

## **The distribution of copper, zinc and manganese in various regions of the brain and in other tissues of children with protein-calorie malnutrition**

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1. The distribution of copper, zinc and manganese in seven regions of the brain, liver, heart, muscle from the abdominal wall, and skin of children was determined by means of atomic absorption spectrophotometry.
2. The results showed significantly lower levels of Cu, Zn and Mn in livers of children with kwashiorkor compared with control subjects. The concentrations of these three elements were higher in livers of children with marasmus than in livers of children suffering from kwashiorkor and not significantly different from those of control subjects.
3. The Cu concentration in muscle obtained from the abdominal wall of subjects with kwashiorkor and the Mn concentration in heart muscle of all groups with protein-calorie malnutrition were significantly lower than the respective values for the control group.
4. There was no significant difference between Cu, Zn or Mn concentration in brain tissues of control subjects and in those with protein-calorie malnutrition.

Severe protein-calorie malnutrition (PCM) is known to produce an imbalance of the major mineral elements of the body. Depletion of potassium and magnesium are often seen in acute cases of kwashiorkor. Recent findings (Hansen & Lehmann, 1969) confirm the fact that zinc and copper concentrations in the serum of patients suffering from protein-calorie malnutrition can be very low.

Warren, Hansen & Lehmann (1969) determined the concentrations of Cu, Zn and manganese in the liver of children suffering from kwashiorkor and compared these findings with those obtained for marasmic children, and children who had died from other causes. This study showed that there was a significant decrease of the concentrations of Zn and Cu in the liver of kwashiorkor patients compared with controls.

As far as we are aware, no observations have been made of the effect of severe PCM on the concentrations of trace metals such as Zn, Cu and Mn in the brain. It seemed to us desirable to investigate whether severe PCM in young children altered the concentration of any of these trace metals. This study might indicate whether reduction in the liver concentrations of Cu and Zn were reflected in the concentrations of these metals in the brain. In this communication the concentrations of Cu, Zn and Mn in various regions of the brain, liver, muscle obtained from the wall of the abdomen, and skin of children who had died from kwashiorkor and marasmus are presented.

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## EXPERIMENTAL

*Material*

Samples of brain, liver, heart, muscle obtained from the wall of the abdomen, and skin were obtained at autopsy from five children with kwashiorkor, three with marasmic kwashiorkor, and six with marasmus. Five children of the same age range that had died of diseases other than kwashiorkor or marasmus and showed no obvious signs of malnutrition were the only subjects available to serve as a control group (Table 1). The criteria for diagnosis of kwashiorkor, marasmic kwashiorkor and marasmus were those outlined by Jelliffe (1966).

Table 1. *Information about the children studied*

Code	Age	Sex	Cause of death
166/67	1 year 5 months	♀	Cardiac failure
170/67	9 months	♂	Collapse of lungs
171/67	1 year 6 months	♀	Pneumonia
236/67	1 year 6 months	♀	Septicaemia
252/67	2 years 4 months	♂	Meningitis
119/67	1 year 6 months	♀	Kwashiorkor
135/67	1 year 5 months	♂	Kwashiorkor
1704/67	1 year 9 months	♂	Kwashiorkor
160/67	2 years	♀	Kwashiorkor
2278/67	4 years	♀	Kwashiorkor
144/67	5 months	♂	Marasmic kwashiorkor
1812/67	1 year 1 month	♂	Marasmic kwashiorkor
1969/67	1 year 1 month	♂	Marasmic kwashiorkor
1620/67	8 months	♀	Marasmus
105/67	1 year 6 months	♂	Marasmus and gastroenteritis
1811/67	2 years	♂	Marasmus
1857/67	1 year 4 months	♀	Marasmus and gastroenteritis
158/67	10 months	♀	Marasmus and rickets
161/67	3 years	♂	Marasmus and rickets

*Preparation of samples and determination of Zn, Cu and Mn*

Throughout the analytical work great care was taken to avoid contamination with metal substances. Borosilicate glassware cleaned in chromic acid and washed with glass-distilled water was used throughout. The liver obtained at post-mortem was washed externally with glass-distilled water and drained on a clean dish, and then a small piece was removed for the determination of the trace metals. This was transferred to a Potter-Elvehjem glass homogenizer and tissue homogenate was prepared from it with the aid of a small volume of glass-distilled water. The method of preparation of the other tissues was similar to the method used for liver. The only modification was that the homogenization step was omitted in heart, muscle and skin samples. The brain was frozen at  $-4^{\circ}$  before dissection, transferred to a Pyrex glass dish and its outer surface washed with metal-free water. The excess water was removed by lightly blotting with clean paper tissues, and small pieces of cortex were dissected out with clean stainless-steel instruments. The brain was then sectioned and small

portions of the deeper structures to be examined were removed with a stainless-steel knife with a thin blade.

The organic material was destroyed and the samples were oxidized by a modification of the nitric-perchloric-sulphuric acid method of Eden & Green (1940). All reagents used were of AR or lead-free grade. Batches of each of the acids used in the destruction of the organic material were analysed for the presence of Cu, Zn and Mn and those with the lowest concentrations of these metals were selected for analytical use. The Zn, Cu and Mn content of each sample was determined by means of a Perkin Elmer 303 atomic absorption spectrophotometer. Reagent blanks were carried out with each set of estimations. Samples of the original tissue homogenate were dried to constant weight at 110° for determination of dry-matter content.

#### RESULTS

The mean concentrations of Zn, Cu and Mn in the various tissues of the four groups of children investigated are presented in Tables 2 and 3. In the PCM groups there was some decrease of Zn, Cu and Mn concentrations in most tissues except skin, brain stem and pons in which the concentrations were higher than in the control group.

##### *Zinc*

The only tissue in which the concentration of Zn was statistically significantly reduced compared with the control group was the liver of children with kwashiorkor ( $P < 0.001$ ). With regard to brain tissues, the mean Zn concentrations in the cortical grey matter of the various groups were significantly higher than the mean Zn concentrations in the subcortical white matter ( $P < 0.001$ ). There was no significant difference in Zn concentration between brain tissues of control subjects and PCM groups.

##### *Copper*

The mean Cu concentrations in liver and muscle were significantly lower in the children with kwashiorkor than in the control group ( $P < 0.001$  and  $P < 0.05$ , respectively). The highest Cu concentrations in the brain were found in the cortical grey matter and basal ganglia. As with Zn, the mean Cu concentration in cortical grey matter of all subjects studied was significantly higher than the means of the corresponding concentrations in subcortical white matter ( $P < 0.01$ ). There was no significant difference in Cu concentration between brain tissue of control subjects and PCM groups.

##### *Manganese*

The mean Mn concentration in the liver was significantly lower in the children with kwashiorkor than in the control group ( $P < 0.05$ ). The mean Mn concentration in heart muscle of all PCM children was significantly lower than the mean value for the control group ( $P < 0.05$ ). There was no significant difference in Mn concentration in brain tissues between control subjects and the PCM groups.

Table 2. Concentrations ( $\mu\text{g/g}$  dry whole tissue) of copper, zinc and manganese in various regions of the brain of children who died as a result of marasmus, marasmic kwashiorkor, kwashiorkor or from other causes (controls)

Tissue	(Mean values with their standard errors; ranges in parentheses)			
	Controls	Marasmus	Marasmic kwashiorkor	Kwashiorkor
		Copper		
Cortical grey matter	16.0 $\pm$ 2.8(9.6-23.9)	13.0 $\pm$ 1.2(10.7-18.2)	12.0 $\pm$ 1.9(8.5-15.3)	14.5 $\pm$ 2.0(10.4-19.4)
Subcortical white matter	8.5 $\pm$ 1.7(5.6-15.1)	8.3 $\pm$ 0.7(6.0-10.7)	9.8 $\pm$ 2.8(5.5-15.3)	6.4 $\pm$ 0.4(4.9-7.4)
Corpus callosum	8.5 $\pm$ 1.7(4.5-13.6)	8.0 $\pm$ 2.3(2.0-15.4)	11.1 $\pm$ 3.0(7.3-14.8)	5.8 $\pm$ 0.4(4.6-6.8)
Basal ganglia	15.2 $\pm$ 2.1(8.5-20.9)	14.1 $\pm$ 1.1(11.8-18.1)	14.4 $\pm$ 4.4(5.5-19.9)	14.4 $\pm$ 5.6(4.4-28.6)
Cerebellum	9.8 $\pm$ 0.9(8.1-12.7)	10.6 $\pm$ 1.0(8.2-14.0)	10.0 $\pm$ 1.2(7.6-11.4)	15.5 $\pm$ 3.8(8.7-30.5)
Pons	5.2 $\pm$ 1.1(2.2-7.5)	7.3 $\pm$ 1.1(3.9-11.2)	9.9 $\pm$ 3.0(5.3-15.6)	5.4 $\pm$ 0.5(3.8-6.9)
Brain stem	9.0 $\pm$ 0.9(6.1-10.6)	10.1 $\pm$ 0.7(8.4-12.1)	16.2 $\pm$ 3.1(10.7-21.4)	14.5 $\pm$ 0.7(13.1-16.2)
		Zinc		
Cortical grey matter	65.3 $\pm$ 3.5(54.8-74.5)	62.6 $\pm$ 4.7(47.0-78.4)	66.8 $\pm$ 9.0(49.6-80.2)	66.5 $\pm$ 3.3(59.5-74.1)
Subcortical white matter	31.0 $\pm$ 1.2(28.2-34.5)	33.3 $\pm$ 4.9(17.4-49.8)	35.9 $\pm$ 0.2(35.5-36.3)	27.0 $\pm$ 0.7(22.3-31.6)
Corpus callosum	38.1 $\pm$ 5.2(29.9-57.5)	35.3 $\pm$ 5.5(19.9-50.0)	45.6 $\pm$ 1.0(44.6-47.1)	25.6 $\pm$ 0.0(25.5-25.8)
Basal ganglia	51.5 $\pm$ 0.9(48.5-54.2)	49.3 $\pm$ 6.9(25.4-65.6)	57.9 $\pm$ 12.4(41.6-82.4)	36.9 $\pm$ 11.8(12.5-64.8)
Cerebellum	62.6 $\pm$ 1.5(59.4-65.6)	49.8 $\pm$ 3.0(38.6-58.6)	57.5 $\pm$ 2.7(54.7-62.8)	51.1 $\pm$ 2.8(45.3-61.8)
Pons	35.6 $\pm$ 2.6(28.8-41.5)	31.2 $\pm$ 4.6(15.8-45.8)	44.0 $\pm$ 2.7(40.0-49.2)	31.5 $\pm$ 1.6(27.0-35.4)
Brain stem	36.5 $\pm$ 2.4(30.3-43.4)	38.0 $\pm$ 3.8(29.2-47.8)	52.0 $\pm$ 8.2(39.6-67.5)	39.7 $\pm$ 1.3(37.6-43.0)
		Manganese		
Cortical grey matter	5.2 $\pm$ 1.0(2.0-8.0)	4.3 $\pm$ 1.3(1.0-9.2)	3.9 $\pm$ 1.0(2.4-5.8)	2.9 $\pm$ 0.9(0.5-4.3)
Subcortical white matter	3.1 $\pm$ 0.6(1.1-5.2)	3.0 $\pm$ 0.9(0.6-5.9)	1.8 $\pm$ 0.3(1.1-2.4)	1.7 $\pm$ 0.3(0.9-2.6)
Corpus callosum	4.2 $\pm$ 1.1(1.1-8.3)	3.4 $\pm$ 1.2(0.4-7.0)	4.1 $\pm$ 0.9(2.9-5.3)	2.2 $\pm$ 0.1(1.8-2.6)
Basal ganglia	3.2 $\pm$ 0.7(1.9-5.8)	4.8 $\pm$ 1.8(1.2-9.4)	3.7 $\pm$ 1.4(1.9-6.5)	2.6 $\pm$ 1.2(0.1-5.5)
Cerebellum	3.4 $\pm$ 0.7(1.4-5.5)	2.0 $\pm$ 0.4(0.9-3.2)	2.0 $\pm$ 0.6(1.0-3.1)	3.2 $\pm$ 0.6(1.9-5.6)
Pons	1.9 $\pm$ 0.2(1.4-2.4)	2.4 $\pm$ 0.7(0.3-4.3)	2.1 $\pm$ 0.5(1.4-3.2)	2.5 $\pm$ 0.4(1.0-3.5)
Brain stem	3.9 $\pm$ 0.9(0.7-5.7)	4.0 $\pm$ 1.1(0.8-6.3)	4.5 $\pm$ 0.7(3.1-5.5)	3.8 $\pm$ 0.4(2.7-4.9)

Table 3. Concentrations ( $\mu\text{g/g}$  dry whole tissue) of copper, zinc and manganese in liver, heart, abdominal wall muscle and skin of children who died as a result of marasmus, marasmic kwashiorkor, kwashiorkor or from other causes (controls)

Organ or tissue	(Mean values with their standard errors; ranges in parentheses)			
	Controls	Marasmus	Marasmic kwashiorkor	Kwashiorkor
		Copper		
Liver	13.8 $\pm$ 1.8(8.7-18.4)	10.6 $\pm$ 2.4(2.3-18.8)	50.8 $\pm$ 34.5(5.7-118.6)	4.8 $\pm$ 1.1(2.1-8.5)
Heart	14.3 $\pm$ 1.2(10.9-16.8)	11.4 $\pm$ 0.9(8.2-14.9)	18.0 $\pm$ 7.7(9.1-33.4)	9.5 $\pm$ 1.1(7.3-13.7)
Muscle	8.1 $\pm$ 1.5(4.4-13.1)	6.8 $\pm$ 2.0(2.2-15.0)	10.5 $\pm$ 4.6(3.9-19.3)	3.4 $\pm$ 0.1(3.1-3.9)
Skin	2.5 $\pm$ 0.4(0.9-3.6)	6.6 $\pm$ 3.6(0.9-22.1)	10.2 $\pm$ 6.9(1.4-23.9)	3.6 $\pm$ 0.5(2.1-5.0)
		Zinc		
Liver	155.6 $\pm$ 4.5(142.1-167.5)	98.8 $\pm$ 26.0(28.6-197.1)	91.1 $\pm$ 44.3(44.2-179.8)	43.4 $\pm$ 6.5(26.8-61.2)
Heart	114.9 $\pm$ 9.3(89.2-145.9)	98.8 $\pm$ 6.7(80.2-128.1)	98.4 $\pm$ 5.1(92.5-108.7)	87.8 $\pm$ 4.2(78.3-99.6)
Muscle	155.2 $\pm$ 13.0(109.9-187.1)	173.0 $\pm$ 17.6(133.1-230.6)	182.0 $\pm$ 7.9(165.9-190.8)	146.6 $\pm$ 19.2(113.5-208.4)
Skin	15.6 $\pm$ 2.6(9.5-22.7)	17.8 $\pm$ 3.4(10.0-31.5)	35.5 $\pm$ 11.0(20.5-57.0)	84.0 $\pm$ 43.8(18.3-229.7)
		Manganese		
Liver	7.1 $\pm$ 0.8(5.3-9.0)	6.3 $\pm$ 1.8(1.5-11.6)	6.1 $\pm$ 4.0(1.4-14.1)	3.8 $\pm$ 1.3(1.9-9.1)
Heart	7.1 $\pm$ 2.7(3.9-18.1)	3.1 $\pm$ 0.9(1.2-6.4)	2.8 $\pm$ 0.6(1.9-4.1)	3.7 $\pm$ 0.9(0.9-6.3)
Muscle	4.8 $\pm$ 1.3(1.4-9.3)	2.1 $\pm$ 0.4(0.7-3.7)	2.2 $\pm$ 0.3(1.6-2.8)	4.2 $\pm$ 0.9(2.1-7.1)
Skin	3.1 $\pm$ 0.4(1.8-4.5)	2.0 $\pm$ 0.3(0.6-2.6)	1.8 $\pm$ 0.8(0.9-3.3)	5.2 $\pm$ 2.0(1.9-11.7)

## DISCUSSION

A general tissue deficiency of Zn, Cu and Mn in subjects with protein-calorie malnutrition has not been unequivocally demonstrated in this study. In kwashiorkor we have confirmed our previous finding of a significant reduction of Zn and Cu in the liver (Warren *et al.* 1969), and demonstrated a lowering of the concentration of Cu in muscle taken from the abdominal wall and of Mn in heart muscle. We were unable to find any reports in the literature on tissue analysis of this kind in subjects with protein-calorie malnutrition, but the concentrations in our control subjects compare fairly well with normal values reported earlier (Warren, Earl & Thompson, 1960; Harding-Barlow, 1961). Small differences between normal trace-element concentrations in tissues reported by different workers are to be expected owing to age differences (Lea & Walker, 1964) and the variety of analytical methods. It is to be expected that the trace-element pattern in organs with a rapid protein turnover will be the most affected in states of protein deficiency. It is of interest that we found this to be so in the liver and not surprising that we found no significant differences in Zn, Cu and Mn concentrations between brain tissues of control subjects and those with protein-calorie malnutrition.

It was of interest to find that the Zn concentration in the skin was not significantly lower in the children with protein-calorie malnutrition than in control subjects. In a previous communication (Hansen & Lehmann, 1969), Zn concentration in the serum was reported to be reduced in children who had ulcerative skin lesions but this was not related to severity of the malnutrition or to rapidity of healing. The repeated therapeutic value of Zn in the healing of wounds and ulcers (Pories, Henzel, Rob & Strain, 1967) may thus be a specific effect connected with the formed elements of the blood in which Zn becomes concentrated rather than an effect on tissue content *per se* (Husain, 1969).

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