LIV. COPPER METABOLISM IN MAN.

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Recent experiments have shown the importance of copper in haemoglobin formation in the animal body, and the wide distribution of copper in plant and animal material has been repeatedly confirmed. There is a lack of quantitative data however on copper metabolism. We have conducted balance experiments with the object of estimating the copper requirement of man, and in addition we have undertaken copper analyses of human cadavers for the purpose of estimating the total copper content of the human body.

Copper metabolism in normal subjects.

Copper balance experiments were carried out during the late spring and early summer on three different subjects, all Chinese, who consumed simple diets of measured copper content. The copper content of Chinese food materials has been reported elsewhere by Adolph and Chou [1933]. White flour (steamed bread) and milk with a small quantity of vegetables were employed for the low copper diet. An increased copper intake was arranged by replacing the milk with increasing amounts of spinach and pork. Copper was determined in food, urine and faeces by the Biazzo method as modified by Elvehjem and Lindow [1928].

Series 1, in which the copper balance was determined on a subject daily for four successive days, was carried out for the purpose of determining how rapidly the body adjusted itself to altered copper intake. Results are shown in Table I. As a result of this preliminary experiment, it was proposed to employ 2- and 3-day metabolism periods in the series following.

In Series 2, three subjects consumed prescribed diets of increasing copper content over four periods. Each period consisted of 4–6 days, the four periods following each other without interval. The metabolism measurements involving the collection of urine and faeces were confined to the last 3 days and in some cases to the last 2 days of each period. Faeces markers were used and the usual precautions in conducting metabolism balance experiments were observed. The iron intake was not controlled.

The urine and faeces were collected directly in glass or porcelain receptacles. The faeces for each collection period were dried over sulphuric acid and ground in a porcelain mortar. For the analyses, 400 ml. samples of urine were evaporated to a volume of 30–40 ml. and then digested with 40 ml. of sulphuric acid in a Kjeldahl flask till colourless. The dried faecal material was treated in a similar manner. The resultant digest was then transferred to a volumetric flask and diluted so that the acid concentration was about 1 %. In carrying out the analyses the copper was separated from iron by precipitation with hydrogen sulphide, with subsequent determination of the copper in the precipitate as outlined by Elvehjem and Lindow [1928]. All analyses were carried out in duplicate.

Table I. *Copper metabolism in man; intake and output averaged in mg. Cu per day.*

Subject	Sex	Age	Weight kg.	Period	No. of experimental days	Cu intake mg.	Cu output (mg.) Urine	Faeces	Total	Balance
					Series 1.					
A	F	36	45·5	I	1	0·66	0·27	2·08	2·35	−1·69
				II	1	0·51	0·16	2·43	2·59	−2·05
				III	1	1·72	0·23	1·92	2·15	−0·43
				IV	1	2·17	0·27	1·22	1·49	+0·68
					Series 2.					
A	F	37	46·5	I	3	0·67	0·21	1·19	1·40	−0·73
				II	3	2·07	0·23	1·67	1·90	+0·17
				III	2	3·40	0·33	4·49	4·80	−1·42
B	M	17	30·2	I	2	1·11	0·20	1·72	1·92	−0·81
				II	3	1·28	0·18	1·81	1·99	−0·71
				III	3	2·63	0·27	1·84	2·11	+0·52
				IV	2	3·69	0·31	6·41	6·72	−3·03
C	F	62	41·2	I	3	1·09	0·15	1·58	1·73	−0·64
				II	3	2·38	0·27	2·14	2·41	−0·03
				III	2	2·85	0·36	2·37	2·73	+0·12
				IV	2	3·39	0·30	2·43	2·73	+0·66

The results, shown in Table I, indicate that equilibrium was reached when the copper intake was about 2 mg. per day.

Copper content of the human body.

Material from five cadavers, all Chinese, two being adults, was obtained from the Department of Pathology of the Peiping Union Medical College, and analyses made of muscle, liver, heart *etc.* In preparing the material for analysis, the tissues were first washed free from blood and the weighed samples then dried in an electric oven at 60° and ashed in an electric furnace at as low a temperature as possible, and the estimation of copper was carried out by the Biazzo method as for food materials. The results are shown in Table II. In Table III, using the data from the two adult cadavers, figures are calculated for the copper content of each tissue, and also for the total copper content of the body. The copper content of blood is estimated at 5 mg. for the entire body.

Table II. *Distribution of copper in tissues of the human body (amounts expressed in mg. per kg. of dry material).*

	Cadaver 1*	Cadaver 2*	Cadaver 3*	Cadavers 4, 5*
Body weight...	5 kg.	5·5 kg.	22·5 kg.	adults
Sex—age ...	F—3 mons.	F—7 mons.	M—12 yrs.	
Heart	111·1	162·0	—	36·4
Liver	207·2	40·8	45·4	60·0
Kidney	262·6	61·0	54·3	48·5
Pancreas	8·1	46·5	12·3	28·0
Spleen	24·4	—	—	19·6
Lungs	48·5	42·4	18·8	17·3
Adrenal	120·4	—	—	—
Muscle	82·3	72·4	12·7	12·6
Bone	—	—	—	6·5

* Cause of death: (1) meningitis, (2) secondary anaemia, (3) nutritional oedema, (4) opium poisoning, (5) aneurism of aortic arch.

Table III. *Estimate of total copper content of adult human body.*

	Copper content of dry tissue mg./kg.*	Average H_2O content of tissue %†	Average total weight of tissue kg.‡	Total copper content mg.
Heart	36·4	79	0·20	1·5
Liver	60·0	70	1·00	18·0
Kidney	48·5	83	0·09	0·7
Pancreas	28·0	75	0·06	0·4
Spleen	19·6	75	0·09	0·4
Lungs	17·3	79	0·80	2·9
Muscle	12·6	76	21·0	63·6
Bone	6·5	30	5·2	23·4
Blood	—	—	—	5·0§
			Total	115·9

* See Table II. † Figures from Oppenheimer, *Handbuch der Biochemie*, **1**.

‡ Figures from standard tables reckoned on basis of 50 kg. man. § Estimated.

Discussion.

The copper balance experiments indicate that equilibrium was reached when the copper intake was approximately 2 mg. per day, and we suggest that this figure represents the daily copper requirement in man. A consideration of the copper content of common foodstuffs, which ranges from 2 to 25 mg. per kg. of dry material, indicates that the average mixed diet readily supplies this amount of copper, particularly if it contains a liberal amount of spinach and similar leafy vegetables.

Inasmuch as copper appears to be associated with iron in haemoglobin formation, it is interesting to compare this figure for copper requirement, 2 mg. per day, with the accepted requirement for iron in man, 15 mg. per day [Sherman, 1932], iron and copper standing in the ratio 7 : 1. Iron and copper requirements per day for the white rat have been accurately determined on the basis of haemoglobin recovery experiments by Hart *et al.* [1928] and Waddell *et al.* [1928], who give the figures 0·5 mg. and 0·06 mg. respectively for iron and copper; these represent an iron-copper ratio of 8 : 1. This ratio is almost identical with that above, and suggests that the outstanding rôle of iron and copper in the human body is expressed in the haemopoietic function.

From the copper balance experiments it is evident that in man copper is mainly excreted through the faeces. This was shown by Filehne [1896] to be the case with dogs and cats, and by Flinn and Inouye [1928] and Lindow *et al.* [1929] to hold also for rats. It is important to note that in spite of changes in the copper intake, our figure for urinary copper remains fairly constant, varying between the limits 0·15 and 0·36 mg. (average 0·25 mg.) per day. Rabinowitch [1933] has recently reported values ranging between traces and 0·7 mg. per day for urinary copper in normal man. The excretion of faecal copper is more irregular, and with two of our subjects a further increase in copper intake was accompanied by a pronounced negative balance and by an apparent breakdown in the storage supply.

Our calculated figure, 115·9 mg., for the copper content of the adult body can be regarded only as a rough estimate. If brain, skin, and other tissues are included this figure should be increased, and it is probable that the total copper content is between 100 and 150 mg. The copper stored in the body is found mainly in the liver, muscles and bones. Our results for copper determinations in adult tissues are slightly higher than those reported by Cunningham [1931].

The higher percentage copper content of certain of the tissues of the two infant cadavers studied is rather marked. A reserve supply of copper in the livers of the newborn has been reported by a number of observers, and discussed by Morrison and Nash [1930], Nitzescu [1931], and Cunningham [1931]. It appears that in certain mammals, including human beings, a reserve storage of both iron and copper is provided to the newborn to insure adequate haemoglobin formation. Cadaver 2, a case of anaemia, exhibits a much smaller amount of copper in the liver. A similar observation is recorded by Morrison and Nash [1930].

SUMMARY.

From copper balance experiments on three adult subjects fed with copper-containing foods, it is shown that the copper requirement in man is approximately 2 mg. per day.

The copper excretion in the urine of the normal individual does not vary appreciably with varied copper intake and averages 0·25 mg. per day.

From analyses of tissues, it is estimated that the adult body contains between 100 and 150 mg. of copper.

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