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Claus Munk Plum*

*The Biochemical Research Laboratory,

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Abstract

By far the majority of studies and speculations on metabolism in man and animals have been concerned with the fate of proteins, fats, and carbohydrates. Even when interest was awakened in enzymes and vitamins, the transformation of the organic substances was still studied. Gradually, however, various methods of the analysis and measuring instruments used have been so perfected that other problems can now be included in the field of biological and medical researches, namely, the significance of inorganic substances for the living organism.

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STUDIES ON "TRACE ELEMENTS" IN THE BLOOD, ESPECIALLY WITH REGARD TO THE OCCURRENCE OF COPPER AND ZINC (A PRELIMINARY PAPER)

Claus Munk PLUM

The Biochemical Research Laboratory, Kolonien Filadelfia, Denmark
(Chief: Dr. Claus Munk Plum)

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By far the majority of studies and speculations on metabolism in man and animals have been concerned with the fate of proteins, fats, and carbohydrates. Even when interest was awakened in enzymes and vitamins, the transformation of the organic substances was still studied. Gradually, however, various methods of the analysis and measuring instruments used have been so perfected that other problems can now be included in the field of biological and medical researches, namely, the significance of inorganic substances for the living organism.

These substances, which can often be demonstrated only as traces, have opened up new fields for our analytical investigations. It is clear today that even substances occurring in insignificant amounts have a vital importance.

Many of different ions have been repeatedly investigated and a number of their functions were clarified early. This is particularly the case with calcium, whose function in the construction of our bones and teeth, in the mechanism of coagulation, and in numerous nerve and muscle processes, etc., is not unknown. Sodium and potassium, as essential ions in the maintenance of the external and internal environments of the cell, have also received a considerable attention. The significance of iron is well known, not merely as a part of the oxygen-transporting haemoglobin of the red blood corpuscles, but also in those enzymes necessary for the oxygen turn-over in the cell. In addition to the above-mentioned substances, numerous others are found in varying amounts, such as copper, cobalt, zinc, manganese, magnesium, molybdenum, cadmium, and fluorine.

In the present paper an account will be given of the significance and occurrence of copper and zinc in blood, with particular attention to the variations found in haematological and neurological diseases.

Copper is found throughout the earth's crust. It has been known for a very long time, for example in alloys such as bronze, which has been used for thousands of years. Salts of copper were employed already in ancient Babylon, in the treatment of various eye diseases.

Table 1. Some figures for iron, copper and zinc in the human body. (Calculated from the literature)

	Daily intake mg	Total amount of metal in human body g	Serum value per μg %	Corpuscle value red cells per μg %	Intracorpuscular protein bound metal in %
Fe ·····	10—25	3—5	120	100000	Haemoglobin 0.34 Fe
Cu ·····	2—5	0.1—0.15	120	100	Haemocuprein 0.34 Cu
Zn ·····	10—15	2	130	1300	Carbonic Anhydrase 0.33 Zn

It has been known for almost two centuries that copper is present in both animal and plant materials. It was assumed, however, that its presence was due to contamination, and not because it was a naturally-occurring substance. It was not until the turn of the century that copper was recognized as an essential constituent of the living organism. After that observation, many workers began to study copper metabolism and its significance in the normal organism, as well as variations in the copper content associated with different pathological states.

The copper content of the normal human organism is about 150 mg of copper. Most of this is found in the liver, kidneys, heart and brain, while only relatively small amounts are found in the muscles, adrenal glands, bones and blood.

In the blood stream, copper is found in both plasma and blood corpuscles. The copper content of whole blood is reported as being around 100 micrograms per 100 ml. Women appear to have somewhat higher values than men.

About 90 per cent of the copper in plasma is bound to proteins, normally to the globulins, in particular to alpha, α_2 to form a compound called *cerulop-lasmin.* Most of the copper in the erythrocytes is likewise bound to proteins, forming a compound called *erythrocuprein.* The biochemical nature of the copper-containing substances found in the other tissues and body fluids is still unknown, but many copper-containing enzymes have been isolated from various tissues, as indicated in Table 2.

Most investigators have been interested only in the amount of copper in serum, but determinations of copper in both whole blood, erythrocytes and leucocytes would also appear to be of some clinical interest.

By far the majority of these investigators agree that under normal conditions, determinations of copper in whole blood give only about 80 per cent of the value of copper found in an equal volume of serum.

There are only few determinations of the copper content of erythrocytes; but some investigators claim to have demonstrated that the copper content in

Table 2. Mammalian Cuproproteins and Cuproenzymes.

Material	Copper %	Mol. Wt.
Cuproproteins:		
Cerebrocuprein I	0.25—0.30	35 000
Erythrocuprein (hemocuprein)	0.32—0.36	33 000
Hepatocuprein	0.31—0.41	35 000
Milk cuproprotein	0.19	?
Cuproenzymes:		
Ceruloplasmin	0.31—0.34	151 000
Cytochrome oxidase	0.006	?
Phenol oxidase (tyrosinase)	0.2	34 000 (110000)
Beta-mercaptopyruvate transsulfurase	0.17	35 000
Butyryl coenzyme A dehydrogenase	0.35	120 000—222 000
Uricase	0.06	110 000

erythrocytes amounts to only approximately 70 per cent of the amount of copper found in an equal volume of serum.

Only few investigators have attempted to determine the copper content of leucocytes. The amount found is very small; determined per 10^9 leucocytes, it is less than one per cent of the amount found in 100 ml serum.

Many factors appear to influence the concentration of copper in tissue, for example diet, age, hormones, pregnancy, and a variety of diseases.

As already mentioned, under normal conditions a greater copper content is found in the blood of women than of men.

During pregnancy, there is a steady rise in the amount of serum copper from the eighth week until term, and thereafter a fall to normal value, reached about the 10th week after delivery. This rise in serum copper content is accompanied by a corresponding rise in the concentration of ceruloplasmin. If the infant's copper content is examined at birth, a very low value is found in the serum, corresponding to a low ceruloplasmin content. The amount of copper which does not take part in the formation of ceruloplasmin, is the same in mother and infant. Not until the second month of life does the infant attain the normal copper content of blood and at the same time the normal content of ceruloplasmin. The interpretation of this must be that the infant is unable to form this complex protein-copper compound during foetal life.

If we now look at the shifts in values noted in various pathological states, we find a variety of data on the copper content of both serum and blood corpuscles. Table 3 shows the values found in our laboratory.

By far the majority of reports on copper metabolism in pathological states come from studies of serum and urine. This obviously does not give a picture

Table 3. The copper content of serum, whole blood, erythrocytes and leucocytes serum in normal and

Diagnosis	n	Serum	Whole blood	Erythrocytes
		Gamma pr 100 ml		
		Average (min-max)	Average (min-max)	Average (min-max)
Controls, male	100	111 (82—176)		
— female	100	122 (80—189)		
— male + female*	94	117 (84—162)		
— — — *	25	118 (87—156)		
— — —	66	117 (92—156)	96 (70—120)	79 (53—107)
Anemia simplex	5	129 (108—149)	108 (88—128)	100 (88—122)
Sec. Anemia (Ulcus ventriculi)	2	131 (109—152)	117 (98—136)	98 (82—114)
Anemia perniciosa	18	129 (109—165)	105 (93—126)	96 (83—116)
Leukemia, acute	2	212 (196—228)	177 (162—192)	125 (72—138)
— chr.	6	186 (129—220)	145 (99—182)	102 (83—121)
Various medical diseases without anemia*	56	118 (75—153)		
Mb. Basedowii	4	196 (122—282)	110 (82—156)	80 (67—102)
Polyarthritis chr.	15	158 (109—224)	123 (92—159)	82 (54—102)
Tuberculosis pulm.	12	115 (79—171)	101 (74—131)	80 (62—102)
Various neurol. diseases*	55	124 (95—162)		
— — — *	66	122 (90—137)		
Epilepsy*	109	133 (86—207)		
—	25	139 (82—216)	112 (70—159)	80 (52—112)
Multiple Sclerosis*	78	124 (92—176)		
— — *	10	117 (103—133)		
— —	22	130 (103—171)	119 (99—131)	111 (87—126)
Psychoses and Neurosis*.	59	119 (85—174)		
— — — .	15	118 (96—172)	95 (72—124)	82 (51—108)
Schizophrenia	10	132 (92—200)	94 (70—136)	80 (56—110)
Cancer canal. dig.	4	168 (147—183)	127 (114—143)	69 (66—81)
— gen. fem.	10	201 (146—249)	154 (126—200)	69 (49—80)
— mammae	3	192 (152—241)	160 (120—201)	114 (106—123)
Graviditas (32—36 wks.).	3	258 (200—312)	173 (144—200)	83 (67—100)
4 weeks post partum	2	130 (122—138)	98 (90—107)	82 (70—91)

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in normal and pathological cases. The ceruloplasmin and copper-oxidase content of pathological cases.

n	Leucocytes Gamma pr 10^9 cells Average (min-max)	n	Ceruloplasmin mg pr 100 ml Average (min-max)	n	Copperoxidase Extinction 610 mμ Average (min-max)
				94	0.281 (0.192—0.405)
		25	37.76 (28.4—58.6)	25	0.278 (0.195—0.399)
60	0.91 (0—1.59)	25	37.80 (28.3—59.4)	25	0.284 (0.191—0.399)
5	0.97 (0.69—1.34)				
2	0.90 (0.34—1.46)				
16	0.98 (0.26—1.51)			12	0.279 (0.142—0.351)
2	0.76 (0—1.52)				
6	0.79 (0—1.09)				
				56	0.293 (0.189—0.395)
4	0.96 (0—14.7)				
11	0.92 (0—1.42)				
12	0.96 (0—1.59)				
				55	0.271 (0.181—0.365)
		66	38.98 (28.0—57.7)	66	0.279 (0.199—0.392)
				109	0.311 (0.190—0.467)
25	0.86 (0—1.57)	25	38.1 (23.2—61.2)	25	0.299 (0.215—0.455)
				74	0.215 (0.144—0.295)
		10	22.68 (18.2—20.6)	10	0.167 (0.148—0.215)
12	0.99 (0.49—1.78)	22	23.36 (18.0—27.6)	22	0.176 (0.132—0.233)
				59	0.274 (0.198—0.425)
15	0.96 (0—1.37)				
10	0.93 (0—1.49)	10	38.82 (26.8—58.9)	10	0.289 (0.180—0.412)
4	0.89 (0.29—1.23)				
4	0.78 (0—0.97)	4	53.12 (40.9—71.0)	4	0.393 (0.276—0.472)
3	1.02 (0.38—1.56)	3	51.0 (40.12—66.84)	3	0.369 (0.255—0.463)
3	0.94 (0.29—1.39)				
2	0.93 (0.65—1.21)				

of the total copper metabolism of the organism, and further studies, elucidating the variations in copper in various tissues, must be awaited, before a complete picture can be formed of the changes in copper metabolism during pathological states.

On the basis of our present knowledge, we may distinguish between states in which there is hypercupraemia, hypocupraemia and a normal level of copper. These evaluations are based on the copper content of the serum, and only the serum. Over and above this are the variations that may occur in copper content of erythrocytes and leucocytes, variations in copper oxidase activity and in the content of ceruloplasmin.

In our laboratory we have been interested during the past few years in these variations in a number of patients suffering from multiple sclerosis, and have also supplemented our material by a number of studies of other neurological diseases. Finally, to elucidate the problem as far as possible, we have also studied the copper metabolism in a number of other diseases. The more extensive the material has become, however, the more variegated has been the picture of copper metabolism, so that the present results must be regarded as only a provisional communication, while the work continues.

We will first examine the state of affairs in hypercupraemia, which is the most usual shift in copper content.

Our studies have confirmed the well-known observations reported in the literature, that the copper content is elevated in acute and chronic infections.

In addition, we could confirm the observation of an increase in copper with increased metabolism. Our results have varied considerably, and we are unable to say whether there is a parallelism between increase in metabolism and increase in amount of copper. This result is in agreement with the observations made by HEILMEYER in 1941.

In most cases, the rise in serum copper was accompanied by a rise in ceruloplasmin content, without an even approximately as great increase in the copper content of erythrocytes or leucocytes.

An examination of the copper content of the blood in patients with malignant tumors showed an increase in serum copper, with normal or at times slightly reduced erythrocyte copper. There was, however, an exception with mammary cancer, in which the same rise in copper was found in both serum and erythrocytes, at the same time with a slight rise in the copper content of the leucocytes.

The serum copper seems to be distributed in the same way as under normal conditions, so that most of it is bound to globulins. There is some discussion as to how this increased amount of copper should be explained. Many consider that it should be interpreted as the reaction of the organism to foreign proteins.

This explanation is used by HEILMEYER in interpreting hypercupraemia in pregnancy, and by BRAUN-STAPPENBECK likewise in malignant tumors. The question is, however, not clarified, for one thing there seems to be no parallelism at all between the duration of the disease, its degree of severity and the serum copper content.

According to the literature, increased copper in the serum is also found in the following diseases: *lupus erythematosus disseminatus*, glomerulonephritis, rheumatoid arthritis and rheumatic fever.

A study of 15 patients with rheumatoid arthritis showed a rise in serum copper and at the same time a rise in both ceruloplasmin content and copper oxidase activity, while the content of copper in the erythrocytes rose only slightly.

The literature reports variations in serum copper and ceruloplasmin content in myocardial infarctions. We have unfortunately had no opportunity of examining this.

In leukaemia, we observed a rise in serum copper, while the erythrocyte copper level values remained around normal. A slight reduction in copper content is found in the leucocytes, although the values found cannot be regarded as statistically significant, both because of the limited material and because of the considerable spread observed in the normal group and the patient material.

The literature reports increased serum copper in a variety of forms of anaemia, being observed frequently in pernicious anaemia, aplastic anaemia and iron-deficiency anaemia. The copper content of the leucocytes is not reported to have altered during these diseases. In our studies of patients with pernicious anaemia we found a slightly increased content of copper in serum, whole blood and erythrocytes, just as this was also found in the leucocytes, but here the percentage increase was not so great as with the erythrocytes. A third of the patients examined, in spite of their rise in serum copper, showed a reduced amount of ceruloplasmin and reduced copper oxidase activity, just as can be observed in patients with multiple sclerosis.

In diseases of the liver, such as portal cirrhosis, both serum copper and ceruloplasmin are found to rise, but in this case it is found that the copper which is not combined in the ceruloplasmin, increases in parallel with the rise in the ceruloplasmin, in contrast to the findings in a number of the previously mentioned diseases.

Increased serum copper was found on examining a number of patients with schizophrenia. This finding confirms a number of previous reports, but there is still some discussion on this increase, as it has not been demonstrated in some other studies, just as discussion continues on whether a rise in the copper oxidase activity takes place at the same time. Our own observations also vary con-

siderably, as a number of the patients examined show quite normal conditions with regard to ceruloplasmin and copper oxidase activity, while others show a clear rise. It should also be emphasized that repeated studies of the same patient at intervals of some weeks have been able to show both normal and increased levels of copper oxidase activity. These findings can be interpreted as signifying that the rises in serum copper and copper oxidase activity are not anything specific for the disease, but that in a patient with schizophrenia the copper metabolism as a whole is out of balance.

In addition to these studies, we have examined a large number of patients with multiple sclerosis. The first investigations were concerned mainly with the determination of total serum copper and ceruloplasmin content, and the results were presented at the Tokyo Haematology Congress in 1960.

These studies were continued later and extended by studies of the copper content of erythrocytes and leucocytes.

A few workers report that a slightly reduced copper oxidase activity is found in multiple sclerosis, while others report normal conditions.

We examined 90 patients with multiple sclerosis and found a reduced copper oxidase activity, compared to the results from 125 analyses in normal subjects.

As a measure of oxidase activity, we used the optical density in all our investigations, determined by HOUCHIN'S method (1958). The average value obtained for our normal material was 0.283, while for the patients with multiple sclerosis it was found to be only 0.204. The difference was shown to be statistically significant. As mentioned before, the copper oxidase activity in cases of pernicious anaemia was found to be decreased in one third of the cases, just as in the patients with multiple sclerosis.

Alongside the determination of copper oxidase activity determinations were made of the ceruloplasmin amount. This was found to be clearly reduced in the patients with multiple sclerosis, while only one third of the patients with pernicious anaemia, as mentioned above, showed a decrease. The average value for the normal material was 37 mg ceruloplasmin per 100 ml, while in patients with multiple sclerosis the average was only 23 mg per 100 ml.

In addition to these studies of serum copper, determinations were made of copper in erythrocytes and leucocytes in 22 patients. Reduced copper amounts were found in the leucocytes in a number of the patients, while the copper in the erythrocytes was found increased.

However, the material in these studies is too small to permit any definite conclusions from the findings, but the investigations are being continued.

As I mentioned previously, cases of hypocupraemia are also found. The most frequently studied disease showing this disturbance in copper metabolism

is Wilson's disease. Unfortunately, I have not myself had the opportunity of examining patients with this disease, as the last patient suffering from the disease in Denmark died about 15 years ago in our hospital. The literature shows that in such cases, a reduction in serum copper of more than 50 per cent may be involved.

Among other diseases in which a reduction in copper amount is found might be mentioned sprue and dysproteinaemia. In addition, a series of observations of reduced copper levels in the serum have been made in "the nephrotic syndrome".

From discussing variations in the amount of one "trace element" in the blood, copper, let us turn to considerations of another of these trace elements, zinc.

Zinc as a pure metal has been known of only relatively recent date, its first description as a chemically pure element being from about 1600. The metal is widespread in the earth's crust, to an amount quoted as 0.004 per cent. The element appears to have vital importance for the growth of both plants and animals.

Interest for zinc in human pathology first arose about 50 years ago, but since then, numerous studies have been made of its occurrence and functions, although it must be admitted that studies of the disturbances in its values which can be found in pathological states are still limited.

One of the best studied compounds of zinc is undoubtedly insulin, but this lies outside the scope of the topic to be discussed here in this paper.

Table 4. Zinc metalloenzymes of known metal content.

Enzyme	Zinc %	Mol. Wt.
Carbonic anhydrase (bovine erythrocytes)	0.1—0.3	30 000
Carboxypeptidase (bovine pancreas)	0.18	13 300
Alcohol dehydrogenase (yeast)	0.18	150 000
Alcohol dehydrogenase (equine liver)	0.18	73 000
Glutamic dehydrogense (bovine liver)	0.2—0.3	1 000 000
Lactic dehydrogenase (rabbit skeletal muscle)	0.07	?
Alkaline phosphatase (porcine kidney)	0.15	?

The human organism contains a total of approximately 2 grams of zinc. The highest concentration is found in the testes, hair and bones; the lowest concentration is found in the brain and blood.

There is thus no relation between the amounts of zinc and copper in the various organs.

Examining the zinc concentration in the blood, it is found in both serum and blood corpuscles. Serum contains 15 per cent of the zinc, found in blood

in the form of two components. The one, the lesser part, about one third of the serum zinc, is probably bound to globulin, while the other, greater part is loosely bound to albumin and represents "the transport zinc".

The other 85 per cent of the zinc is found in the erythrocytes.

The leucocytes also contain a certain amount of zinc. A protein can be extracted from the leucocytes, containing about 0.3 per cent zinc. This amounts to 80 per cent of the zinc in the leucocytes. An enzymatic compound is involved, but "the nature of its enzymatic activity is not known". All that is known, is that its function differs considerably from that of "carbonic anhydrase". So far, no fewer than 7 zinc metallo-enzymes have been separated from animal tissues, as shown in Table 4. As mentioned, our present knowledge of these substances is very limited, but the observations which have been made appear to confirm that metal enzyme complexes are involved, rather than metallo-enzymes.

Much of the information available on zinc metabolism comes from studies of the zinc content of the blood.

In spite of the scanty literature and the considerable variation between the observations of different workers, it may be stated with more or less certainty, as also confirmed by our own observations, that under normal conditions, about 85 per cent of the zinc in the blood is found in the erythrocytes, while 2—3 per cent is found in the leucocytes, and the remainder, about 10 per cent, is found in the serum. The analyses also show that no definite difference can be demonstrated in the zinc content between men and women, as was the case with copper. Variations in the zinc concentration of the blood can be found, just as with copper, but these variations do not always run parallel between the two elements.

Reduced zinc values can often be found in acute and chronic infections. A clear relation is seen between the rise in temperature and the fall in zinc level, so that the lowest zinc values are found with the hightest temperature.

Studies of a number of patients with malignant tumors showed likewise a reduction in serum zinc combined with a significant fall in the amount of zinc in the erythrocytes and a quite slight fall in the leucocyte content. The fall seemed most pronounced in the cases of mammary cancer, in which the most pronounced rises in copper content were found.

In the analysis of blood from patients with simple anaemias, we have been unable to demonstrate definite variations in the serum zinc content, as reported by other authors, while in our studies of cases of pernicious anaemia, we have observed a clear fall in the serum zinc, erythrocyte zinc and leucocyte zinc.

The blood of patients with multiple sclerosis also shows a clear reduction in the zinc content of serum, erythrocytes and leucocytes, just as in pernicious anaemia,

Table 5. The zinc content of serum, whole blood, erythrocytes and leucocytes in normal and pathological cases.

Diagnosis		Serum	Whole blood	Erythrocytes	Leucocytes
		Gamma pr 100 ml			Gamma pr 10^9 cells
		Average (min-max)	Averagc (min-max)	Average (min-max)	Average (min-max)
Controls, male	25	131 (80—169)	686 (512—802)	1312 (1058—1688)	29 (17—46)
— female	25	129 (78—160)	605 (507—739)	1210 (1045—1463)	30 (16—51)
Anemia simplex	5	121 (61—179)	625 (492—776)	1241 (1012—1493)	27 (16—43)
— perniciosa	11	103 (59—141)	541 (456—614)	1198 (1027—1304)	22 (12—31)
Leukemia acute	2	88 (45—121)	392 (296—470)	1014 (820—1204)	10 (7—13)
— chr.	6	100 (55—130)	433 (319—483)	1126 (873—1216)	14 (6—21)
Tuberculosis pulm.	12	129 (85—151)	652 (541—752)	1292 (1072—1422)	30 (16—51)
Various neurol. diseases	15	131 (61—152)	651 (556—842)	1281 (1156—1402)	29 (15—39)
Epilepsy	10	139 (97—181)	729 (582—879)	1369 (1172—1690)	34 (21—53)
Multiple Sclerosis	12	112 (60—133)	593 (478—663)	1178 (957—1283)	25 (12—34)
Psychosis et Neurosis	11	133 (78—162)	699 (567—783)	1371 (1137—1524)	30 (16—39)
Schizophrenia	5	135 (83—157)	651 (561—772)	1231 (1127—1462)	29 (15—50)
Cancer uteri	10	106 (65—146)	576 (512—658)	1278 (1166—1392)	22 (10—31)
— mammae	3	98 (60—124)	521 (472—567)	1149 (1072—1226)	20 (9—30)

During pregnancy, there is a clear reduction in the zinc concentration in the mother, while the foetal blood shows a high concentration. This is the exact opposite of the findings with copper.

The possibilities of significant interactions among the trace elements in human and animal physiology, have not received the attention which they undoubtedly deserve, in spite of the considerable body of knowledge indicating the importance of such interactions in the nutrition of plants.

A few studies have been undertaken on the effects of one element in counteracting the toxic effects of excessive intakes of another, such as copper in zinc and molybdenum toxicity, and on the influence of comparatively massive intake of one element on the absorption of another, such as phosphate on iron and manganese uptake. Until recently, however, little evidence has been presented to suggest that there is an interrelationship between the amounts of the trace elements required by animals and human beings, or between their metabolism within the body, at physiological and pathological levels.

Among the earliest of such physiological interactions to be revealed was that between iron and copper. Both these elements are concerned, as one of their functions, with hematopoiesis, so that a deficiency of one will clearly limit the requirement of the other. It is usually stated that copper is necessary for the iron utilization in blood formation, but iron must also be necessary for copper utilization, at least to the extent that copper is concerned in hematopoiesis. It is well-known from studies carried out on animals that an inhibiting effect of molybdenum on copper retention is markedly influenced by the level of inorganic sulphate. From this it will be clear that the studies of copper metabolism and copper requirements are of little value unless the molybdenum and inorganic sulphate levels in the diet are known. Further it has been shown in animals that the copper requirement is greatly affected by the level of the dietary zinc and so on. As mentioned, however, most examinations and experiments are carried out on animals, and so far we know very little about the interaction of the trace elements in human pathology. In any case it is obvious that considerable caution should be exercised in the interpretation of nutritional and metabolic studies of the trace elements, in which attention is restricted to a single element only.

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