CCLXXIV. THE EXCRETION OF COPPER IN URINE AND FAECES AND ITS RELATION TO THE COPPER CONTENT OF THE DIET.

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IN a previous paper the present writer [1934] described a method for the determination of copper in blood. The reaction employed was the formation of a yellow-coloured complex of copper with sodium diethyldithiocarbamate which could be extracted with amyl alcohol. The amyl alcohol layer was removed and compared in a colorimeter with a standard similarly prepared. This reaction is extremely sensitive, it being possible to detect 1 part of copper in 10 million parts of solution. Using this technique human blood was found to contain 0.185–0.230 mg. Cu per 100 ml.

So far only one investigation of the copper content of urine appears to have been made. Rabinowitch [1933] obtained values of a trace—0.41 mg. Cu per litre for the copper content of normal urine. This writer stated that addition of copper to the diet definitely increased the amount excreted in the urine, but did not publish any analyses of the copper content of the faeces or the daily diet. In the present investigation the copper contents of the daily diet and faeces were determined as well as that of the urine.

METHODS.

1. *Collection of urine and faeces.* The urine and faeces were collected during periods of 6–21 days. The daily urines were made up to a constant volume and a composite sample prepared. The faeces were dried on a steam-bath and weighed, ground up and stored in sealed bottles.

2. *Diet.* An average half-daily sample of the diet (with the exception of butter) was weighed. After drying on a steam-bath, it was weighed, ground up and stored in a sealed bottle.

TECHNIQUE.

In the determination of copper in blood, the proteins were precipitated with trichloroacetic acid and the copper determined directly in the filtrate. Naturally such a procedure would be impossible with urine, faeces or a food. Ashing of some form is necessary. These materials contain large amounts of calcium phosphate which tends to precipitate during the estimation since the reaction is carried out in alkaline solution. In the past investigators have carried out a preliminary separation of the copper as sulphide in acid solution. This procedure is time-consuming and needs very careful attention to ensure accurate results. The present writer has found that the addition of sodium citrate prevents the precipitation of calcium phosphate without interfering with the reaction. A copper-free sample of sodium citrate was used throughout the investigation.

Throughout the investigation 0.01 mg. Cu was used as the standard since the colour value obtained in this region presents sharp comparison in the colorimeter. After each determination the unknown extract was shaken with a solution of sodium cyanide, and in each case it was noted that the colour disappeared, *i.e.* the colour was due to copper only. This precaution is necessary since bismuth gives a yellow colour with sodium diethyldithiocarbamate and is soluble in organic solvents. The reaction with bismuth is far less sensitive than that with copper—1 mg. of bismuth gives less colour than 0.01 mg. of copper. Bismuth is not likely to be present often in material derived from experiments such as these. Manganese gives a pink to dirty brown colour soluble in organic solvents. Manganese occurs in foodstuffs and faeces in higher concentration than copper. The present writer has noted that just as in the case of iron, the presence of pyrophosphate in an alkaline medium prevents any reaction between manganese and sodium diethyldithiocarbamate so that the metal is not extracted. Manganese therefore must form un-ionised complexes with pyrophosphate in the same way as iron.

Urine. 50–100 ml. of urine were measured into a 300 ml. Kjeldahl flask and boiled almost to dryness with 5 ml. concentrated nitric acid. 2 ml. of concentrated sulphuric acid were then added, and the heating was continued until the nitric acid was driven off. The remainder of the organic matter was finally destroyed by the addition of perchloric acid and further heating. The digest was made up to a known volume with distilled water and a volume containing about 0.01 mg. Cu taken for analysis (usually equivalent to 50 ml. urine). 5 ml. of 20 % sodium citrate were added, followed by 2 ml. of 4 % sodium pyrophosphate, and the solution was made alkaline to litmus by the addition of ammonia. 5 ml. of amyl alcohol were added, followed by 0.5 ml. 2 % sodium diethyldithiocarbamate, and the whole was vigorously shaken. The amyl alcohol layer was removed, passed through a filter-paper to remove any suspended particles of water and compared in a colorimeter with a standard containing 0.01 mg. Cu prepared similarly.

Faeces. 1 g. of dried faeces was heated in a Kjeldahl flask with 5 ml. of concentrated sulphuric acid and 15–25 ml. perchloric acid until all the organic matter was destroyed. When the excess of perchloric acid had been removed, the mixture was cooled and diluted with distilled water to 100 ml. If the digest contained any white insoluble matter it was diluted to about 20 ml. with distilled water, 1 ml. concentrated hydrochloric acid was added and the whole heated until solution was complete, after which it was diluted to 100 ml. with distilled water. A volume containing about 0.01 mg. Cu (usually about 10 ml.) was taken for analysis, which was carried out similarly to that described under urine. In the case of faeces 5 ml. of 4 % sodium pyrophosphate were added during the estimation instead of 2 ml. as used in the case of urine.

Diet. Owing to their low concentration of copper, samples of diet were ashed in silica basins by a dry method. It was found that when samples of such diets were ashed in silica basins, low results and poor recoveries of added copper were obtained. On the other hand it was found that urine and faeces could be ashed by this method to give satisfactory results. These materials usually gave a large amount of ash, consisting mainly of phosphates, and the silica of the basin did not appear to be attacked. When diets are ignited, the ash is usually very much smaller in comparison and the silica of the basin is invariably attacked even when the temperature is kept as low as possible. When the sample of diet was mixed with sodium phosphate the silica of the basin did not appear to be attacked and consistent results and accurate recoveries of added copper could be obtained.

The following was the method adopted for the determination of the copper contents of the diets. A sample of 10 g. of the dried diet *plus* the appropriate amount of butter was placed together with 5 g. of copper-free sodium phosphate in a silica basin and ignited at as low a temperature as possible. Final traces of carbon were destroyed by the addition of concentrated nitric acid and further heating. The ash was dissolved in distilled water containing 5 ml. concentrated hydrochloric acid and the solution diluted to 50 ml. A sample containing about 0.01 mg. Cu was taken for analysis which was carried out as described under urine. It was usual to add 5 ml. 4 % sodium phosphate as in the case of faeces.

Copper-free reagents were used throughout this investigation and all filter-papers were washed first with dilute acid and then with distilled water.

From the results shown in Table I, it will be seen that copper added to urine, faeces or samples of diet could be estimated quantitatively by the technique just outlined.

Table I. *Recovery of copper added to urine, faeces or diet.*

		Initial copper content mg.	Copper added mg.	Total copper mg.	Copper recovered mg.
Urine	1	0.216	(a) 0.1	0.301	0.085
			(b) 0.2	0.400	0.184
			(c) 0.3	0.530	0.314
	2	0.160	(a) 0.1	0.262	0.102
			(b) 0.2	0.368	0.208
			(c) 0.3	0.454	0.294
Faeces	1	0.104	0.2	0.300	0.196
	2	0.109	0.1	0.211	0.102
	3	0.083	0.1	0.179	0.096
	4	0.081	0.2	0.296	0.215
	5	0.091	0.2	0.310	0.219
	6	0.088	0.2	0.292	0.204
Diet	1	0.086	0.10	0.180	0.094
	2	0.102	(a) 0.10	0.210	0.108
			(b) 0.20	0.300	0.198
			(c) 0.40	0.506	0.404
	3	0.096	(a) 0.20	0.288	0.192
			(b) 0.50	0.595	0.499

In Table II are shown the average daily excretions of copper in the urine and faeces during the periods stated of 17 cases. The average daily copper contents of the diets are also shown. Several preparations of the same half-day diet were made and the average copper content taken. It will be noted that several of the cases show the same copper content of the diet—these were on the same diet. Cases 1–14 had no organic disease while cases 15 and 16 had subacute nephritis of the nephrotic type. Case 17 had carcinoma of the stomach and was only able to take fluids. The diets consumed by Cases 1–14 were well varied and were normal mixed diets.

It will be seen that, with the exception of No. 17, within the limits of experimental error total excretion balances the intake. The copper contents of the diets were found to be slightly higher than when calculated from the analyses of food-stuffs by Lindow *et al.* [1929]. The normal intake of copper would appear to vary from 2 to 2.5 mg. *per diem* on an average. As would be expected No. 17 shows a marked loss of copper—the loss being chiefly in the faeces. The fact that the total amount of copper excreted was 0.63 mg. *per diem* suggests that the minimum amount required *per diem* to preserve copper equilibrium may be in the region of this figure.

Table II.

Case	No. of days of collection	Av. daily excretion of copper in the urine mg.	Av. daily excretion of copper in the faeces mg.	Total daily excretion of copper mg.	Av. daily content of copper in diet mg.
1. Normal	6	0.29	2.60	2.89	2.72
2. Fracture	15	0.36	1.98	2.34	2.44
3. Fracture	13	0.52	1.88	2.40	2.44
4. Fracture	21	0.45	2.08	2.53	2.42
5. Fracture	21	0.50	2.15	2.65	2.42
6. Fracture	20	0.36	2.08	2.44	2.42
7. Normal	7	0.38	2.18	2.56	2.41
8. Normal	10	0.33	2.07	2.40	2.41
9. Normal	9	0.29	2.00	2.29	2.41
10. Normal	14	0.30	1.93	2.23	2.34
11. Fracture	16	0.44	1.70	2.14	2.29
12. Fracture	20	0.22	1.83	2.05	2.22
13. Normal	12	0.21	1.67	1.88	2.12
14. Amputation of leg	18	0.18	1.56	1.70	1.89
15. Nephritis	8	0.32	1.24	1.56	1.43
16. Nephritis	8	0.16	1.00	1.16	1.21
17. Carcinoma of stomach	8	0.11	0.52	0.63	0.21

In Table III are shown the copper contents of human urines as obtained by the present writer.

Table III.

	No. of cases	Minimum	Maximum	Average
1. Copper excretion in mg. per litre	16	0.08	0.48	0.18
2. Copper excretion in mg. <i>per diem</i>	24	0.12	0.52	0.28

These agree very closely with the figures obtained by Rabinowitch [1933].

SUMMARY.

1. The copper contents of the urine and faeces of 17 persons have been determined and compared with the copper contents of the diets.
2. Human urine contains 0.08–0.48 mg. of copper per litre.
3. An average daily diet would appear to contain 2–2.5 mg. of copper.

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