118. THE ZINC AND COPPER CONTENTS OF THE ORGANS AND TISSUES OF CHINESE SUBJECTS

BY WILLIAM GEORGE ELFORD EGGLETON

From the Henry Lester Institute of Medical Research, Shanghai

(Received 15 March 1940)

It is now a recognized fact that the two metals Zn and Cu are with a few doubtful exceptions universally present in biological material. The published data show some disagreement regarding the actual amounts to be expected. For example, Scott & Fisher's [1938] value for the Zn content of pancreas is 10 times as great as values reported by Lutz [1926] and by Hattori [1929], and the values for the Cu content of various organs reported by White (quoted by Herkel [1930]) and by Chou & Adolph [1935] are considerably higher than those reported by Herkel [1930], Cunningham [1931] and Tompsett [1934]. The results reported by some authors have been based on analyses of only a few samples, while the analytical technique employed is in some instances open to question.

Comparison with the amount of a metal in the blood will show whether or not it is concentrated in an organ or tissue, while comparison of the relative amounts of two metals present in an organ and in blood will show if either is preferentially retained. This last-mentioned point of difference may help to reveal a special property or function.

It is not known whether biological relationships exist between Cu and Zn although it is known that both form compounds with proteins. Only Bodansky [1921] has determined both metals in the same material.

Methods

The post-mortem material used was in no case more than 24 hr. old. In at least 12 cases samples were obtained within 6 hr. of death. After transportation to the laboratory in sealed jars to prevent water loss the specimens were prepared for analysis by removing adherent fat and connective tissue and cutting them into small pieces. A representative sample was dried for analysis and the remainder weighed and dried to constant weight. Some workers have adopted the technique of washing the organ or tissue free from blood regardless of the possibility that other soluble materials may be removed.

The "dithizone" method of Sylvester & Hughes [1936] was used for isolating the two metals from the ashed material. The Zn was determined iodimetrically by the method of Lang [1929; 1933] and the Cu colorimetrically with diethyldithiocarbamate after McFarlane [1932]. Minor modifications have been described elsewhere by Eggleton [1940].

Provided that a temperature of 450° is not exceeded, dry ashing does not result in loss of Zn or Cu. Wet ashing involves the use of relatively large quantities of acid and alkali which cannot be freed sufficiently from traces of Zn and Cu which are always present, and analyses cannot be performed accurately on small quantities of material owing to the disproportionately high blanks.

The oedematous condition of several cases under examination made it necessary always to express results in terms of dry matter. Greater uniformity could be obtained by expressing the results in terms of fat-free dry matter since

fat also can serve as a diluent in some tissues. However, this was not considered sufficient justification for the extra labour involved in what was at this stage a general rather than a detailed investigation.

Results

The blood of patients under treatment for beriberi, nutritional oedema and general malnutrition, whose pre-hospital diets are characterized by a small amount of protein and a larger amount of carbohydrate, has been shown by Eggleton [1940] to contain less Zn and somewhat more Cu than the blood of individuals whose diet approximates to normal. The relevant values obtained are summarized below for reference.

Table 1. *Zn and Cu contents of blood, mg./kg. dry matter*

	No. of cases	Zn	Cu	Zn/Cu
Foreign (well nourished)	4	30.5	7.3	4.2
Chinese (well nourished)	4	32.3	8.1	4.0
Chinese (ill nourished)	55	18.0	9.9	1.82

In continuation of this study it was primarily desired to compare the Zn and Cu contents of the organs and tissues of subjects coming within these two dietary categories. The pre-hospital dietary history of beriberi subjects can be inferred, that of other patients is often difficult to assess with accuracy, whereas that of accident cases can be guessed only from the appearance of the body at death. These difficulties together with the existence of secondary complications in most cases made it impossible to obtain a satisfactory comparison. For this reason the original intention was abandoned and data were collected from all cases that came to the autopsy table.

The Zn and Cu status of human organs and tissues

The figures for Zn here reported agree reasonably well with those reported by Rost & Weitzel [1919] for brain, liver and muscle, by Lutz [1926] for brain, liver, kidney and testicle and by Hattori [1929] for brain, liver, kidney, pancreas and heart. Figures for liver published by Raoult & Breton [1877], van Itallie & von Eck [1913], Herkel [1930] and Lundegaardh [1934] are also in agreement. The figures of Cristol [1923] for liver and spleen and of Bertrand & Vladescos [1913] for testicle are relatively high whereas those of Hattori [1929] for spleen are relatively low. The value found by Simakov [1936] for pectoral muscle is also relatively low.

Where comparisons are possible the figures for Cu agree satisfactorily with those reported by Bodansky [1921] for brain, by Herkel [1930] for liver, kidney, pancreas, spleen and thyroid, by Cunningham [1931] for brain, liver, kidney, pancreas and spleen and by Tompsett [1934] for brain, liver and kidney, spleen and pancreas. The figures reported by Chou & Adolph [1935] for liver, kidney, pancreas, spleen, heart, lung and muscle are all considerably higher, as are the figures given by White (quoted by Herkel [1930]) for liver, kidney, pancreas, spleen and heart.

The mean values obtained are in good agreement with those published by the majority of other workers, which, in view of the varied nature of the cases under consideration, is of some significance, for it suggests that "normal" mean values can be derived approximately from a series of hospital cases chosen without

Table 2. *Distribution of Zn and Cu in the organs and tissues of Chinese subjects, mg./kg. dry matter*

Sex and age	Cerebrum		Cerebellum		Liver		Kidney		Suprarenal		Pancreas		Spleen		Heart		Lung		Muscle	
	Zn	Cu	Zn	Cu	Zn	Cu	Zn	Cu	Zn	Cu	Zn	Cu	Zn	Cu	Zn	Cu	Zn	Cu	Zn	Cu
F 04	55*	12.0*	60*	8.2*	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
F 9	47*	14.2*	54	27.9	116	31.2	218	7.4	—	—	216	6.3	74	5.9	79	10.7	76	5.3	—	—
M 13	—	—	—	—	—	—	—	—	—	—	121	—	—	—	—	—	—	—	—	—
F 16	—	—	—	—	—	—	—	—	—	—	116*	12.4	—	—	—	—	—	—	—	—
F 17	33*	18.1*	45*	37.8*	186*	33.3*	122	—	—	—	—	—	61*	5.6	88*	18.0	—	—	—	—
M 18	46*	16.0*	60*	21.8*	545	48.1	245	15.0	106	6.8	135	4.6	72	8.5	80	11.3	149	10.0	307	7.3
F 19	—	—	—	—	178	32.4	68	11.6	—	15.3	169	14.2	80*	13.1*	118	18.6	—	—	—	—
F 25†	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
M 26	—	—	—	—	347	32.7	257	30.1	89	8.6	215	7.6	68	3.5	113	11.2	43	8.3	206	4.0
M 28	41	19.3	58	27.1	260	21.7	248	8.2	76	14.3	158	11.0	71	8.9	136	20.4	63	13.5	121*	7.7*
M 30	47*	18.9*	62*	29.3*	—	—	—	—	—	—	—	—	—	—	—	—	—	—	283	9.2
M 31	44*	15.7*	54*	26.2*	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
M 32	—	—	—	—	562	93.4	260	12.3	97	9.0	146	11.5	66	8.0	86*	8.1	62	12.1	181*	6.5
M 32a	38	19.3	55	25.3	285	68.6	157	8.3	106*	26.7*	—	—	79	13.3*	—	—	71	8.2	—	—
M 33	53	—	69	—	123	—	137	—	—	—	—	—	—	—	66	—	—	—	—	—
M 34	51*	18.2*	54*	25.4*	164*	53.2*	160*	14.1*	—	—	—	—	87*	8.1*	139*	12.7*	—	—	—	—
M 35	44*	21.0*	56*	38.2*	172*	26.7*	179*	46.1*	—	—	115*	7.8	76*	5.9*	87*	15.3*	—	—	—	—
M 38	55*	24.7*	55*	35.9*	169	25.5*	89*	11.6*	—	—	44	9.7	85	8.1*	86	18.2*	—	—	—	—
M 40	34*	23.0	42*	48.8	183*	99.9*	155*	13.9*	—	—	78	—	69*	4.7*	84*	13.4	—	—	—	—
M 43	42*	18.1*	51*	22.3*	190*	15.3*	209	7.6	72*	6.8*	128	4.1	62	4.2	92	9.4	57	6.5	267	5.1
M 45	32*	17.0*	51*	26.8*	226*	34.9*	314*	18.7	—	—	93	—	73*	6.4*	93*	17.0	—	—	—	—
M 48	43	—	53	—	162	—	146	—	—	—	—	—	—	—	126	—	—	—	—	—
M 51	40*	17.2*	46	22.6*	308	47.1	239	9.2	40	11.2	160	8.8	64	9.4	—	—	—	—	—	—
M 58†	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
F 60	—	—	—	—	154	18.4	149	10.0	72	5.4	139	8.2	62	4.8	105*	8.7	49	10.9	164	8.2
M?	39	17.9*	60	34.9	399	23.9	238	4.6	98	10.0	132	7.0	70	5.7	118	13.7	43	7.0	234	4.4
??	32	17.0	53	30.4	166	17.1	129	10.2	64	4.1	—	—	73	5.8	112*	7.8	60	7.0	271	5.2
Mean Zn and Cu	43	18.1	55	28.8	245	40.2	186	14.1	82	10.7	135	8.7	72	7.2	100	13.4	67	8.9	226	6.4
Mean ash % in d.m.	6.13	—	6.93	—	4.13	—	4.27	—	3.24	—	4.62	—	5.03	—	3.99	—	4.64	—	—	—
Mean ratio Zn:Cu	2.38	—	1.91	—	6.1	—	13.2	—	7.7	—	15.5	—	10.0	—	7.5	—	7.5	—	35.3	—

The thyroids of M 32a, F 60, M? and ?? contained respectively, 116, 168, 105* and 71 p.p.m. Zn and 24.6 7.1, 3.3* and 5.9 p.p.m. Cu. The thymus of M 13 contained 121 p.p.m. Zn and that of M 40 49 p.p.m. Zn. The testicles of M 18 and M 32a contained 54 and 69 p.p.m. Zn and 6.3 and 19.4 p.p.m. Cu respectively. The dura mater of M 33 contained 56.8* p.p.m. Zn and 7.7* p.p.m. Cu; the choroid plexus and sciatic nerve contained respectively 57.1 and 45.5 p.p.m. of Zn. The dura of M 35 contained 18.4 and 4.3 p.p.m. of Zn and Cu respectively and the gall bladder and bile fluid contained 31.3 p.p.m. and 14.6 of Zn respectively. The choroid plexus and sciatic nerve of M 48 contained 99.6 and 24.3 p.p.m. of Zn respectively.

* Figures marked with an asterisk have been confirmed by duplicate analyses at least.

† Sample taken from living subject at amputation.

special regard to pathological condition. It is probable that the observed variability arises more from differences in dietary habit than from pathological conditions, except in so far as chronic disease may influence the quantity or quality of food ingested.

Cerebrum and cerebellum. In the present investigation 17 brains were analysed for the Zn and Cu contents of both cerebrum and cerebellum. The results show that cerebellum contains more Zn and Cu than cerebrum, that both contain on the average less Zn than any other organ or tissue examined, that both, but especially cerebellum, contain larger amounts of Cu than any other organ or tissue examined with the single exception of liver and that the Zn and Cu contents of brain shows only a small range of variation. The amount of Zn in the dry matter of brain is less than twice the amount of Zn in the dry matter of blood, whereas the amount of Cu is 3-4 times as great; the ratio Zn/Cu is much smaller than in any other organ or tissue, even liver. From these facts it is concluded that Cu is selectively concentrated by these organs, particularly by the cerebellum, though the reason is not understood.

Suprarenal, spleen, lung, thymus and testicle. These all contain, in terms of dry matter, about twice as much Zn as blood, but the amount of Cu in these organs is of the same order as in blood. Zn is therefore selectively concentrated to a similar extent in all these organs.

Heart muscle and voluntary muscle. There is a marked difference between voluntary muscle and involuntary muscle in respect of Cu and Zn contents. In voluntary muscle (*Pectoralis major* and *Rectus abdominalis*) the mean Zn content is high (226)—as high as in hair, teeth, liver and kidney. The mean Cu content is low (6.4) and is not significantly different from the amount in blood. In heart muscle the mean amount of Zn (100) is less than half that present in voluntary muscle but the mean Cu content (13.4) is more than twice as much. Whereas only Zn is concentrated in voluntary muscle both metals are concentrated in heart muscle.

Liver. Although usually greater in liver the amounts of Zn and Cu are more variable than in any other organ or tissue examined. A high Cu content does not necessarily accompany a high Zn content or *vice versa*. However the ratio Zn/Cu in liver is on the average approximately the same as in blood, which indicates that both metals are in general concentrated in unchanged proportions.

Kidney. This organ also shows considerable variability in Zn and Cu contents. Although both metals are concentrated the ratio Zn/Cu is twice that of blood, indicating that Zn is concentrated in the kidney to a greater extent than Cu.

Pancreas. The Zn content of this organ is occasionally comparable with that of liver and kidney. The amount of Cu, however, is seldom greater than that in blood. Zn therefore is selectively concentrated.

Thyroid. The four results obtained suggest that Zn is normally concentrated in the thyroid, whereas Cu is not.

Influence of nutrition on Zn and Cu contents

Of special interest (Table 3) is the high Zn content, and possibly also high Cu content, of the liver and kidney in those cases which were obviously ill nourished. This is taken to indicate that under starvation conditions these two organs are so depleted of fat, glycogen and other matter with which these metals are not normally associated, that the concentration is increased in terms of residual dry matter. This effect is more noticeable with Zn than with Cu and in the liver than in the kidney. It is probable that the proteins with which the Zn and Cu occur in combination may be regarded as being always in a state of saturation in

Table 3. *Zn and Cu contents of liver and kidney in well and ill nourished subjects*

Well or fairly well nourished patients	Ash % d.m.	Zn		Cu		Ratio Zn/Cu
		p.p.m.d.m.	% in ash	p.p.m.d.m.	% in ash	
		Liver				
F 17	3.64	186	0.51	33.3	0.092	5.6
M 34	3.41	164	0.48	53.2	0.156	3.1
M 38	4.42	169	0.38	25.5	0.058	6.6
F 60	3.79	154	0.41	18.4	0.049	8.4
F 19	4.07	178	0.44	32.4	0.080	5.5
M 28†	4.93	260	0.53	21.7	0.044	12.0
Mean	4.04	185	0.46	30.8	0.080	6.9
Ill nourished patients						
M ?	5.04	399	0.79	25.9	0.051	15.4
M 26	3.53	347	0.98	32.7	0.093	10.6
M 51	4.33	308	0.71	47.1	0.109	6.5
M 32	4.66	562	1.21	93.4	0.200	6.0
M 18	4.62	545	1.18	48.1	0.104	11.3
Mean	4.44	432	0.97	49.4	0.112	10.0
Well or fairly well nourished patients						
Kidney						
F 17	4.59	122	0.27	—	—	—
M 34	3.71	160	0.43	14.1	0.038	11.4
M 38	3.57	89*	0.25	11.7	0.033	7.6
F 60	3.67	149	0.41	10.1	0.028	14.8
F 19	3.39	68*	0.20	11.6	0.034	5.9
M 28†	4.11	267	0.54	8.2	0.020	30.0
Mean	3.84	143	0.35	11.1	0.031	13.9
Ill nourished patients						
M ?	4.79	238	0.50	4.6	0.010	51.7
M 26	3.36	257	0.77	30.1	0.090	8.5
M 51	3.62	239	0.66	9.2	0.025	26.0
M 32	4.03	260	0.65	12.3	0.031	21.1
M 18	5.32	245	0.46	15.0	0.028	16.3
Mean	4.22	248	0.61	14.2	0.037	24.8

* Nephritis.

† In spite of the clinician's description it would appear that M 28 should be regarded as poorly nourished.

respect of these metals, so that the amount found is in this sense an inverse measure of the degree of fatty infiltration conditioned by the diet. Although analyses were not made it would appear likely on this basis that the Zn content of an organ is positively correlated with its N content. It is not possible to make suitably accurate comparisons with other organs and tissues owing to paucity of data but the indications are that they are affected only slightly in this respect.

DISCUSSION

It is frequently stated that certain organs "accumulate" metals, the implication being that the organ has an affinity for the metal which is accumulated for a specific purpose. Foetal liver is said to accumulate Zn and Cu, an argument advanced in support of the belief that these metals are indispensable to life.

This is no argument in itself, for the higher concentration may be due to the fact that the amount of some major constituent of the liver normally present

and of which these metals are independent, is deposited in the liver to an increased extent after birth, thereby lowering their concentration in terms of dry matter. If metals are associated in organic combination with proteins then the other constituents with which they are not associated will act as diluents to varying degrees.

Eggleton [1940] has reported the lowered Zn content and slightly increased Cu content of the blood in acute, subacute and chronic beriberi and in cases of protein deficiency and nutritional oedema. It was thought that this condition might be reflected in the Zn and Cu contents of the organs. However, this is not so. The explanation would appear to be that, whereas a low Zn intake such as occurs in malnutrition involving low protein, produces a low Zn level in the blood, the liver and kidney are depleted of fats, cholesterol, glycogen etc. to a relatively greater extent in the aggregate than of protein under these conditions, so that the concentration of Zn and Cu is increased in terms of residual dry matter. However, it is still not understood why blood Cu should be increased while blood Zn is decreased.

It is considered that little information is to be gained by studying the changes in concentration of isolated metallic constituents of organs in various pathological or nutritional states unless they are considered in conjunction with changes in other relatively more bulky constituents. Nevertheless, the figures here reported have some value, for it is necessary to know the highest concentration of a metal which may be reached under all conditions except where the metal in question has been ingested in unnatural quantities. For this reason it is important to consider the amount present relative to the total ash content or to the amount of some other metal known to be a regular constituent.

The results show that whereas the Zn contents of all tissues studied are in general higher than that of the perfusing blood, the Cu contents are higher only in liver, brain and possibly also in kidney and heart. Why other organs should not have their proportionate share of Cu is not clear, unless it be that there is some important difference in respect of the compounds with which these metals can unite.

SUMMARY

An investigation into the distribution of Zn and Cu in the human body, involving over 300 Zn and 200 Cu analyses of 13 different organs and tissues from 26 Chinese subjects of both sexes, ranging in age from 156 days to 60 years and dying from various causes, has shown that both metals are always present. Comparison with the average amounts of these metals present in the dry matter of blood shows that Zn is concentrated in all organs and tissues examined, but that Cu is concentrated only in liver, brain, kidney and heart in order of descending magnitude.

Cerebrum and cerebellum contain less Zn and, with the exception of liver, more Cu than any organ or tissue examined. Cerebellum contains more Zn and Cu than cerebrum.

The increased concentration of Zn and Cu in the liver and kidney observed in cases of malnutrition is thought to be due to the relatively greater withdrawal of the fats and carbohydrates from these organs, than of the proteins with which the metals remain in association.

I am indebted to Dr P. B. Wilkinson of the Medical Dept., Hongkong, to Dr C. L. Dale of St Luke's No. 2 Hospital, Shanghai and to my colleague Dr L. S. Kau for supply of autopsy material and for access to their records.

REFERENCES

- Bertrand & Vladescos (1913). *C.R. Acad. Sci.* **173**, 176.
Bodansky (1921). *J. Biochem.* **48**, 361.
Chou & Adolph (1935). *Biochem. J.* **29**, 476.
Cristol (1923). *Bull. Soc. Chim. Biol., Paris*, **5**, 23.
Cunningham (1931). *Biochem. J.* **25**, 1267.
Eggleton (1940). *Chin. J. Physiol.* **15**, (1) (in the Press).
Hattori (1929). *Chiyo-Bioin Nempo*, No. 4.
Herkel (1930). *Beitr. path. Anat.* **85**, 513.
Lang (1929). *Z. anal. Chem.* **79**, 161.
—— (1933). *Z. anal. Chem.* **93**, 21.
Lundegaardh (1934). *Naturwissenschaften*, **22**, 572.
Lutz (1926). *J. Industr. Hyg.* **8**, 177.
McFarlane (1932). *Biochem. J.* **26**, 1022.
Raoult & Breton (1877). *C.R. Acad. Sci.* **85**, 40.
Rost & Weitzel (1919). *Zbl. Biochem. Biophys.* **21**, 440.
Scott & Fisher (1938). *J. clin. Invest.* **17**, 725.
Simakov (1936). *Biokhimiya*, **1**, 685.
Sylvester & Hughes (1936). *Analyst*, **61**, 734.
Tompsett (1934). *Biochem. J.* **29**, 480.
Van Itallie & von Eck (1913). *Arch. f. Pharmazie*, **251**, 51.