MINOR ELEMENTS IN SOIL AND THEIR EFFECT ON FORAGE AND FEEDING CATTLE WITH SPECIAL REFERENCE TO THEIR SIGNIFICANCE AS TRACERS OF EVOLUTION OF SOME COMPLEX ORGANIC STRUCTURES: AS DETERMINED BY APPLICATION OF GOLDSCHMIDT’S LAW

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§1. INSECURE FOUNDATION OF PRESENT KNOWLEDGE OF INTERRELATION OF TRACE ELEMENTS

There are elements like Fe and Al which are required in ‘minor’ proportions for plants but nevertheless present in major proportions in soils, while the reverse is true for elements like N. This shows that the entry of those elements into the plants is not entirely governed by the law of mass action. There are elements like Co and I which are present in forage simply because they are available in the soil, serving no essential purpose for themselves although essential for feeding cattle. This means that this entry is neither exclusively governed by any known biological function of these elements in the absorbing organism. There are also elements like Se and F which though not so poisonous to plants are toxic to animals. Mn, Fe and Cu are essential to both plants and animals, while B is essential only for plants. Mo, on the other hand, although essential for some plants, soon reaches toxic limits for animals. K and Mg are present in major quantities in plants, while their quantitative importance is taken up by Na and Fe in animals. There is a curious relation between Mo causing ‘teart’ disease in animals, and Co causing ‘pining’ in sheep observed in eastern Rose-Shire. There are some running down or wasting diseases of animals which were cured by Cu and Co although the herbage showed no such deficiency. Much information in this regard is desirable that can show the causes for these curious interrelationships and antagonisms between the different elements. The above facts only show that the quantity which enters the organism is not exactly according to what is available in the medium nor is it entirely limited by any specific function in that organism. In the words of Watson
and Smith (1946) "there seems to be a curious interaction or antagonism between the trace elements and consequently the indiscriminate use of mixture should be avoided until our information is better founded".

There is need therefore to review the position from the point of view of newer chemistry of those elements based on ionic or atomic radii and covalency or co-ordination theory which are really fundamental and to see if these considerations can co-ordinate and put on a better foundation at least some of the seemingly conflicting facts noted above regarding the interaction and function of the different elements. Baudisch (1945) applied concepts of magneto-chemistry in early attempts at understanding the biological functions of trace-elements but the concepts of crystal chemistry do not seem to have been applied to this field so far.

§2. POSSIBLE STEPS IN THE EVOLUTION OF SOME OF THE ESSENTIAL COMPLEX ORGANIC STRUCTURES

Comparing for example the essential structure of (1) the respiratory pigments like the cytochromes, (2) haemoglobin and (3) the chlorophylls, it is found that they are all metal derivatives of porphyrins to which are co-ordinated two more nitrogen atoms of a protein, with similar X-ray patterns and differences only in side chains.* The iron in the universally present respiratory pigments like the cytochromes, which are the most primary structures in the embryo and evolution (Barcroft), can change from the ferrous to ferric or vice versa, the substituents in the porphyrins and the proteins being such that the corresponding change in the valencies and double bonds can be reversibly communicated from one end of the molecule to another and finally from one substrate to another. The evolution of other substances like haemoglobin or chlorophylls from these primary structures can be pictured in two important steps, either firstly a metallic substitution and secondly then by a change in the side chains, or in a way in which the order of these two steps is reversed. It is proposed to show in this discussion that the first of these two alternatives is more natural as in that way the second of the two steps can be shown as a consequence of the first which in its turn can be taken to conform to the well-known law in geochemistry established by the pioneering work of Goldschmidt (1944) and his school. This deals with the replacement of one ion or atom by another in the framework of minerals and clays, and postulates that one ion or atom can replace another only if their radii agree within 10 per cent. of

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* One difference between cytochrome and the rest is that it has the iminazole N of the protein satisfying the co-ordination number of the metal while the others have not got them in a position to satisfy it.
each other. These premises, it will be shown, are sufficient to explain many of the peculiarities and relationships between the trace elements noted in Section I.

§3. EVIDENCE FOR THE IONIC REPLACEMENTS IN THE BUILD UP OF COMPLEX ORGANIC SUBSTANCES

In the experiments of Rama Moorthy and Desai (1946) an iron injection into one half of a chlorotic tobacco leaf showing symptoms due to an iron deficiency, leaving the other half as the strictest possible control resulted in the quick appearance of colour and chlorophyll in that half as was to be expected, accompanied curiously enough by a significant decrease in the absolute Mn content (as for example from 31.3 to 4.3 part per million on dry matter basis) following the increase in the iron content due to injection. This is (direct) evidence for the rôle of ionic interchanges in the build up of chlorophyll by the plant.

§4. EVOLUTION OF HÆMOGLOBIN AND THE FUNCTION OF Cu AND Co IN CURING ANÆMIA

The cytochromes may be pictured as $\overset{\text{N}}{\overset{\text{Fe}^3}{\text{N}}}$ in which the iron is held by co-valency from 4 N atoms of the porphyrin on the same plane with two iminozole N atoms of the protein on either side (top and bottom). It can only change from ferrous to ferric or vice versa under the stimulation of the substrate, but it cannot add additive compounds as all the six co-ordination bonds are strongly held by six N atoms (Theorell, 1941). There is also an equilibrium between the ferrous and ferric compound, by reversibly changing which it acts as donor or receptor in the oxidation-reduction reactions connected with respiration. The side chains and double bonds are, however, such that they reversibly react to the consequences following this oxidation and reduction of iron and this may simply be represented as follows:—

$$\overset{\text{N}}{\overset{\text{Fe}^3}{\text{N}}} \Leftrightarrow \overset{\text{N}}{\overset{\text{Fe}^2}{\text{N}}} \quad (\text{radius} = 0.83 \text{ Å}) \quad \Leftrightarrow \overset{\text{N}}{\overset{\text{Fe}^3}{\text{N}}} \quad (\text{radius} = 0.67 \text{ Å}) \quad (1)$$

It will be seen according to Goldschmidt's law applied to biology that Fe$^2$ with radius 0.83 Å can be replaced by Mg$^2$ (0.78), Mn$^2$ (0.91), Zn$^2$ (0.83), Cu$^2$ (0.80) and Co$^2$ (0.82) while Fe$^3$ (0.67) can be replaced by Mn$^3$ (0.70), Co$^3$ (0.65) and Mo$^4$ (0.68).
Suppose that in the course of evolution or development of the embryo, the respiratory cytochromes come into contact with solutions containing Cu²⁺. The latter will replace Fe²⁺ of the cytochrome and the effect is to shift the equilibrium in equation (1) to the left and then get continuously replaced by Cu²⁺ till the reduction of Cu²⁺ complex to the Cu⁺ complex opposes the formation of Fe²⁺ complex from the Fe³⁺ complex.† The effect is the attainment of a thermodynamic equilibrium between the two couples.

If such a state of affairs happens to the copper prophyrin formed by substitution, there will be a further increase in the size of the central atom (Cu⁺⁺ radius 0·96) creating a force throwing away the N atoms, primarily affecting the relatively weaker bonds attached to the iminazole nitrogen which can be rotated in such a way as to allow of other atoms coming in for satisfying the co-ordination number of the centre atom. Thus the first effect of substitution by Cu in a cytochrome molecule is to orient the iminazole nitrogen bonds so far away that the new molecule can form additive compounds with other molecules (like O₂ in the case of hæmoglobin and CO₂ in the case of chlorophylls). This may be the reason for the essentiality of copper both for plants and animals. The other effect is that the molecule which could adjust to a changing valency of 2 to 3 of the central atom has to adjust to a further reduction in the valency of the central atom to 1. This after the lapse of some time obviously means certain changes and rearrangement of the double bonds and side chains and hence the molecule may not be able to retain that reversibly labile nature it possessed in the cytochrome molecule. If now Co²⁺ (0·82) is also present, it can replace Cu²⁺ (0·80) which is in dynamic equilibrium between Fe²⁺ complex on one hand and Cu⁺⁺ complex on the other and prevent the irreversible changes associated with copper going too far. It may be mentioned here that this Cu²⁺ complex is not exactly identical with that first formed from cytochrome but is the one with the necessary strain resulting from its dynamic equilibrium with its own couple containing the cuprous complex. Subsequently this Co²⁺ can be replaced by Fe²⁺ to form the hæmoglobin which can unlike the parent cytochrome form additive compounds with O₂ without undergoing oxidation but resembles the cytochrome more than the chlorophyll does. It may be that this Co complex is a stable intermediate and can be isolated. Smith (1945) isolated a Co containing anti-pernecious anæmia factor whose constitution is not yet established.

† One of the chief factors determining the frequency and extent of the different changes is the oxidation and reduction potential involved.
§5. FUNCTION OF Mo IN PLANTS AND ANIMALS AND ITS ANTAGONISM WITH COPPER AND COBALT

Suppose instead of Cu and Co as above, there was Mo in solution \( \ldots \text{Mo}^4(0.68) \) can replace \( \text{Fe}^3 \) instead of \( \text{Fe}^2 \) and unlike in the case of \( \text{Cu}^2 \), the equilibrium in equation (1) of the cytochrome will shift to the right. \( \text{Mo}^4 \) has a higher co-ordination number of 8, a higher valency, smaller diameter and greater affinity for nitrogen compared to iron. The result of substitution by Mo is that the two valencies from 4-N atoms of the porphyrin mesomerically holding Fe have to accommodate Mo with four valencies and therefore such a strain in the molecule is induced that what was previously functioning as a respiratory pigment has to change the nature and reversibility in its side chains and bonds and the molecule begins to fix nitrogen. This explains the essentiality of Mo (Burk; Bortells, 1939) for biological nitrogen fixation. As the change induced by Cu and Co which are necessary for hemoglobin formation is just opposite in direction compared to the change induced by Mo, this gives a clue as to how an excess of Mo in the feed can cause anæmia or 'teart' disease noted in sheep in Somerset and Worcester and how Cu acts as corrective for this purpose.

The antagonism between copper and lead found in the Swayback disease of animals is however slightly different in nature. \( \text{Pb}^4 \) with a radius of 0.84 Å can replace Fe in competition with \( \text{Cu}^2 \); because of its greater valency and co-ordination number \( \text{Pb}^4 \) can produce at least part of the effects described under \( \text{Mo}^4 \) in the matter of fixing additive compounds. The effects however do not last long as on reduction \( \text{Pb}^4 \) changes to \( \text{Pb}^2 \) with a diameter of 1.32 Å and because of the accompanying expansive force described under \( \text{Cu}^+ \), the capacity for additive compound formation is enhanced. But this takes the molecule to a situation where this \( \text{Pb}^2(1.32) \) can be easily exchanged by the mobile monovalent K (\( \gamma = 1.33 \) Å) with its greater solubility and capacity to ionise, in addition to the rearrangements made necessary when the side chains and bonds are to adjust to the new valency of the central atom. Hence there is a great tendency for the molecule to decompose. These effects can however be counteracted by Cu which not only can initiate its own chain of reactions in competition with \( \text{Pb}^4 \) but can also replace \( \text{Pb}^4 \) from the complex even in the initial stages using the replaced \( \text{Pb}^4 \) for oxidising any \( \text{Cu}^+ \) in the medium. Thus it is that Cu is cure for the Pb induced swayback.

§6. BUILDING UP OF THE CHLOROPHYLL AND THE FUNCTION OF Mn AND Mg IN IT

If after the action of Cu, \( \text{Mn}^2(0.91) \) be present it can replace \( \text{Cu}^{+1} \) before the side chains and double bonds lose their accommodating power for the
changing valencies of the central atom, although due to the changes associated with copper, liability now can be expected only under external excitation (like the action of light) and at this stage this ability could be kept up by the action of Mn which can change valencies just like the Fe atom. If at this stage Mg$^2+$ (0.79) replaces Mn$^3+$, the result even under external stimulation is that, although the side chains and double bonds do adjust to a possible reduction in the valency of the central atom, they find that the new central atom (Mg) can no longer change its valency and have therefore to reduce the additive compounds like Co$_3$ held by this Mg in co-ordination. This may be how chlorophyll gets its functions and abilities compared to the parent cytochromes.

If, however, excess of Mn be present, it might prevent Cu doing its part as the oxidation potentials involved in the reactions Mn$^3+$ = Mn$^4+$ + $\bar{\epsilon}$, Cu$^+ = Cu^2+ + \bar{\epsilon}$, are respectively $-1.51$ and $-0.167$ volts. This can be remedied by more iron, which may form more of the cytochrome to start with and excreting the un-utilisable Mn compounds.

§7. SULPHUR–SELENIUM RELATIONSHIP

Biologically sulphur is an essential constituent of proteins and hair especially. If, however, Se which can easily replace S by virtue of their chemical relationship, ionic or atomic radii, substitutes S in part, it will break the S-cycle by virtue of its greater metallic nature and will therefore be biologically harmful, resulting in bad wool production. Se is not so toxic for plants either because the metabolism of S, producing wool, is not important in plants or that in plants, Mo$^6+$ or Mn$^4+$ or B or other element specifically present might counteract the action of Se in a way at present not so clear.

DISCUSSION AND CONCLUSION

The picture of the complex molecules in the living cell like the cytochromes herein presented is that they are arranged and act as sieves, the walls of which are made of nitrogen atoms, the thickness of the walls being made up of the porphyrin and the protein. Hence the importance of ionic radii for the admission and retention of the different metallic ions in these sieves. Although no base exchange of the type shown by Gedroitz in soils and clays is indicated which is mainly a surface phenomenon, what is expected is only the ionic interchange of the type shown by Goldschmidt in the minerals formed out of the magma. This interchange which is a phenomenon of the interior is expected to be valid only a short while during and after the formation of the original molecular pattern. That is why the metallic porphyrins extracted from the living cell do not so easily exchange...
their metal ions because during extraction they lose their original arrangement in the living cell. There is however some evidence on the effect of the substitution of Mn for Fe in the hæmins (Theorell, 1945). But from analogy with the extracted metallo porphyrins, those in the living cells cannot be expected to be so slow in exchange. For once the principle of exchanges is conceded, the frequency of exchanges with Fe, Cu, Mn and Mg, etc., needed for example in the formation of chlorophyll in the plant must be as frequent as the formation of chlorophyll itself in the plant, which must be sufficiently high indeed!

The frequency of this ionic interchange is determined by (1) energy considerations, (2) agreement with ionic or atomic radii and (3) periods of time course of plant reactions or periods of "Chemical-physical sensitivity". To these Prof. H. Lundegardh in a private communication suggests the "addition of obstacles founded in the complicated structure of the protoplasm" resulting in the selectivity or "favouring the absorption of certain ions and impeding others, although the barriers break in a pathological way when these elements are present in toxic concentrations. The importance of ionic interchange" in exchange processes had been emphasised by Lundegardh (1946). The idea presented in this paper of a combined action of different heavy metal atoms of similar dimensions in certain physiological process gets experimental support in a very recent article published subsequent to writing of this paper on the activation of phospho-glucomutase by metal ions wherein the experimental proof for the combined action of two metal atoms for this enzyme reaction is provided by Stickland (1949). Thus his theory not only explains and correlates a number of experimental facts regarding ionic antagonism and their essentiality for certain important biological reactions but also provides a possible link in the probable evolution of some of the essential complex organic structures in the living organism. This theory also indicates future possibilities of experimentation, as well as possible extension to cover other metallo complexes in the organism other than porphyrins. For example, Pb is expected to supplement Mo for nitrogen fixation where as Cu or Cu and Co are expected to hinder or antagonise the effect of Mo.

Goldschmidt's law also states that amongst those metals that so agree in radii within 10-15\% of each other, those that have a greater bonding strength get a preference over the rest, concentrations remaining the same. This bonding strength increases with the valency and decreases with the radius of the ion. The normal permeability of an ion in an organism is therefore determined by the condition that its radius should agree within
10-15% of the space in the lattice of the complex organic structures in the organism that are capable of accommodating a metal ion, while the avidity with which it is absorbed depends on the bonding strength of the ion and its concentration in the medium. These then should determine whatever specificity that is attached to the biological distribution of these trace-elements along with the fact that the organism should be able to perpetuate itself in the presence of that ion in order to show its distribution, and for the normal type of co-ordination, this avidity gives rise to the normal Mellor and Maley series of ionic preferences.

SUMMARY

There are many gaps in our knowledge of the biological interrelationships and antagonisms of the trace elements. For example, there are certain animal deficiency diseases of Cu and Co which plant analyses for these elements do not reveal unless there is an understanding of the elements which antagonise them and of those related.

The application of Goldschmidt's law to the replacements of atoms and ions in complex crystal structures (in the form that such replacements are possible only when the size of the atoms concerned agree within 10% of each other) coupled with a knowledge of the changes in valency and oxidation reduction systems involved, has been shown in this paper to elucidate many of these relationships and antagonisms.

The essentiality of Cu and Co for haemoglobin formation, the cause of anaemia due their deficiencies and how they are antagonised by Mo are shown, as also the essentiality of Mo for N fixation and the antagonism between Cu and Pb in the sway-back diseases. The essentiality of Fe, Cu, Mn and Mg for chlorophyll formation and the possible steps in the evolution of chlorophyll and haemoglobin from cytochrome by the combined action of intermediary metal atoms have been traced along with the biological antagonism between Se and S. A consequence of this is the greater importance of Mo for leguminous plants compared to others with a tendency for the deficiency in cattle of Cu in countries where legumes and the like are extensively grown.

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