

Availability of some trace elements

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'Availability' may be defined as 'how much is available from the amount present'. According to this definition, I shall begin with a short survey of the trace element contents of feeds. After this I shall deal with the utilization of the trace elements of feeds by animals and factors influencing it. Finally I shall try to demonstrate some examples of the dynamics of absorption.

Trace element contents of feeds

Meadow hay serves as the base of nearly all cattle rations. The question is how much the trace element content of farm-produced fodder contributes to the

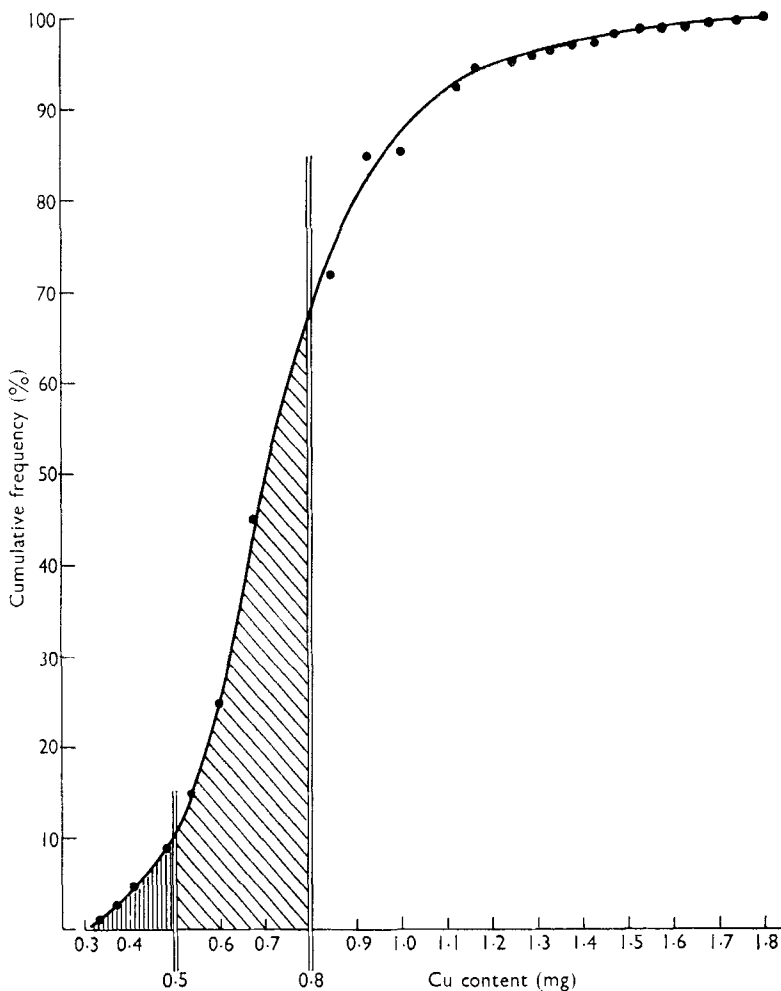


Fig. 1. Cumulative frequency distribution (%) of the copper content of meadow hay (mg/100 g dry matter).

requirement of livestock. Fig. 1 shows the copper contents that we found in more than 200 samples of hay from different areas of Southern Germany (Kirchgessner, 1959a, 1961a).

We calculated the cumulative frequency distribution by adding the numbers of samples of each class of Cu contents, expressing these values as percentages of the total number of samples and plotting them on the ordinate, the corresponding class being plotted on the abscissa (Fig. 1). This cumulative frequency shows that 10% of the samples contained less than 0.5 mg Cu/100 g meadow hay and nearly 70% contained less than 0.8 mg. Depending on the requirement, more than half of all samples did not provide an optimum supply of Cu for cattle. This statement can be made not only for hay but also for other farm-produced feed (Kirchgessner, 1961b).

The occurrence of cobalt follows a similar pattern. Assuming a requirement of 6 or 8 $\mu\text{g Co}/100\text{ g}$ dry matter of feed, 55–70% of all samples does not cover the requirement of cattle (Kirchgessner, 1959a, 1961a).

Another aspect of this investigation is the wide range of distribution of the values. In order to find some of the reasons for these variations we examined the effects of botanical composition and of time and method of harvesting on the trace element content of meadow grass (Kirchgessner, 1955, 1957a, 1957b; Wöhlbier & Kirchgessner, 1957a; Kirchgessner, Merz & Oelschläger, 1960).

At first we compared the trace element contents of fifty different grasses, legumes and other pasture plants. The results are presented in Fig. 2 on the basis of a value of 100 for the grasses. The Cu content of the pasture plants other than grasses and legumes was about 60% higher than that of the grasses; the legumes ranged between. The differences of the Co contents were still more pronounced. Pasture plants other

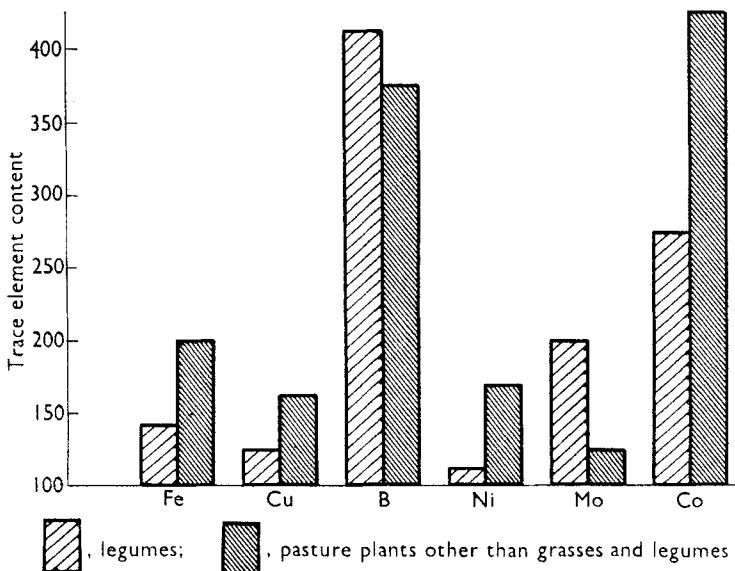


Fig. 2. Trace element contents of legumes and pasture plants other than grasses and legumes, the content of grasses being taken as 100.

than grasses and legumes contained four times the amount in the grasses. The situations for the other trace elements were similar, as shown in the figure.

The stage of growth can also definitely affect the trace element contents of meadow grass. Fig. 3 illustrates the effect of stage of growth on the contents of cobalt, zinc, copper, and nickel of three grasses. The content of each element on 3 May was taken as 100.

Time of cutting did not appreciably affect the Co content. However, the contents of zinc, copper, and nickel fell to 60, 40 and 20%, respectively, with advancing growth. The decrease of Cu content was correlated with the decrease of protein

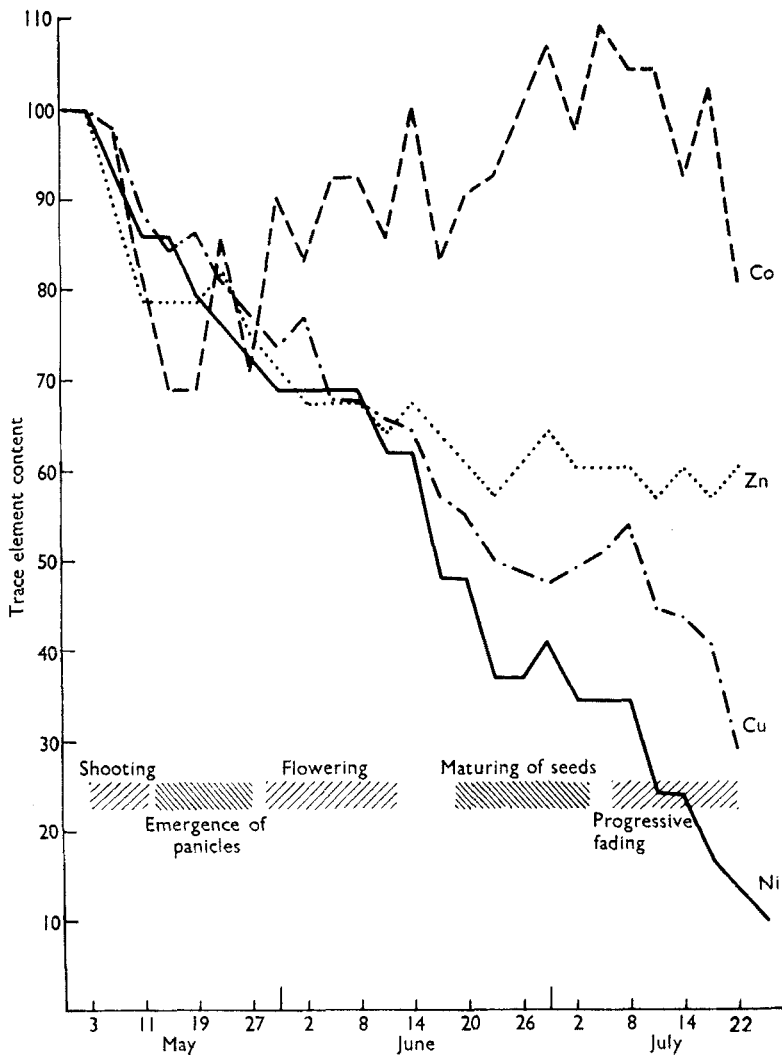


Fig. 3. Effect of stage of growth on the trace element contents of some grasses, the content on 3 May being taken as 100.

content. It would be of some interest to prove if there exists a relation to the occurrence of Cu-protein complexes in herbage.

The trace element contents of meadow hay may also be substantially affected by the methods of drying. Field curing causes heavier losses of Cu and Co than does curing on racks.

These investigations were not limited to Cu and Co. They included the trace elements manganese, zinc, iodine, molybdenum, and fluorine as well as the non-essential elements aluminium, nickel, chromium, and partly also lead, boron, and arsenic. The same trace elements were determined in commercial foodstuffs (Fenner, 1956; Wöhlbier & Kirchgessner, 1957*b*). Nearly all commercial foodstuffs contain more Cu than home-grown fodder does.

A survey of the occurrence of trace elements in feeds must also deal with milk, the food of calves. Normal milk contains very small amounts of trace elements. The first milking of colostrum contains two and a half times more Cu, and the value for Mn is four times higher. Values for I, Zn and Co range between these values (Kirchgessner, 1957*c*, 1959*b*). Trace element concentration in normal milk is very low. The cow's food, however, contains much more. In our investigations only 0.4% of the Co intake appeared in the milk and about 10% of the intake was retained by the animal. Similar relations occur with Zn and Cu. Only 1-3% of both elements was excreted in milk, whereas retention ranged from 10 to 20%.

Trace elements in metabolism

Values for trace element contents of feeds are necessary but of limited use if the utilization of these elements remains obscure. For studying trace element metabolism absorption studies are of great value. Hitherto we have used balance trials, because laboratories were lacking for isotope work. To avoid contamination of feed, urine and faeces by contact with metals, metabolism cages lined with plastic were constructed which are described elsewhere (Kirchgessner, Munz & Oelschläger, 1960). Some information on trace element metabolism is provided by the distribution of their excretion. For growing pigs this distribution was fairly constant, independent of the experimental conditions. Of the total excretion 95-98% was found in the faeces, but only 1-3% of Mn, Al, Zn, Cu and Co, and 4-6% of Ni and Cr excretion were found in the urine. In contrast to this distribution of excretion one-third of the total Mo and F excreted appeared in the urine (Kirchgessner, Munz & Oelschläger, 1960; Kirchgessner & Oelschläger, 1961; Kirchgessner & Weser, 1963; Kirchgessner, Weser & Friesecke, 1963; Kirchgessner, Weser, Friesecke & Oelschläger, 1963).

Retention of trace elements, however, varies more than their excretion and distribution because absorption and retention are affected by several factors. Very important factors are the interrelationships between individual elements as well as their interrelationships with organic compounds during absorption and retention. We tried to investigate such interrelations for trace elements in a number of balance trials with cattle and particularly with pigs.

Calcium and copper interrelation. The utilization of Cu by cattle is affected by a number of factors. Tompsett (1940) found that the retention of Cu may be influenced

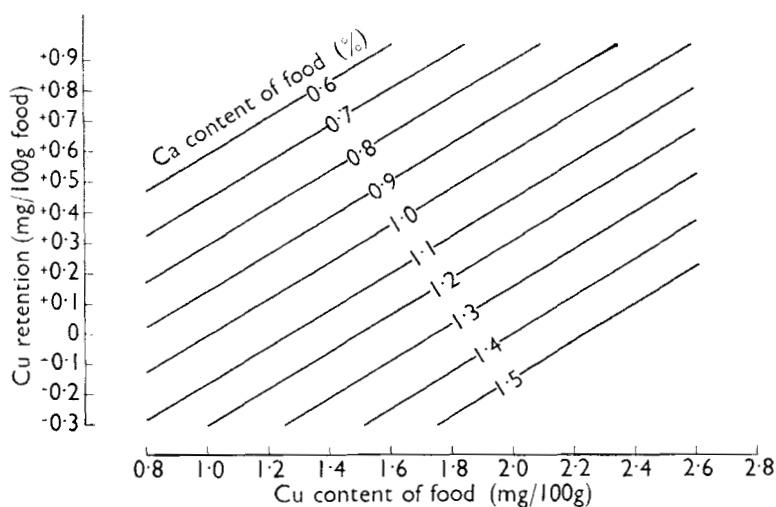


Fig. 4. Effect of copper and calcium intake on Cu retention by cattle.

by the Ca content of the feed. Dick (1952, 1953, 1954) demonstrated that supplementary calcium carbonate greatly decreased Cu utilization by sheep. Our experiments with dairy heifers confirmed this interrelationship between the Ca content of the ration and the retention of Cu (Fig. 4, Kirchgessner, 1959c). Increasing the Ca content of the feed considerably diminished Cu retention even though the Cu content of the feed remained constant. For instance, when the Ca content of a ration, containing 1 mg Cu/100 g, increased from 0.7% to 0.95%, the Cu retention of heifers decreased from 40% to 0. On the other hand, the absorption of Cu by pigs was not prevented by a calcium carbonate supplement (Kirchgessner, Munz & Oelschläger, 1960). The reasons may be derived from the following findings in *in vitro* experiments. The speed of dialysis or diffusion of Cu is constant in acid ranges up to pH 5.5. With decreasing concentration of hydrogen ions the dialysis rate decreases very much. As, however, the concentration of hydrogen ions is much higher in the stomach of pigs than in the abomasum of cattle, even addition of basic compounds does not affect the Cu absorption by pigs.

Crude fibre and molybdenum interrelation. Another interrelationship exists between the crude fibre content of the feed and Mo retention. In balance trials heifers showed an average increase in the Mo retention by about 10 µg/kg feed, when the crude fibre content of the feed increased by 1% (Kirchgessner, 1959c).

Calcium and zinc interrelation. Interrelations in the trace element metabolism of pigs are of particular interest in respect to the role of Ca in parakeratosis. Parakeratosis of pigs is supposed to be caused by Zn deficiency, which is said to be enhanced by increase in Ca content of the ration. According to Newland, Ullrey, Hoefler & Luecke (1956, 1958), Scharrer (1955) and Liebscher (1956), increased Ca uptake is supposed to prevent retention of Zn. We examined this relation in balance experiments with growing pigs (Kirchgessner, Munz & Oelschläger 1960). A calcium

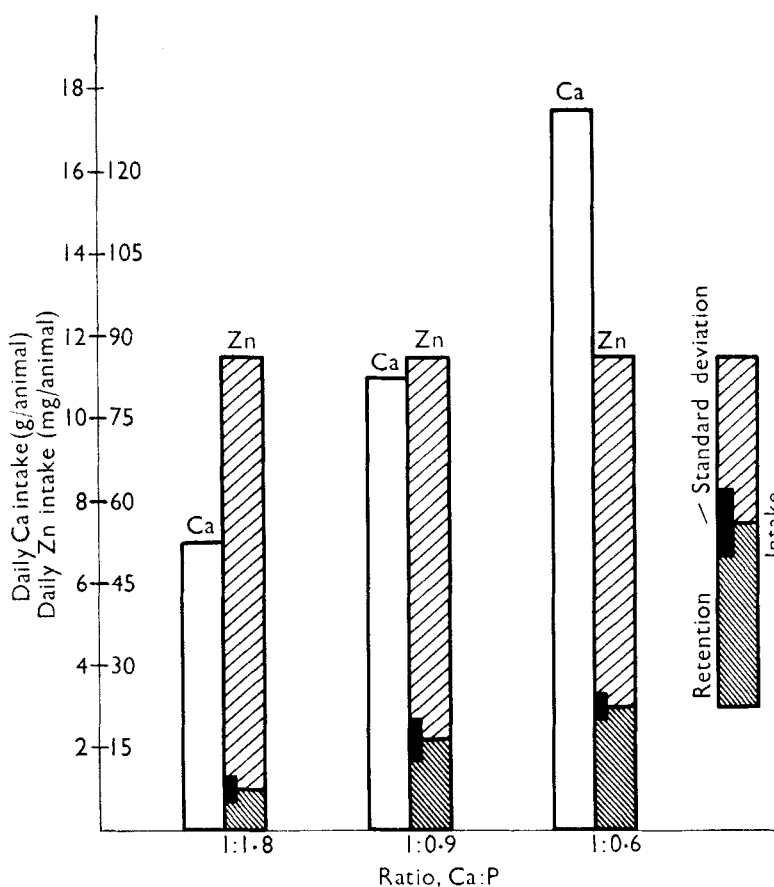


Fig. 5. Effect of calcium intake on zinc retention by growing pigs.

carbonate supplement was designed to double or to treble the Ca intake. The results of those trials, graphically presented in Fig. 5, demonstrate that Zn retention at a constant Zn level of the feed was not decreased but increased by the higher dietary Ca levels. The groups with Ca supplements showed in parallel with the increased Zn retention a lower urinary excretion of Zn. This proves absence of relation between Ca and Zn in the gastro-intestinal tract. This is in full agreement with the results of Lewis, Hoekstra & Grummer (1956, 1957) and Lewis, Hoekstra, Grummer & Phillips (1956) who found higher Zn contents of the liver, kidney, and hair at higher dietary Ca levels.

We confirmed the interrelation between Ca and Zn by examining thirty samples of cow's liver. Here, too, Ca content and Zn content were closely positively correlated (Kirchgessner, 1962). Finally we tried the reverse and gave pigs different Zn supplements. Whereas in the former experiments Zn retention rose with an increased Ca uptake and retention, now Ca retention was increased in the group given additional Zn and retaining more Zn (Kirchgessner & Oelschläger, 1961).

Interrelations between trace elements and vitamins and antibiotics

Interrelations do not occur only between individual elements but also between trace elements and organic compounds, particularly vitamins and antibiotics which may enter the digestive tract as exogenous ligands. With the vitamins, we examined particularly relations between pyridoxine and the different trace elements (Kirchgeßner, Friesecke & Oelschläger, 1962).

In eighteen balance trials we used growing pigs to study the effect of antibiotics on the metabolism of trace elements (Kirchgeßner, Oelschläger & Munz, 1960). The daily retentions of Co and Zn were more than doubled whereas Cu retention was increased threefold by an antibiotic supplement. Furthermore, higher amounts of Mn and Fe were retained by the animals receiving antibiotics, but differences in the deposition of Al, Ni, Mo and Cr were not significant. These results are only valid for certain stages of growth. Similar results were obtained with poultry.

Dynamics of absorption

Results of balance experiments in trace element metabolism may be useful in planning diets for animals, but they do not reveal the mode of action of the interrelations observed. Therefore we began to investigate some aspects of the dynamics of trace element absorption with Cu as an example (Kirchgeßner & Weser, 1964; Weser & Kirchgeßner, 1964b).

The inorganic or organic ligands of the ingesta are able to form Cu compounds that are sparingly soluble or stable complexes which may seriously affect Cu absorption. Copper hydroxide may be mentioned as an example of the importance of sparingly soluble Cu compounds. In a similar way absorption of Cu as present in sulphides of mono- or di-valent Cu may be considerably reduced. CuS is hardly absorbed at all. Most of the other inorganic Cu compounds, however, show such a high solubility and the formation of sparingly soluble compounds is hardly to be expected. Mostly, Cu ions occur as aquo complexes or as soluble chelated complexes.

In vitro experiments were designed to provide some information on complex formation and ion association of Cu ions. We applied the technique of Brintzinger (1935) and of Jander & Spandau (1939, 1941), who used the dialysis coefficient instead of the diffusion coefficient as criterion, to investigate the rate of the Cu diffusion through cellulose or animal tissue membranes. We applied Cu concentrations similar to those found in feeds and in the digestive tract of animals. The Cu concentration of the dialysate was determined polarographically using the Kemula electrode (Weser & Kirchgeßner, 1964a).

Table 1. *Dialysis coefficients of copper in presence of some inorganic anions*

Added anion (10^{-3} moles/l.)	Dialysis coefficient λ (10^{-3} h $^{-1}$)
Without addition	7.9
SO $_4^{2-}$	7.9
H $_2$ PO $_4^-$	7.9
HPO $_4^{2-}$	0.5
P $_2$ O $_7^{4-}$	1.0
(PO $_3^-$) $_n$	1.2

Table 1 shows the effect on the dialysis of Cu of some inorganic ligands naturally occurring in feeds or in the digestive tract. The dialysis coefficient λ indicates the velocity of Cu dialysis through a cellulose membrane (Weser & Kirchgessner, 1964b). Proton-accepting anions (HPO_4^{2-}) as well as chelating agents (di- and polyphosphates) depressed the Cu dialysis coefficient but the anions SO_4^{2-} and H_2PO_4^- did not. Addition of cations showed no effect. These results were confirmed by dialysis through an animal tissue membrane.

The velocity of Cu diffusion may also be influenced by organic ligands. The diet is mainly composed of polymer organic compounds which are broken down into oligomer or monomer fragments during the passage through the digestive tract. The fragments may form stable complexes with Cu aquo ions and depress the velocity of Cu diffusion. Table 2 presents the Cu dialysis coefficients in presence of some organic ligands. Short-chain fatty acids as well as sorbose did not show any effect. Amino acids with high association constants for Cu delayed Cu dialysis. Though free citric acid was without any effect, the dialysis coefficient was depressed by sodium citrate.

Table 2. *Dialysis coefficients of copper in presence of some organic ligands*

Addition (5×10^{-2} moles/l.)	Dialysis coefficient λ (10^{-3} h^{-1})
Without addition	9.5
Acetic acid	9.5
Propionic acid	9.3
L(-)-sorbose	9.5
Citrate	5.8
Proline	4.7
Histidine	3.7

These and some other dialysis experiments may thus be summarized: the velocity of Cu diffusion is greatly diminished by complex-forming agents. Hence the question arose, whether these findings could be reproduced in the living animal. To examine the absorption rate of several Cu compounds in vivo we chose the rat liver test which has been used by Schultze, Elvehjem & Hart (1934), Schreier, Kretz & Yang (1957), Mills (1955, 1956) as well as by Dutt & Mills (1960). We gave weanling female rats a purified diet deficient in Cu for a depletion period of 4 weeks. The course of depletion, was checked at weekly intervals. At the end of the depletion period the Cu content of the rat livers was as low as $3.5 \mu\text{g/g}$ dry matter. Now we gave by stomach tube different Cu compounds dissolved in water to groups of four animals each. The animals were killed 24 h later and the Cu concentration of the livers was determined. The results are given in Table 3. Highest liver storage resulted from the administration of Cu aquo ions. The polyphosphate complex and the serine Cu complex were poorly absorbed. Thus the results were in agreement with our in vitro experiments. Because the animals were starved before the treatment, the concentration of complex-forming ligands in the stomach and in the upper part of the small intestine was low, and the hydrated Cu ions could pass and be absorbed quickly. The complex-bound Cu ions moved more slowly and were less efficiently absorbed.

Table 3. *Copper contents of livers of rats given by stomach tube a single dose of a Cu compound dissolved in water*

Compound	Total Cu content (μg)
None	8
Copper sulphate	13
Copper polyphosphate	8
Copper (serine) ₂	7

The breakdown of the ingesta leads to a higher concentration of complex-forming ligands in the digestive tract. Hence varying results were to be expected if the Cu compounds were added to the diet. We administered the various Cu compounds mixed with the food continuously for 2 weeks. The results are presented in Table 4.

Table 4. *Copper content of livers of rats given Cu compounds continuously in the diet for 2 weeks*

Compound (5×10^{-5} moles Cu/kg diet)	Total Cu content (μg)
None	10
Cu—sulphate	30
Cu—polyphosphate	28
Cu—citrate	38
Cu—(proline) ₂	31

Unlike in the stomach-tube experiment, a more efficient absorption of the Cu aquo ion could not be observed. Obviously a rapid absorption of the hydrated Cu ions was prevented by a high concentration of complexing agents. The small and stable copper citrate complex, however, was least affected in the rate of diffusion by additional complex-forming agents and could be absorbed most efficiently. These results partly agree with experiments of Mills (1954, 1956) who found an increased rate of Cu absorption after giving organic complexing agents from herbage extracts with high constants of Cu association. The results of Havinga & Bykerk (1947) and of Schreier *et al.* (1957) may also be interpreted in this way. On the other hand, the rapid absorption of stable complexes is certainly limited, for instance by the molecular size.

But even those transition metals that are bound in large complexes can be made available for absorption. This is possible by displacing the metal ion of the complex by another metal ion. If the environment of a complex provides a surplus of metal ions with higher association constants than that of the complex-bound metal ion, the less firmly bound metal ion may be set free and can be absorbed as aquo ion.

We examined the effect of a surplus of metal ions with high constants of complex association in balance experiments with growing pigs receiving extremely high supplements of copper sulphate (Kirchgessner & Weser, 1963). Additional dietary Cu increased the retention (Table 5) of the metal ions Fe^{3+} , Zn^{2+} , Mn^{2+} , Co^{2+} . We suppose that these metal ions have been displaced from macromolecular complexes

Table 5. *Daily retention of trace elements by growing pigs when copper sulphate was added to the diet (4×10^{-3} moles Cu/kg diet)*

Group	Cu (mg)	Fe (mg)	Zn (mg)	Mn (mg)	Co (μ g)
Control	1	0.3	6	1.3	10
CuSO ₄ · 5H ₂ O	30	12	8	2.4	38

by the highly complexing hydrated Cu ions and thus could be more efficiently absorbed and retained.

We tried to confirm this hypothesis by experiments in vitro. We suspended 9.3 g of egg albumin in 2 l. 0.15 M-KCl and added Fe, Zn, Mn, and Co in physiological amounts. The results are stated in the first column of Table 6. The experiments were repeated under the same conditions and 0.18 m-mole CuSO₄ · 5H₂O was added. It may be seen from the second column of Table 6 that the coefficients of dialysis of all the elements added were increased by the Cu supplement.

Table 6. *Dialysis coefficients of trace elements with or without addition of copper to a protein-containing solution*

Element	Dialysis coefficient λ (10^{-3} h^{-1})	
	No Cu added	Cu added
Fe	1.6	1.7
Zn	3.6	4.8
Mn	2.2	4.4
Co	1.7	17.9

Belonging to the strongest complex-forming metals of the first transition group, Cu displaced the less firmly bound metals from the macromolecular protein complexes (Kirchgessner & Weser, 1963).

I hope our work may represent a small contribution to the knowledge of the role of complex reactions in the metabolism of trace elements. Yet, it is my conviction that the number of unsolved problems has increased rather than decreased. For the rest, I hope to have emphasized that an appropriate use of trace elements in the feeding of animals has to be based on profound understanding of the metabolic mode of action.

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Factors which affect the availability of magnesium

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The incidence of hypomagnesaemia in ruminants has stimulated interest in the study of magnesium absorption from the alimentary tract. Under normal circumstances, the absorption of Mg is far from complete, so that the substantial endogenous excretion of Mg into the digestive tract which occurs in ruminants (Storry, 1961) can be a serious drain on the Mg reserve of the animal under conditions of impaired Mg absorption. Since man secretes relatively less saliva and intestinal juice than ruminants he is better equipped to exist on low Mg intakes. However, if absorption is impaired, as in the malabsorption syndrome, a negative Mg balance and hypomagnesaemia are often found (Hanna, Harrison, MacIntyre & Fraser, 1960).

The simplest way to account for endogenous Mg is to assume that it is secreted into the digestive tract distal to the absorptive region. However, Care and van't Klooster (1964) have observed in the sheep that under normal dietary conditions the