of standard zinc wave by ACTH (8 *x* Armour) 0.24 mg/ml (%)
0.1 *M* NaOAc · HOAc	4.64	18
0.1 *M* NH_4OAc	6.46	8
0.1 *M* N-ethyl morpholine acetate	8.18	0
0.1 *M* Na glutamate	4.63	22

* Corticotropin B of Brink *et al. J. Am. Chem. Soc.,* **74**, 2120 (1952).

In order to correlate the zinc-binding reaction with biological activity, a standard procedure for carrying out the measurement was adopted, and numerous samples of ACTH (the potency of which had been determined by the hypophysectomized rat method [*1*]) were subjected to zinc-binding determination. The data were assembled in the Klotz (*2*) function of pm/x versus

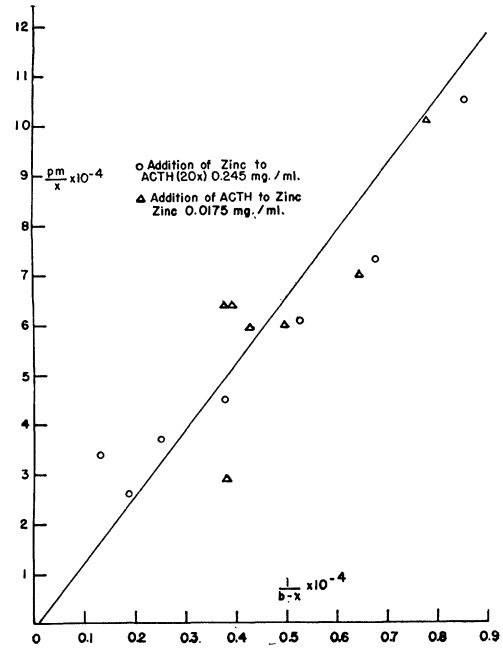

FIG. 1. Binding of zinc ion by ACTH in 0.1 *M* acetate buffer, pH 4.64.