CHANGES PRODUCED BY PROTEIN MALNUTRITION ON THE CONCENTRATION OF DEOXYRIBONUCLEIC ACID IN HUMAN LIVER[1]

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Abstract

The content of deoxyribonucleic acid of samples of liver obtained from children at autopsy under standardized conditions was determined. There were higher concentrations in the livers of malnourished children, but there was not a significant difference associated with a distinction between kwashiorkor and marasmus.

Previous work (1, 2) has shown that various types of protein deficiency produced a significant increase in the average deoxyribonucleic acid (DNA) content of isolated rat liver nuclei, and secondarily an increase in the DNA concentration of the liver homogenates.

Since the main purpose of our studies is to clarify the metabolic changes produced by protein deficiency in humans, it was decided to determine the DNA concentration of the livers of children who had died of protein–calorie malnutrition. It was our aim to determine whether the changes found in the human material would correspond to those previously described in the livers of rats subjected to protein-deficient diets.

Samples of livers obtained from autopsy material under standardized conditions were analyzed for DNA. The results showed an increase in the DNA concentration in livers of malnourished children, in accord with the previously published experiments on rats. No significant differences could be detected between the increases in DNA concentration produced by the two clinical types of protein–calorie malnutrition in children, kwashiorkor and marasmus (3).

Material and Methods

Samples of liver were obtained through the Division of Pathology of the Institute of Nutrition of Central America and Panama (INCAP) at the Roosevelt Hospital. Their selection was restricted as follows. They were obtained only from children between the ages of 1 month and 4 years, who had died with a clinical diagnosis of protein–calorie malnutrition. This diagnosis had to be confirmed afterwards by postmortem pathological study. To avoid the effect of treatment on the composition of the liver, only those subjects that had been in the hospital for no more than 24 hours were accepted. Only samples that were collected during the first 8 hours after the death of the

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patient were used for the study, since previously it had been shown in animal experiments that during this period no significant change in the DNA concentration is produced.

A sample of liver of approximately 5 g was obtained through a small incision immediately after the body was taken to the morgue. It was kept frozen at −70 °C until the time of analysis, which was usually within 1 month. Previous animal experiments had shown that neither freezing and thawing, nor keeping the livers frozen at −70 °C for as long as 1 month, produced any changes in the DNA concentration.

At the time of processing, the samples were thawed at 4 °C and weighed. A 10% homogenate was made in cold distilled water with a Waring Blendor in a cold room. The samples in which excess fibrous tissue prevented proper homogenization were discarded. Aliquots in duplicate were taken for determinations of dry weight and the DNA content. DNA was extracted with 5% trichloroacetic acid at 90 °C, and quantitatively determined by the colorimetric method of Dische (4, 5). Total nitrogen was determined by the micro-Kjeldahl technique described by the Association of Official Agricultural Chemists (6).

The subjects under study were divided into three groups.[3] In the first were children who had died of causes not related to nutritional conditions.[4] The weights and heights of these children were within normal limits according to the studies of Falkner (7); therefore this group was used as a control. In the second were children who had died of the marasmus type of protein–calorie deficiency (clinical diagnosis confirmed by post-mortem study). In this group, the weight deficit varied from 20 to 50% and the height deficit from 0 to 20% of the control. In the third group were children who had died of the kwashiorkor type of malnutrition. In this group the weight deficit varied from 15 to 60% and the height deficit from 0 to 17% of the control.

Results and Discussion

The results of the analyses of the liver samples from these three different groups are presented in Table I. It is evident that protein deficiency in children produced an increase in the DNA concentration of the liver. The normal values found in malnourished children when the results are expressed per unit of dry weight can be explained by the differences in water content of the livers (76.2% in kwashiorkor, 74.2% in marasmus, and 81% in the control[5]), which cancel the differences between groups.

If it is assumed that the only difference between the liver of a malnourished child and that of a normal child of the same age is the size, and therefore that the relative composition of both organs is the same, the ratio of the total liver

[3]The relatively small number of cases in each group is due to the extreme difficulty in obtaining autopsy material to meet the conditions imposed on our samples, especially for the control group.

[4]The age of the control group was determined by the availability of autopsy material and not by choice.

[5]The relatively high water content of the control group is due to the higher water content of the livers of the youngest children.

TABLE I

Changes in deoxyribonucleic acid concentration produced by protein deficiency in the livers of children

	Deoxyribonucleic acid content of liver					
	Mg per g of fresh tissue		Mg per g of dry tissue		Mg per mg of liver nitrogen	
Cases	$\bar{x}$	S.D.	$\bar{x}$	S.D.	$\bar{x}$	S.D.
Well nourished (4)	4.7	1.0	25.2	2.7	0.25	0.02
Malnourished						
Marasmus (5)	7.1	1.8	26.7	4.4	0.34	0.14
Kwashiorkor (7)	6.1	0.3	25.7	4.4	0.37	0.10

NOTE: The figures in parentheses represent the number of cases studied.

weight of a malnourished subject to that of a control should be the same as the ratio of the total amounts of a liver constituent for the two subjects. If the latter ratio is found to be different from the former, it could be concluded that the content of the constituent in question in the liver of the malnourished child is different from that in the liver of the normal child. This type of calculation has the advantage that it reveals changes in the composition of the organ with respect to the total mass, regardless of any other changes that might have occurred in other constituents.

The DNA content of the human livers analyzed was calculated as described and the results are presented in Table II. The increase in the DNA content can be considered not only with respect to the total mass of the organs but also with respect to the total nitrogen content.

TABLE II

Changes in the livers of malnourished children from cases of Kwashiorkor and Marasmus

	Kwashiorkor		Marasmus	
Ratios	$\bar{x}$	S.D.	$\bar{x}$	S.D.
$\frac{\text{Liver weight (malnourished)}}{\text{Liver weight (well nourished)}}$	2.4	2.1	3.4	4.9
$\frac{\text{Total nitrogen (malnourished)}}{\text{Total nitrogen (well nourished)}}$	2.1	1.7	2.6	2.9
$\frac{\text{Total DNA (malnourished)}}{\text{Total DNA (well nourished)}}$	3.3	2.3	5.7	8.0

NOTE: DNA = deoxyribonucleic acid.

When the ratios[6] corresponding to marasmus/kwashiorkor were calculated, the following results were obtained: total nitrogen of liver, 1.5 ± 0.5; total liver weight, 1.6 ± 0.9; total DNA of liver, 1.7 ± 1.0. It is noteworthy that there is no great difference between the DNA content of the livers in the two clinical types of protein–calorie malnutrition. However, the similarity of the

[6]The ratios are given as means ± standard errors.

DNA concentration in the two forms of protein–calorie malnutrition should not be interpreted as meaning that the overall metabolic disturbance in both clinical conditions is the same. Further studies have been initiated to gather direct information on the effects of reduced cytoplasm content and of increase in the proportion of polyploid-type nuclei on the increased DNA content of the liver in both conditions.

In Table III the analytical results are presented according to the age of the children, since it has been demonstrated that there is an increase in the DNA

TABLE III

Changes in deoxyribonucleic acid concentration produced by protein deficiency in the livers of children according to age

		Deoxyribonucleic acid content of liver		
Cases	Ages	Mg per g of fresh tissue ($\bar{x}$)	Mg per g of dry tissue ($\bar{x}$)	Mg per mg of liver nitrogen ($\bar{x}$)
Well nourished	1–4 months (3)	5.1	26.4	0.27
	2.5 years (1)	3.6	21.6	0.21
Malnourished	3–17 months (10)	6.2	27.1	0.38
	2 to 4 years (2)	6.6	24.2	0.36

NOTE: The figures in parentheses represent the number of cases studied.

content of the liver with age (8). Here also, it can be noticed that protein deficiency produced an increase in the DNA concentration of the liver.

Attempts to determine the nuclear ploidy by measuring nuclear diameters on histological sections (9) failed because of extreme shrinking and distortion of the nuclei after dehydration and staining of the preparations.

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