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Relationships between copper, zinc and iron in the plasma, soft tissues and skeleton of the rat during Cu deficiency

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1. The effect of dietary copper deficiency on the distribution of Cu, zinc and iron between plasma, various soft tissues and bone was investigated in weanling male rats.

2. The concentration of Cu decreased in plasma, liver, kidney and femur, and the concentration in plasma correlated with that in all three organs. The total amount of Cu in the liver was reduced over the whole depletion period and a net loss from kidney also occurred over a shorter period, indicating that liver and, to a lesser extent, kidney both provide a mobilizable reserve of Cu.

3. Animals in galvanized cages developed Cu deficiency more rapidly than similar rats in stainless-steel cages owing to Zn aggravating the depletion. Zn accumulated in the liver and femur of Cu deficient rats, particularly when they were housed in galvanized cages.

4. Cu-deficient animals accumulated Fe in the liver, but had reduced concentrations in plasma, kidney and spleen. The hypertrophy of the heart and bone-marrow observed in Cu-deficient rats appeared to be secondary to the anaemia resulting from this impaired mobilization of hepatic Fe.

The importance of the liver in copper metabolism is well established. In addition to its functions in ceruloplasmin synthesis and the excretion of Cu through the bile (Hazelrig, Owen & Ackerman, 1966; Owen & Hazelrig, 1966), the liver also appears to act as a storage organ for the metal through its relation with the direct-reacting fraction of Cu in the serum (Dempsey, Cartwright & Wintrobe, 1958). In the rat, as in man and most other species, the Cu concentration is high in the liver of newborn animals but decreases with maturity (Brückmann & Zondeck, 1940; Cunningham, 1946; Lorenzen & Smith, 1947) and the concentration is further reduced in animals having a low dietary intake of the metal (Schultze, Elvehjem & Hart, 1936; Baxter & Van Wyk, 1953; Wintrobe, Cartwright & Gubler, 1953).

However, other organs, particularly the heart and kidneys, also contain high concentrations of Cu (Underwood, 1962) and, although the concentration in bone is only moderate, the skeleton contains about 23% of the total body Cu in both adult humans and rats (Lindow, Peterson & Steenbock, 1929; Chou & Adolph, 1935). The present investigation was therefore undertaken to examine the effect of dietary intake on the distribution of Cu between the blood plasma and the liver, kidney and femur of the rat. Exploratory measurements of zinc concentrations in the first experiment suggested an effect of Cu on Zn metabolism within certain tissues and the study was accordingly broadened to include the secondary effects of Cu deficiency on the distribution of Zn and iron between the same organs.

* For reprints.

EXPERIMENTAL

Animals and diets

Weanling male albino rats of the Wistar strain, weighing about 50 g, were allocated randomly to the required number of groups and housed three in a cage. The cages were previously washed with a solution of the disodium salt of ethylenediaminetetra-acetic acid (EDTA) (4 g/l) and thoroughly rinsed with deionized water to remove any traces of Cu. The rats were given synthetic diets, the amount of food provided for all animals being restricted to that consumed by the least hungry cage of rats in the Cu-deficient group. Glass-distilled water that had been subsequently deionized was provided *ad lib.*

Diets were prepared by mixing (g/kg): casein 200; sucrose 660; arachis oil 80; cod-liver oil 20; salt mixture 40 containing the main minerals and salts of trace elements, and purified vitamins. They were similar in composition to those described previously for depletion experiments with other minerals (Heaton & Anderson, 1965) except that the casein of low-vitamin content (Fisons Ltd) was further purified by washing three times with a solution of EDTA (Apgar, 1968), then three times with deionized distilled water; the casein was separated by centrifugation and finally freeze-dried at 15°. Mineral salts of Analar grade were used whenever possible and sucrose was of Laboratory Reagent grade. The control diet contained (parts/10^6): 50 Cu, 12 Zn and 180 Fe. A Cu-deficient diet containing 0·4 parts/10^6 was obtained by omitting cupric chloride from the salt mixture and diets containing 2 and 10 parts/10^6 Cu were prepared by reducing the amount of cupric chloride in the salt mixture, the composition of the diets being otherwise unaltered.

Expt 1. Four groups of rats were kept in galvanized cages and received diets of different Cu content for 48 d. Group A consumed the Cu-deficient diet (0·4 parts/10^6), B and C had diets low in Cu (2 and 10 parts/10^6 respectively), and D ate control diet (50 parts/10^6). A fifth group (E) consisted of animals killed at the beginning of the experiment.

Expt 2. Two groups of rats, each initially containing forty-two animals, were housed in cages constructed entirely of stainless-steel and given identical amounts of Cu-deficient (0·4 parts/10^6) and control (50 parts/10^6) diets for up to 126 d. Twelve similar rats were killed at the beginning of the experiment, and batches of animals from both groups were killed at intervals throughout the experimental period, each animal being taken from a different cage within the group.

Blood was obtained from the tail when required during an experiment; at the end of the experimental period, rats were killed by exsanguination from the heart while they were under diethyl ether anaesthesia, the heparinized plasma being separated immediately. The liver, kidneys, femurs and humeri were removed rapidly, the individual organs from each animal being stored separately in glass containers at −20° until required for analysis.

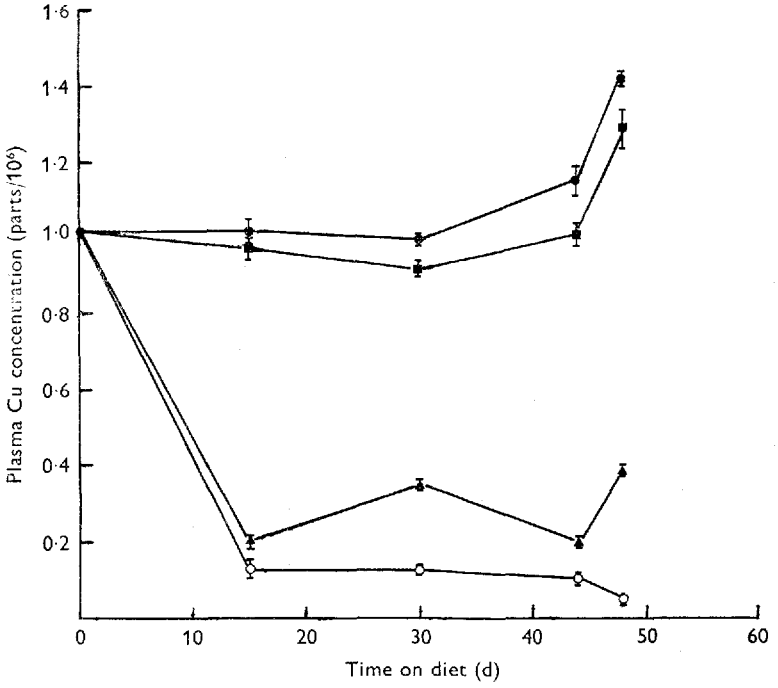

Fig. 1. Copper concentrations in the plasma of rats receiving dietary Cu at different levels. ○, 0·4; ▲, 2; ■, 10; ●, 50 parts/10⁶. The vertical bars represent the standard errors of the mean.

Preparation of tissues for analysis

Plasma was deproteinized with hydrochloric and trichloroacetic acids as described by Gubler, Lahey, Ashenbrucker, Cartwright & Wintrobe (1952).

Soft tissues were wet-ashed in a nitric acid–perchloric acid mixture. Bone was scraped free from adhering tissue after immersion for a moment in hot, deionized, distilled water. A complete femur was dried to constant weight at 105° in a small porcelain crucible, transferred to a micro-Kjeldahl flask and wet-ashed with nitric and perchloric acids.

The perchloric acid was of Aristar quality and other reagents used in the preparation and analysis of tissues were of Analar grade whenever possible.

Analytical methods

Cu and Zn were determined in deproteinized plasma and solutions of tissue ash by atomic-absorption flame photometry. Fe in tissue ash was also determined by atomic-absorption analysis, but the Fe content of plasma was measured colorimetrically by its reaction with tripyridyl, and the haemoglobin in blood was estimated as cyanmethaemoglobin (Dacie & Lewis, 1963).

The statistical significance of differences was assessed by Student's *t* test, standard errors being calculated from values for individual animals within the same group because differences between cages were no greater than variations between rats in the same cage.

Table 1. *Copper and zinc concentrations in the liver, kidneys and femurs of rats with different Cu intakes for* 48 *d*

(Mean values with their standard errors; numbers of animals in parentheses)

		Concentration (parts/10^6 in dry matter)									
		Liver				Kidneys				Femurs			
		Cu		Zn		Cu		Zn		Cu		Zn	
Group	Dietary Cu concentration (parts/10^6)	Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE
A (10)	0·4	3·61	0·49***	129	17	10·2	0·7***	108	7	4·85	0·34	500	43
B (10)	2	13·7	0·55***	157	6***	17·3	0·6***	118	8	5·14	0·27	500	20**
C (10)	10	15·6	0·80*	130	7	22·3	0·9	127	5*	5·78	0·55	438	32
D (11) Control	50	17·9	0·54	118	4	24·1	1·3	110	4	5·71	0·58	398	24
E† (10)		32·9	6·3***	116	8	20·4	0·7*	—	–	8·04	0·48**	361	14

Values significantly different from control: * $P < 0·05$; ** $P < 0·01$; *** $P < 0·001$.
† This group consisted of animals killed at the start of the experiment.

Table 2. *Total amounts of copper and zinc in the liver, kidneys and femurs of rats with different Cu intakes for* 48 *d*

(Mean values with their standard errors; numbers of animals in parentheses)

		Amount of metal (μg)									
		Liver				Kidneys				Femurs			
		Cu		Zn		Cu		Zn		Cu		Zn	
Group	Dietary Cu concentration (parts/10^6)	Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE
A (10)	0·4	6·3	0·6***	216	26*	2·98	0·20***	30·3	2·0	2·59	0·15*	26·5	2·1
B (10)	2	16·1	0·7**	194	12**	4·68	0·19***	32·6	2·8	3·36	0·22	32·5	1·2**
C (10)	10	21·1	1·4	171	15	6·55	0·35	37·3	2·2	3·87	0·31	28·9	2·2
D (11) Control	50	23·5	1·0	149	4	6·45	0·34	33·3	1·8	3·73	0·42	25·8	1·7
E† (10)	—	15·1	1·6	62	2	2·10	0·12	12·1	0·6	1·09	0·07	4·9	0·2

Values significantly different from control: * $P < 0·05$; ** $P < 0·01$; *** $P < 0·001$.
† This group consisted of animals killed at the start of the experiment.

Table 3. *Dimensions of humeri from copper-deficient and control rats after* 48 *d on diet*

(Mean values with their standard errors for twenty-two bones/group)

	Value				
	Cu-deficient		Control		
Dimension	Mean	SE	Mean	SE	*P* value
Length (mm)	23·4	0·1	23·3	0·1	>0·5
Total width (mm)	1·99	0·03	2·04	0·02	>0·1
Width of spongy bone (mm)	1·23	0·03	1·10	0·03	<0·01
Ratio of width, spongy bone:total bone	0·619	0·012	0·544	0·016	<0·001

RESULTS

Expt 1. *Effect of dietary intake on the distribution of Cu in the body*

Anorexia developed in the Cu-deficient rats after 36 d, but the mean final body-weights of 134, 147, 152 and 146 g for animals in groups A, B, C and D respectively were not significantly different. Rats in group A were visibly anaemic after 26 d and this was confirmed by measurements of the blood haemoglobin concentration, which declined to 100 g/l by the 15th d and between 50 and 60 g/l from the 30th d onwards; none of the other experimental groups had values outside the control range of 120–160 g/l. Coarsening of the coat and loss of hair accompanied the anaemia in deficient animals during the later stages of the experiment, and enlarged hearts, livers, kidneys and spleens were found in most animals of this group when they were killed.

The Cu concentration in the plasma of rats in groups A and B fell rapidly during the first 15 d of the experiment but then remained relatively constant although a further decline occurred in group A during the final week of depletion (Fig. 1). No significant difference from the control value was observed in animals of group C.

The concentrations of Cu in the liver and kidneys were decreased in rats of groups A and B, but the concentration in the femurs was not significantly reduced (Table 1). The control animals (D) had much lower concentrations of Cu in the liver and femurs than animals killed at the start of the experimental period (E), presumably owing to changes during the maturation process.

Comparison of the total amounts of Cu in the liver, kidneys and femurs of each rat showed that they increased progressively in animals of groups A, B and C (Table 2), but the amounts in animals of group C were not significantly different from those in control rats (D), suggesting that a dietary Cu concentration of 10 parts/10^6 was adequate to meet the requirements for tissue growth under the conditions of this experiment. Approximately 58% of the Cu originally present was lost from the liver of animals in group A during the depletion period, but in the kidneys and bone of these rats, as in all three tissues from animals in other groups, the total amount of Cu present in the complete organ increased during the experimental period.

The bones of Cu-deficient rats were translucent in appearance and the humeri from animals in groups A and D were radiographed, and the dimensions of the bones were measured from the radiographs with a travelling microscope. The length and total

Table 4. *Copper, zinc and iron concentrations in the liver, kidneys and femurs of rats given Cu-deficient* (0·4 *parts*/10^6) *and control* (50 *parts*/10^6) *diets*

(Mean values with their standard errors)

		Concentration (parts/10^6 in dry matter)															
		Liver						Kidneys						Femurs			
		Cu		Zn		Fe		Cu		Zn		Fe		Cu		Zn		Fe	
Time on diet (d)	No. of rats	Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE
								Control rats											
0	12	26·6	2·8	76	5			21·4	1·9	215	26	412	61	8·80	0·85	392	56	0·129	0·014
12	6	66·4	10·7	133	5	536	29	22·1	3·6	116	13	347	39	6·76	0·35	203	11	0·104	0·011
20	6	69·9	17·2	120	8	392	45	22·5	3·6	118	8	374	41	6·60	0·25	201	10	0·097	0·009
32	6	48·7	7·9	120	5	459	41	17·6	2·1	98	6	310	65	6·39	0·34	204	17	0·085	0·005
41	6	33·5	4·0	113	6	593	20	21·7	1·8	105	4	302	19	7·06	0·49	195	14	0·096	0·004
53	4	31·1	4·4	115	4	567	58	21·7	1·3	107	13	343	36	6·26	0·24	184	9	0·083	0·007
126	6	17·2	0·9	117	1	789	42	23·4	0·8	122	4	445	20	6·02	0·31	215	11	0·086	0·009
								Cu-deficient rats											
12	6	17·5	1·8**	120	10	543	27	14·1	1·8	118	13	285	29	6·04	0·24	202	38	0·113	0·011
20	6	11·2	0·9*	106	9	480	36	11·5	1·7*	95	14	283	48	5·67	0·32*	193	36	0·092	0·019
32	6	8·1	0·9***	147	10	677	74*	12·4	0·8*	103	13	270	18	5·73	0·26	225	31	0·089	0·012
41	6	11·0	0·6***	136	19	704	106	12·9	1·4**	117	13	288	28	5·13	0·28**	193	19	0·081	0·009
53	4	9·2	0·6*	150	25	928	90*	10·1	1·7**	100	6	294	54	5·42	0·14*	215	7*	0·074	0·003
126	6	2·2	0·3***	111	4	1054	76*	9·3	1·0***	123	11	272	29***	5·11	0·28	187	19	0·068	0·008

Values significantly different from control: * $P < 0·05$; ** $P < 0·01$; *** $P < 0·001$.

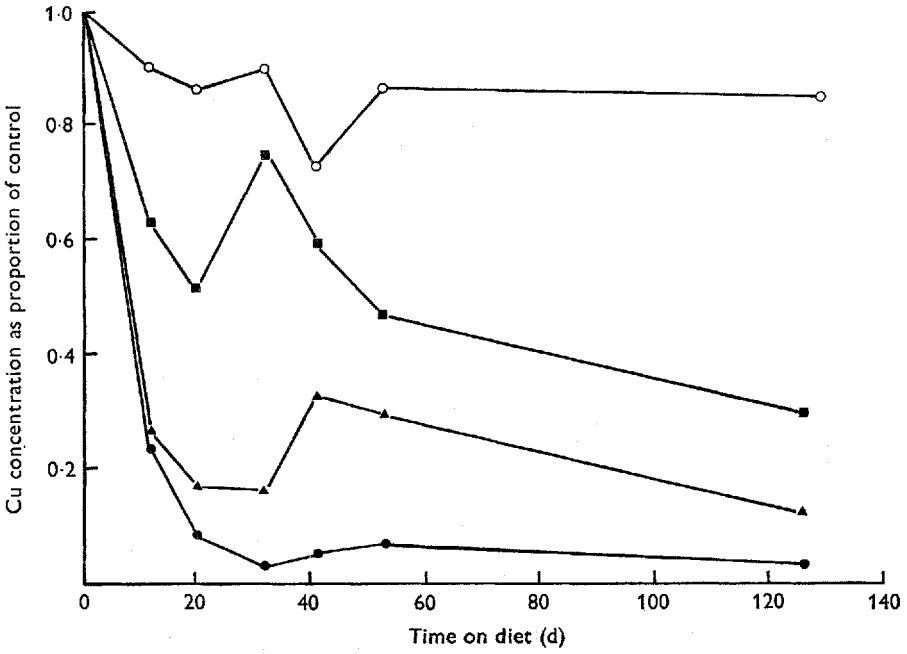

Fig. 2. Copper concentrations in tissues from Cu-deficient rats, expressed as a proportion of that in control animals of the same age. ○, femur; ■, kidney; ▲, liver; ●, plasma.

width of the bones were the same in both groups of rats (Table 3), but in deficient animals the width of the spongy bone and marrow cavity was increased at the expense of compact bone. A negative correlation was found between the haemoglobin concentration in the blood and the ratio of spongy bone: total shaft width in the humeri of these rats ($r = -0{\cdot}777, n = 12, P < 0{\cdot}001$).

Expt 2. Sequence of changes in distribution during Cu deficiency and repletion

The Cu-deficient rats in this experiment did not develop anorexia or lose bodyweight compared with control animals, as observed in Expt 1. Although the blood haemoglobin concentration was slightly depressed in deficient animals during most of the experimental period, it was only after 116 d depletion that the value dropped to 100 g/l and anaemia developed. Histological examination of blood indicated that the anaemia was of the hypochromic type. There was hypertrophy of the heart in the final group of deficient animals compared with controls killed at the same time (heart weights $0{\cdot}420 \pm 0{\cdot}031$ and $0{\cdot}290 \pm 0{\cdot}006$ g respectively, $P < 0{\cdot}01$).

The Cu concentration in the plasma of deficient animals fell rapidly to 0·27 parts/10^6 during the first 12 d, then more slowly to about 0·07 parts/10^6 after 70 d and remained at this level for the rest of the experimental period; in control animals the concentration remained between 1·00 and 1·26 parts/10^6. The concentration of Cu in liver, kidney and bone decreased (Table 4), but in each organ the decrease was most severe during the first 20 d. Interpretation of these changes in Cu concentration is, however, complicated by variable effects due to maturation of the animals. The concentration in the liver of control rats rose to a maximum after 12–20 d, when the

Table 5. *Total amounts (μg) of copper, zinc and iron in the liver, kidneys and femurs of rats given Cu-deficient (0·4 parts/10⁶) and control (50 parts/10⁶) diets*

(Mean values with their standard errors)

		Amount of metal (μg)															
		Liver						Kidneys						Femurs			
		Cu		Zn		Fe		Cu		Zn		Fe		Cu		Zn		Fe	
Time on diet (d)	No. of rats	Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE
									Control rats										
0	12	15·8	1·8	44	1			2·90	0·32	28·1	10·9	56	11	1·30	0·14	58	8	0·024	0·004
12	6	51·9	9·3	103	8	420	38	4·03	0·57	21·4	2·1	63	5	2·12	0·14	64	5	0·033	0·004
20	6	39·5	9·5	91	7	397	35	4·26	0·57	22·7	1·9	72	8	2·34	0·18	71	6	0·034	0·003
32	6	47·2	7·7	116	8	452	65	4·50	0·62	24·8	2·2	76	12	2·95	0·29	94	11	0·039	0·004
41	6	38·4	6·8	129	13	675	67	5·36	0·79	23·1	3·9	76	11	4·08	0·49	110	7	0·054	0·003
53	4	29·8	2·1	161	20	760	109	6·54	0·79	31·8	3·1	100	8	4·63	0·50	141	8	0·054	0·007
126	6	35·6	2·2	241	6	1632	104	11·7	0·5	60·7	1·5	224	17	6·54	0·53	232	14	0·094	0·013
									Cu-deficient rats										
12	6	11·6	1·5**	79	9	370	46	2·33	0·28*	19·9	2·8	40	6	1·78	0·13	59	5	0·033	0·004
20	6	11·3	0·8*	106	7	489	44**	2·52	0·36*	20·9	3·1	64	12	2·13	0·15	73	15	0·035	0·008
32	6	9·3	1·6***	148	16	799	141*	3·52	0·40	28·5	4·1	76	9	2·65	0·25	107	18	0·042	0·007
41	6	13·7	1·0**	168	21	881	136	3·18	0·34*	29·5	3·3	72	5	2·97	0·21	95	9	0·047	0·005
53	4	11·1	0·9***	181	34	1104	82*	2·66	0·54**	26·0	2·2	91	18	3·45	0·10	137	7	0·047	0·003
126	6	5·3	0·6***	290	14*	2762	230**	4·45	0·71***	57·9	7·0	127	16**	5·23	0·47	194	28	0·071	0·012

Values significantly different from control: * $P < 0·05$; ** $P < 0·01$; *** $P < 0·001$.

Table 6. *Copper concentrations (parts/10^6) in plasma and in dry matter of liver, kidneys and femurs after repletion for 4 and 10 d*

(Mean values with their standard errors: $n = 5$ for animals repleted for 4 d, $n = 4$ for other groups)

	4-d repletion		Control		10-d repletion		Control	
Tissue	Mean	SE	Mean	SE	Mean	SE	Mean	SE
Plasma	0·87	0·03**	1·05	0·04	1·02	0·04	1·04	0·33
Liver	14·0	0·84*	19·0	1·2	13·5	0·4	17·7	1·5
Kidneys	28·2	3·4	32·2	1·8	26·8	2·9	25·8	2·3
Femurs	5·10	0·33	6·09	0·24	5·70	0·08	5·72	0·47

Values significantly different from control: * $P < 0·05$; ** $P < 0·01$.

animals were between 35 and 43 d old, and then decreased continuously with increasing age; in femurs the concentration fell during the first 12 d and then remained constant, but in kidney the concentration of Cu appeared to be unaffected by the maturity of the animal.

Examination of the Cu concentration in tissues of deficient animals, expressed as a proportion of that in controls of the same age, to allow for the effect of maturation, showed an appreciable although temporary rise in the concentration in kidney after 32 d depletion and in liver after 41 d depletion (Fig. 2). Positive correlations were found between the concentrations in the plasma and the liver ($r = 0·656$, $n = 17$, $P < 0·01$), kidneys ($r = 0·696$, $n = 17$, $P < 0·001$) and femurs ($r = 0·567$, $n = 17$, $P < 0·05$) throughout the depletion period. The Cu concentration in the spleen of deficient animals ($1·21 \pm 0·22$ parts/10^6 dry weight) was also lower than in controls ($7·09 \pm 0·30$ parts/10^6) when measured at the end of the experiment.

The total amounts of Cu in different organs varied considerably during the depletion period. Cu was lost from the liver whereas the amount in bone increased progressively despite the depletion (Table 5). A small amount of Cu was also lost from the kidneys during the first 12 d, after which the amount present generally increased. There was, however, a suggestion of a further temporary loss from the kidneys between 32 and 53 d, which coincided with a temporary increase in the amount in liver. In control animals the total amounts of Cu in all organs increased considerably, although the amount in the liver was maximum after 12 d and then tended to decrease.

The effect of repletion with Cu salts was investigated by transferring nine deficient rats to control diet on the 80th day. Five of these rats were killed after 4 d repletion and the remainder after repletion for 10 d, four animals which had received control diet throughout being killed on each occasion. The Cu concentrations in plasma and liver had risen appreciably after 4 d repletion but were still significantly lower than in control animals (Table 6) although this difference was abolished after repletion for 10 d. There appeared to be a more rapid response in the kidney, where the Cu concentration in experimental animals was not significantly different from that of controls after 4 d repletion.

Changes in Zn and Fe distribution during Cu deficiency

Measurement of Zn concentrations in Expt 1, when the animals were kept in galvanized cages, showed an accumulation of the metal in the liver and femurs of animals on suboptimum Cu intakes. This was demonstrated by a rise in both the concentration (Table 1) and total amount of Zn (Table 2) in these organs and was particularly evident in the animals of group B. However, more extensive measurements in Expt 2, when the animals were in stainless-steel cages, showed no significant effect of Cu deficiency on the concentration of Zn in either plasma, liver, kidney or bone (Table 4), but the total amount of Zn in the liver was consistently higher in Cu-deficient than in control animals although the difference was statistically significant only in the final group (Table 5).

The concentration of Fe in the plasma of Cu-deficient rats was found to be severely depressed when measured on the 32nd and 126th days of Expt 2, the value being only $0{\cdot}40 \pm 0{\cdot}08$ parts/10^6 in deficient animals compared with $1{\cdot}76 \pm 0{\cdot}19$ parts/10^6 in controls at the end of the experiment ($P < 0{\cdot}001$). This decrease was accompanied by an increase in the concentration and total amount of Fe in the liver from about the 32nd day onwards, and a corresponding decrease in the Fe content of the kidney was observed towards the end of the depletion period (Tables 4 and 5). The Fe concentration in the spleen was also decreased from 1368 ± 81 parts/10^6 dry weight in control animals to 586 ± 61 parts/10^6 in the final batch of Cu-deficient rats ($P < 0{\cdot}01$).

DISCUSSION

Storage of Cu in liver and kidney

The two experiments described in this paper were designed to investigate in a complementary manner the effect of dietary Cu on the metabolism of the element and the sequence of changes during the development of Cu deficiency. The concentrations of Cu in the liver and kidneys of rats depleted for 126 d in Expt 2 (Table 4) were lower than those observed after 48 d depletion in Expt 1 (Table 1), indicating that a more severe deficiency was produced in Expt 2. Varying degrees of depletion were produced in different tissues; the concentration fell most rapidly and severely in the plasma, followed by the liver and kidney, with only a comparatively small change in the femur (Fig. 2).

Measurement of the total amount of Cu in an organ provides the most convincing evidence for mobilization of the element from that organ, because it excludes dilution due to growth which can be the cause of a fall in concentration. The total amount of Cu in the livers of deficient animals fell by about 60% during the depletion period in both experiments (Tables 2 and 5), confirming that the liver acts as a storage organ which can liberate considerable amounts of Cu to other tissues during deficiency (Dempsey *et al.* 1958; Dowdy, 1969; Owen & Orvis, 1970).

The kidney also appeared to have a previously unrecognized function in the storage of Cu. A correlation was observed between the Cu concentrations in kidney and plasma, and the concentration in kidney showed a temporary rise between the 20th and 32nd

days of depletion, which coincided with a period of rapid loss from liver. Moreover, a considerable loss of total Cu from the kidney occurred during the 5th and 6th weeks of depletion, when mobilization from the liver appeared to be halted and the organ even showed a temporary rise in total Cu (Tables 4 and 5) that was associated with a small rise in the plasma Cu concentration. A similar temporary rise in the Cu concentration of liver during a period of depletion was observed previously by Dempsey *et al.* (1958) but the reason for it was not explained.

When Cu-deficient animals were transferred to the control diet the kidneys appeared to become replete rather more rapidly than the liver (Table 6) and this observation, together with the greater incorporation of radioactive Cu into the kidneys than the liver of deficient rats reported by Schultze & Simmons (1942), confirms the relation between Cu in the plasma and the kidney. The kidney therefore appears to have a significant function in the storage of mobilizable Cu, but the fact that the total amount of Cu in the organ increased during the depletion period indicates that it is subsidiary to storage in the liver. As the concentration of Cu in the kidney, unlike that in the liver, was not influenced by the state of maturity of the rats (Table 4), measurement of kidney Cu may provide the most reliable means of assessing the severity of Cu depletion, especially in young animals.

Although the concentration of Cu in the femur was also correlated with that in plasma throughout the depletion period, the decrease in bone concentration was much less than in either liver or kidney, and it appeared that the amount in bone varied with the plasma concentration in a non-selective manner.

Relations between Cu, Zn and Fe metabolism

Cu deficiency developed much more slowly in the second experiment than in the first, as judged by the delayed appearance of anaemia and the higher Cu concentration in liver after a similar time-interval (Tables 1 and 4), although a more severe depletion was eventually produced. Anaemia was detectable after 2 weeks in Expt 1, as found by Mills & Murray (1960), but in Expt 2 it required nearly 4 months to produce a similar fall in blood haemoglobin concentration, as observed by Owen & Hazelrig (1968). Both experiments were conducted with rats of the same strain, sex and size, that consumed diets of identical composition and grew at the same rate, and it therefore appears that the difference must have been due to the use of galvanized cages in the first experiment and steel cages in the second. Rats housed in galvanized cages would have a higher and more variable intake of Zn than those in Expt 2, and this may have aggravated the Cu deficiency both by competing with the very small amount of dietary Cu for absorption in the intestine (Van Campen & Scaife, 1967; Starcher, 1969; Van Campen, 1969) and by increasing the urinary excretion of Cu (Duncan, Gray & Daniel, 1953).

Zn also appeared to have an additional effect on haemoglobin synthesis or breakdown, because deficient animals in Expt 1 had lower blood haemoglobin concentrations than those at the end of Expt 2, despite the lower Cu concentrations in the liver and kidneys of the latter animals. This conclusion is supported by the finding that

high levels of dietary Zn lowered the haemoglobin concentration in animals with normal blood Cu levels (Grant-Frost & Underwood, 1958).

The effect of Cu depletion on Zn metabolism, appeared to vary with the relative intakes of the two metals. In Expt 1, Zn tended to accumulate in the liver and femur of rats with suboptimum intakes of Cu, suggesting that competition between the two metals similar to that observed in the intestine (Starcher, 1969; Van Campen, 1969) may also occur in other tissues. However, more extensive studies in Expt 2, when Zn intake was carefully regulated, failed to confirm this suggestion, although a non-significant tendency for Zn to accumulate in the liver of Cu-deficient rats was still observed. It appears, therefore, that Zn has a greater influence on Cu metabolism than Cu exerts on Zn, both under physiological conditions and in the pathological state of Zn toxity studied by O'Dell (1967).

The decreased concentrations of Fe found in the plasma, kidney and spleen of Cu-deficient rats appeared to be caused by an impaired mobilization of Fe from the liver, which resulted in the accumulation of Fe in that organ (Tables 4 and 5) as previously reported by Marston, Allen & Swaby (1971). In the Cu-deficient pig, Fe also accumulated in the duodenal mucosa and reticulo-endothelial system owing to a similar impairment in transport from these tissues to the plasma (Roeser, Lee, Nacht & Cartwright, 1970). The grossly enlarged hearts found in both our experiments, together with the hypertrophy of the bone-marrow, liver, kidney and spleen observed in the first experiment all appear to be secondary to the anaemia resulting from this impaired utilization of Fe, which was probably aggravated by Zn in the first experiment as discussed previously. The observation that the heart hypertrophied more than the spleen in our studies, when the reverse is found in Fe-deficiency anaemia (Gubler, Cartwright & Wintrobe, 1957), is probably due to Cu deficiency also impairing cytochrome oxidase activity in the heart (Dreosti, 1967).

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