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Biologically active metals in human tissues. I. The effect of age and sex on the concentration of copper in aorta, heart, kidney, liver, lung, pancreas and skeletal muscle.

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Key terms: age; aorta; biologically active metal; concentration of copper; copper; heart; human tissue; kidney; liver; lung; pancreas; sex; skeletal muscle

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Biologically active metals in human tissues

I. The effect of age and sex on the concentration of copper in aorta, heart, kidney, liver, lung, pancreas and skeletal muscle

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VUORI, E., HUUNAN-SEPPÄLÄ, A. and KILPIÖ, J. O. Biologically active metals in human tissues: I. The effect of age and sex on the concentration of copper in aorta, heart, kidney, liver, lung, pancreas and skeletal muscle. *Scand. j. work environ. & health* 4 (1978) 167—175. Autopsy specimens of aorta, heart, kidney, liver, lung, pancreas and skeletal muscle were collected from 86 accident victims. The copper concentration in each tissue was determined with atomic absorption spectrophotometry. The descending order of the tissues in respect to copper concentration was: liver, heart, kidney, pancreas, lung, muscle, and aorta. No significant difference was found in the copper levels of samples from male and female autopsies. When the effect of age on the average copper concentration was studied, liver and kidney showed a decreasing concentration up to maturity, the copper concentration in pancreas and skeletal muscle showed a continuous decline with increasing age, and there was no clear-cut effect of age on the copper concentration of heart, lung and aorta. According to the results the Finnish population does not differ, on the average, from other populations with respect to tissue copper concentrations.

Key words: copper, human tissues.

Copper is an essential trace element for animal life. Animal experiments with copper deficient diets have shown that the main symptoms caused by copper deficiency are anemia, vascular abnormalities, abnormal keratinization and depigmentation of hair and wool, difficulty in parturition, neonatal ataxia, abnormalities in bone formation, myocardial fibrosis, demyelination of the central nervous system and gastrointestinal disorders (2, 6, 27). Copper is known to act as a cofactor for many enzymes, it is an integral part of some proteins, and it is also found in some naturally occurring pigments (6, 27).

Since copper deficiency can lead to so many pathological conditions, it is quite natural that the role of copper in the genesis of human diseases has been widely discussed (2, 27), especially in respect to chronic illnesses such as cardiovascular diseases (13, 21). However, human adults have never been reported to suffer from dietary copper deficiency (28).

From earlier reports it is evident that there is a geographical and racial variation in the copper concentrations of at least some human tissues (22). However, with few exceptions the results concerning the content of copper in human tissues are based on studies with a very limited number of samples. Besides only a few authors have tried to assess the effect of age (9, 11,

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22) and sex (14) on the copper concentration of human tissues.

When the possible role of trace elements in the etiology of diseases is studied, it is essential to know the average level of the trace elements in the tissues of healthy subjects. Only one study has been published on this topic in Finland (8). According to Forssén (8) the copper concentrations of various tissues in the Finnish population seem to be lower than those reported elsewhere. Recently it has been reported that during the last five decades the copper content of the average Finnish diet has diminished (12). The morbidity and mortality due to cardiovascular diseases are extraordinarily high in Finland; e.g., of all European countries Finland has the highest annual rate of acute myocardial infarctions in males aged 20—60 years (29). In addition, it has been proposed that the beneficial effect of copper is to maintain the elasticity of the blood vessels (13). This proposal has led to the suggestion that a low copper supply might be one of the factors causing chronic cardiovascular diseases in Finland (12).

In order to gain more information on the copper content of human beings in Finland and on the effect of age and sex on the copper concentration of human tissues, we have calculated the copper concentration of the aorta, heart, kidney, liver, lung, pancreas, and skeletal muscle of 86 subjects who died accidentally.

MATERIAL AND METHODS

Autopsy specimens of aorta, heart, kidney, liver, lung, pancreas, and skeletal muscle were collected from 96 humans who had died in an accident. The autopsies were performed at the Department of Forensic Medicine, University of Helsinki, during the autumn of 1973 and the spring of 1974. Most of the subjects had died abruptly. Ten subjects were excluded later — five because they had stayed alive for more than 24 h in the hospital, two because they were known to have had diabetes mellitus, one because he had suffered from hypopituitarism, one because he had suffered from rheumatoid arthritis since the age of 3, and one because he had died of myocardial infarction. In these cases the disease or the medical treatment before death may have caused disturbances in trace-element metabolism. Thus 86 cases were included in the statistical analysis, 64 males and 22 females. The age and sex distribution of the series is presented in fig. 1.

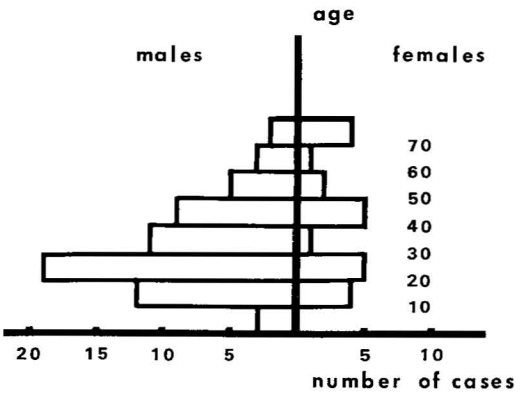

Fig. 1. Age and sex distribution of the subjects.

The specimens for analysis were taken during the autopsy. Samples of aorta were taken from areas where atherosclerotic changes, if present, were not prominent. Specimens of m. pectoralis major were taken to represent skeletal muscle, and liver, lung, pancreas and kidney samples were taken to represent the parenchyma of each organ. A division along the bronchial tree was not made in the lung samples. Kidney samples were taken as a segment which represented both cortex and medulla in equal proportions. All samples were put into polyethylene bags and taken to the laboratory for deep freezing (—20°C) immediately after autopsy. The procedure of taking and preparing samples was carefully tested so that contamination would be prevented.

Approximately 2 g of the tissue samples were weighed in covered 15 ml silica crucibles. The samples were dried overnight at 105°C and then carbonized in a muffle furnace at 250°C for 4—5 h. The dry ashing commenced at 450°C and took approximately 48 h to be completed. In the middle of the ashing procedure the crucibles were allowed to cool, and 0.1 ml of concentrated nitric acid (Suprapur®, Merck) was added. The acid was evaporated at 250°C, and the temperature

was then raised again to 450°C. When needed, the treatment with nitric acid was repeated.

The ash was dissolved with 2 ml of 4M hydrochloric acid (Suprapur®, Merck) with gentle warming. The crucibles and covers were rinsed several times with small volumes of deionized water and finally diluted to 10 ml with deionized water.

The copper concentrations of the ash solutions were then determined with flame atomic absorption spectrophotometry (Perkin-Elmer, model 300). The instrument was calibrated according to the manufacturer's recommendations.

The accuracy and precision of the method were tested with standard reference material no. 1577, bovine liver, produced by the National Bureau of Standards (NBS), Washington, D.C. Our mean result from eight determinations was 199 μg/g with a range of 190—203 μg/g. The certified value for copper is 193 $\pm$ 10 μg/g as given by the NBS.

The mean dry weights (of wet weight) and ash weights (of dry weight) of the tissues studied are presented in table 1.

Table 1. Dry weight and ash weight of the material.

Material	Dry weight (% of wet wt.)		Ash weight (% of dry wt.)	
	Mean	SD	Mean	SD
Aorta	28.3	2.8	4.4	1.9
Heart	22.0	4.0	4.7	0.9
Kidney	20.4	2.6	5.4	0.8
Liver	28.9	4.0	3.8	0.8
Lung	20.4	2.7	5.4	1.0
Muscle	25.2	4.2	4.4	0.8
Pancreas	26.1	7.8	4.6	1.3

RESULTS

The copper concentrations of the analyzed tissues are shown in table 2 for all the subjects, and separately for the males and females. The distributions were quite normal in the heart and kidney samples, but they were skewed in the rest of the specimens, for which they tended towards lower values. If the median values are examined, the females have lower copper concentrations for every tissue analyzed, except aorta, but the differences between the two sexes were not remarkable.

It can be seen (table 2) that the concentration of copper is the highest in the liver samples, and then in descending order in the heart, kidney, pancreas, lung, and muscle samples, and is the lowest in the aorta. Figs. 2—8 show the effect of age on the concentrations of copper, as well as the ranges in each tissue analyzed. The concentration of copper in liver (fig. 5) and kidney (fig. 4) decreases up to maturity, while that in pancreas (fig. 8) and skeletal muscle (fig. 7) show a continuous decline with increasing age. No clear-cut effect of age on the copper concentration of the heart (fig. 3), lung (fig. 6), or aorta (fig. 2) can be seen. In table 3

Table 2. Number of cases and tissue copper concentrations (μg/g of dry weight) of males, females and all subjects.

Material	Males				Females				All subjects			
	Number of cases	Copper concentration			Number of cases	Copper concentration			Number of cases	Copper concentration		
		Median	Mean	SD		Median	Mean	SD		Median	Mean	SD
Aorta	64	4.40	5.26	2.40	20	4.50	4.55	1.50	84	4.40	5.09	2.23
Heart	64	17.3	17.3	4.65	22	16.9	16.7	2.97	86	17.2	17.2	4.28
Kidney	62	13.3	13.7	3.11	22	12.8	13.7	4.21	84	13.2	13.7	3.40
Liver	64	22.7	23.8	9.51	22	20.4	21.7	6.65	86	22.3	23.3	8.88
Lung	63	6.80	7.40	2.80	22	6.50	6.75	2.70	85	6.60	7.23	2.77
Muscle	64	5.70	6.21	3.09	22	4.50	5.95	4.39	86	5.30	6.14	3.44
Pancreas	64	6.90	7.52	3.45	21	6.20	6.60	2.24	85	6.70	7.30	3.21

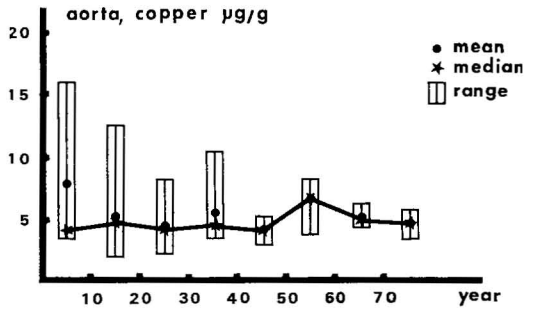

Fig. 2. Median copper concentration of aorta as a function of age. (µg/g, dry weight)

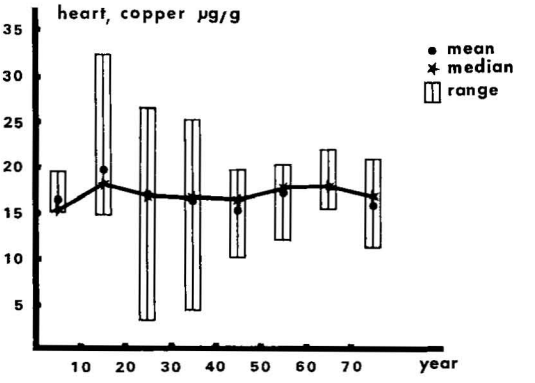

Fig. 3. Median copper concentration of heart as a function of age. (µg/g dry weight)

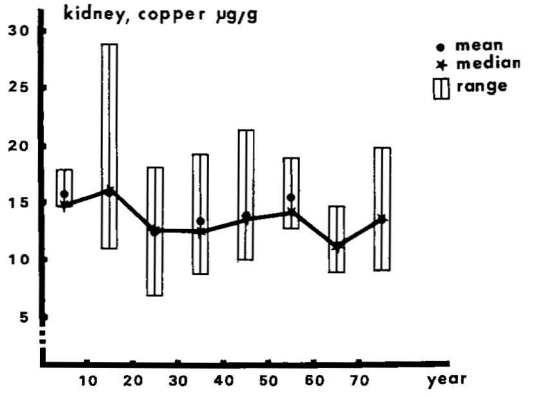

Fig. 4. Median copper concentration of kidney as a function of age. (µg/g, dry weight)

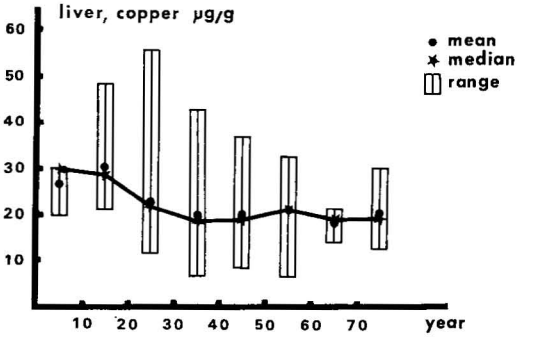

Fig. 5. Median copper concentration of liver as a function of age. (µg/g, dry weight)

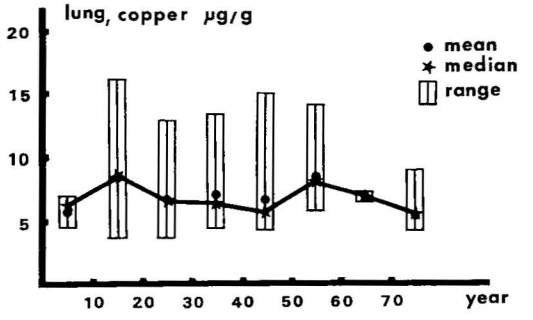

Fig. 6. Median copper concentration of lung as a function of age. (µg/g, dry weight)

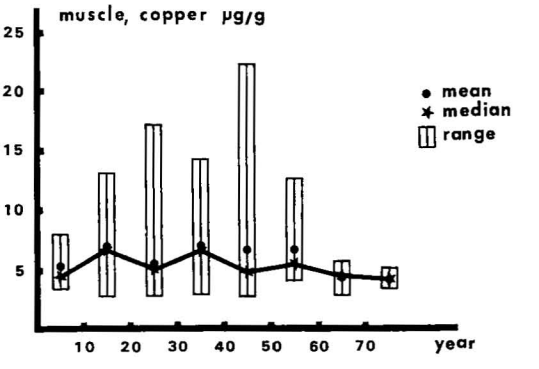

Fig. 7. Median copper concentration of muscle as a function of age. (µg/g, dry weight)

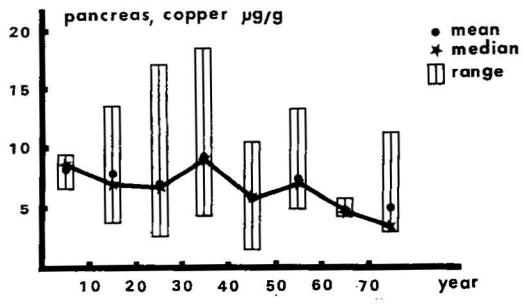

Fig. 8. Median copper concentration of pancreas as a function of age. (µg/g, dry weight)

the concentration of copper measured in liver in the present study is compared with the corresponding concentrations reported in earlier studies. Table 4 shows a comparison of our results with those of earlier studies also for tissues other than liver. To make the comparison possible our results have been converted to give micrograms per gram in ash weight according to the percentages given in table 1.

DISCUSSION

When one attempts to assess the levels of trace-elements in human beings, there are several difficulties, e.g., obtaining samples which represent a normal healthy population. Most authors have taken samples for analysis from subjects who have died abruptly in accidents, but they have also included subjects with known diseases (1, 9, 25). Even subjects with dissimilar diseases have been used as control groups in studies concerning tissue copper concentrations in certain other diseases (11, 14, 15). In our study we collected samples from autopsy subjects who were obviously healthy prior to death and who at autopsy were verified to have been killed accidentally. Most subjects had died instantly and all within 24 h of the time of the accident. Deaths caused by violence were about three times more common among males than females in Finland in 1973 (3). This phenomenon is the main reason for the preponderance of males in our material, since the subjects who died in accidents involving violence and were sent for autopsy to the Department of Forensic Medicine were included in our study in the order of autopsies performed.

Table 3. Mean copper concentration of human liver according to different authors. (µg/g dry weight)

Mean (µg/g)	Number of cases	Method [a]	Reference	Geographic area
28	199	ES	Butt et al. 1954 (1)	United States
28	634	C + S	Griffith et al. 1954 (9)	United States
25 [b]	148	ES	Tipton and Cook 1963 (24)	United States
20 [b]	6	NAA	Parr and Taylor 1964 (19)	United States
15 [b]	4	C + S	Cartwright and Wintrobe 1964 (2)	United States
23	7	NAA	Todd et al. 1967 (26)	England
35	40	NAA	Smallwood et al. 1968 (23)	England
33	8	NAA	Danielsen and Steinnes 1970 (5)	Norway
15 [b]	36	AAS	Morgan 1972 (15)	United States
24	92	ES	Indraprasit et al. 1974 (11)	United States
30	19	AAS	McKenzie 1974 (14)	New Zealand
26	16	AAS	Evenson and Anderson 1975 (7)	United States
23	86	AAS	Present study	Finland

[a] AAS = atomic absorption spectroscopy; C + S = chemical or spectrographic analysis; ES = emission spectroscopy; NAA = neutron activation analysis.

[b] Calculated value to give micrograms per gram of dry weight on the assumption that the mean ash weight of dry weight and dry weight of wet weight average 3.7 % and 35 %, respectively (24).

Table 4. Comparison of copper concentrations of tissues in different geographic areas. (μg/g ash weight)

Geographic area	Aorta	Heart	Kidney	Liver	Lung	Muscle	Pancreas	Method [a]	Reference
United States									
Median	—	—	—	—	—	—	—	C + S	Griffith et al.
Mean	—	446	377	746	247	—	—		1954 (9) [b]
Cases	—	137	201	199	72	—	—		
United States									
Median	90	350	260	510	120	74	140	ES	Tipton and Cook
Mean	97	350	270	680	130	85	150		1963 (24)
Cases	103	140	143	148	141	136	138		
United States									
Median	—	—	—	—	—	—	—		Cartwright and Wintrobe
Mean	—	273	182	392	—	75	—	C + S	1964 (2) [b]
Cases	—	5	5	5	—	5	—		
Africa									
Median	110	320	270	700	150	—	150	ES	Tipton et al.
Mean	120	350	400	870	170	—	140		1965 (25)
Cases	16	43	48	45	44	—	6		
Far East									
Median	170	350	410	1200	230	—	180	ES	Tipton et al.
Mean	250	410	700	1700	420	—	260		1965 (25)
Cases	65	62	66	67	69	—	58		
Near East									
Median	150	290	340	710	200	—	180	ES	Tipton et al.
Mean	200	320	510	990	350	—	190		1965 (25)
Cases	15	20	31	33	33	—	26		
Switzerland									
Median	58	340	200	700	130	—	160	ES	Tipton et al.
Mean	110	350	240	810	130	—	140		1965 (25)
Cases	5	8	9	9	7	—	4		
Finland									
Median	40	240	140	340	60[c]	0[d]	90	X-ray	Forssén
Mean	—	—	—	—	—	—	—		1972 (8)
Cases	18	20	19	20	19	19	19		
New Zealand									
Median	—	—	—	—	—	—	—	AAS	McKenzie
Mean	—	—	320	656	155	—	—		1974 (14)
Cases	—	—	19	19	10	—	—		
Finland									
Median	100	366	244	587	122	120	146	AAS	Present study
Mean	116	366	254	613	134	140	159		
Cases	84	86	84	86	85	86	85		

[a] AAS = atomic absorption spectoscopy; C + S = chemical or spectrographic analysis; ES = emission spectroscopy; X-ray = X-ray fluorescence spectrometry.
[b] Calculated values to give micrograms per gram of ash weight by using mean values of percentages for ash weight of wet weight and ash weight of dry weight given by Tipton and Cook (24).
[c] Middle lobe.
[d] < 20 μg/g.

There are several reports in the literature on changed tissue copper concentrations in patients with certain diseases. For example, it has been reported that the concentration of copper is low in the aortas and high in the hearts of atherosclerotic patients (21). High liver copper concentrations have been noted in Wilson's disease,

primary biliary cirrhosis, other types of intrahepatic cholestasis, large duct obstruction, and in chronic hepatitis (23). Elevated hepatic copper content has also been reported in subjects with ischemic heart disease, if the patients were not suffering simultaneously from diabetes or hypertension (16). No differences in liver copper levels have been found between subjects who died from hypertensive disease or neoplastic disease and subjects dying from other causes (14). However, these findings are not uniform; a decreased liver copper concentration has been reported to occur in hypertensive patients (11). Low hepatic copper content has been reported in patients with a hepatic neoplasm or with diabetes mellitus (20). Normal hepatic copper content has been reported in other types of carcinomas (16), and no significant difference has been found between the copper concentrations of normal and malignant tissues (5).

When the role of trace elements in various diseases is discussed, it is not only essential to know the concentrations of these elements in tissues under normal and disease conditions, but also to try to interpret that information into the known role of trace elements in the metabolic processes. For example it has been reported that the copper content is low in atherosclerotic aortas (21), and, at the same time, it is known that copper deficient pigs and chickens develop abnormal elastin (6), due to reduced amine oxidase activity caused by copper deficiency. Abnormal elastin contains less desmosine and isodesmosine and has fewer cross-linkages than normal elastin. This phenomenon was first noted in copper deficient animals, but also human elastin of aorta and skin from a copper deficient patient (Menkes's disease) showed the same structural abnormalities (4, 17, 18). This same situation might exist in atherosclerotic aortas where copper concentrations have been reported to be low (21). It should be remembered, however, that the analysis and interaction of the findings from grossly sclerotic aortas are highly difficult. Copper metabolism is also known to be changed in patients with rheumatoid arthritis (10).

Although the literature on the copper content of human tissues is extensive, only a few studies have evaluated the effect of age (9, 11, 22) and sex (14). In our study the order of tissues with respect to descending copper concentrations was liver, heart, kidney, pancreas, lung, muscle and aorta. This order is the same as that reported earlier (8, 24). According to the median values in our study, females have lower copper concentrations for every tissue investigated, except the aorta. However, when the mean values are examined, there are no significant differences between the sexes. Neither did McKenzie find differences in copper concentrations in male and female autopsies (14).

No effect of age could be found on the copper concentration of heart, lung and aorta. The copper concentration of pancreas and skeletal muscle showed a decrease with increasing age. The copper concentration of liver and kidney showed a decrease up to maturity. Our results differ from those reported by Schroeder et al. (22) in two main respects. Schroeder and coworkers demonstrated a considerable decline in the copper concentration of liver and aorta in their two oldest age groups, but we did not. According to Indraprasit et al. (11) the trace-element content of kidney cortex showed the most apparent relationship to age; copper concentration reaches a peak in the age group 40—60 years and then subsequently decreases. Griffith et al. (9) noticed elevated liver copper concentrations in children, and in the age group 40—60 years the copper concentrations were above the mean of the total material. In our material the copper concentrations of aorta and lung in the age group 50—60 years were somewhat higher than the mean of all the age groups.

According to Underwood (27) liver copper concentrations are sensitive to low copper intakes, and determinations of hepatic copper are useful aids in the diagnosis of copper deficiency. It is also known that in certain diseases copper accumulates in high quantities in the liver (20, 23). As can be seen from table 3, the liver copper concentrations found in our study seems to be quite comparable with earlier data from different geographical areas. Table 4 allows an even more comprehensive comparison of tissue copper

concentrations between the present study and previous ones. However, cautious interpretation is needed since different analytical procedures have been used. All authors do not give the age structure of their material, and the number of cases in their studies vary. Moreover, the interval between the first and the latest report presented in table 4 is more than 20 years. Besides, some materials comprise not only healthy persons who had died in accidents, but also chronically ill, hospitalized patients (1, 7, 9, 11, 14, 16, 25).

Our results do not agree with those previously presented by Forssén. According to her results the Finnish population has a below-average tissue concentration of copper (8). The difference is probably due to the limited number of cases investigated in the earlier study, as well as to differences in the methodology. Forssén used a less sensitive method (X-ray fluorescence spectrometry), and thus only 66.3 % of the samples were above the detection limit for copper.

Our results are based on accidentally killed healthy subjects, and the tissue concentrations presented reflect normal copper metabolism as closely as possible. This information is pertinent when certain chronic diseases are studied in Finland in the future, and the role of copper as a possible etiologic factor is evaluated. Although the present results indicate that, on the average, Finns do not suffer from copper deficiency, there remains a possibility that subjects with chronic diseases, e.g., cardiovascular disease, may have a marginal or relative copper deficiency. But this seems rather improbable to us since the human body as an organism is capable of regulating certain basic metabolic processes. In addition when the mechanism regulating copper intake does not function, the copper accumulates in certain target organs, especially in the liver (23). If the copper tissue concentrations of those subjects excluded because of known disease from the statistical analysis in this study, i.e., two cases of diabetes mellitus, one case of myocardial infarction, one of juvenile rheumatoid arthritis and one of hypopituitarism, are compared with the corresponding concentrations of the rest, no differences are observed; the copper concentrations of all the excluded cases were well within the ranges found for normal subjects.

If a deficiency of any trace element has a role in the etiology of a disease in man, the changes in metabolism caused by this deficiency are neither instant nor total, due to the fact that the deficiency is not absolute but only marginal (or partial), and thus the changes in body metabolism (enzyme synthesis and activity, transport processes, etc.) brought about by this kind of deficiency, i.e., marginal absorption and utilization, can only lead to clinical manifestations of a chronic nature.

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