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Effect of Protein Malnutrition on the DNA Content of Rat Liver [1,2,3]

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ABSTRACT The constancy of the DNA concentration in normal tissues has suggested the possibility of using this cellular component as a fixed point of reference to express the results of tissue analysis. In the field of nutrition, the constancy of this concentration in the liver has been questioned in those cases in which the rats have been fed protein-deficient diets. Some investigators who have studied the average DNA content of the isolated liver cell nucleus under conditions of protein deficiency, have not been able to show any significant increase. On the other hand, histochemical studies as well as DNA analysis of whole liver homogenates of the same type of animals have demonstrated a significant increase in the average DNA content of the nucleus and in the DNA concentration of the liver, respectively. In the present study, weanling and young adult rats were fed protein-deficient diets and the average DNA content of the isolated liver cell nuclei, as well as the DNA concentration of the whole liver homogenate, were studied. The results obtained strongly support previous observations that protein-deficient diets produce in the rat a significant increase in the average DNA content of the liver cell nucleus and in the DNA content of the liver.

It has been shown that the deoxyribonucleic acid (DNA) content of diploid nuclei in interphase is a constant quantity for all the cells of a given animal or plant species (1–4). Based on this observation, it has been proposed (3, 5–8) that the DNA concentration of a given organ be used as a point of reference to demonstrate any changes in the concentration of other cellular constituents induced by the experimental manipulation of the animal.

In the last decade, there has been much controversy in the field of nutrition with respect to the constancy of the average DNA content of the liver cell nucleus under conditions of protein deficiency. Thomson et al. (3), McIndoe and Davidson (9), Fukuda and Sibatani (10) and Campbell and Kosterlitz (11–13) have all reported that no significant increase in the average DNA content of the liver cell nuclei can be demonstrated when these organelles are isolated from the livers of rats fed protein-deficient diets. On the other hand, Ely and Rose (14) and Lecomte and DeSmul (15), using histochemical methods, have shown that a significant increase in the DNA content of the liver cell nucleus occurs when the rats are fed nitrogen-free or protein-deficient diets. Moreover, studies carried out on total liver homogenates (16–18) showed also that there is a slight increase in the DNA concentration of the liver of protein-malnourished rats.

The present study was carried out in an attempt to clarify this controversy, and because of the importance that the results may have on the elucidation of the effect of protein malnutrition on cellular composition and metabolism.

MATERIAL AND METHODS

One group of young adult rats (190 to 250 g) and one group of weanling female rats (35 to 45 g) of the Sprague Dawley strain were used in this study. Each group was divided into subgroups having a similar average weight. The animals were placed in individual cages with raised wire-screen bottoms. Four of the subgroups of adult rats were then fed each of the following diets, ad libitum, for 3 weeks: a) 20% casein (vitamin-free);[4] b) 5% zein;[5] c) 5% total corn protein (as ground yel-

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[4] "Vitamin Free" Casein, Nutritional Biochemicals Corporation, Cleveland.
[5] Obtained from Nutritional Biochemicals Corporation.

the weanling rats, no significant increase in the average DNA content of the nuclei could be demonstrated.

DNA concentration in total liver homogenates. Table 2 shows the results of the analysis of the total liver homogenates. In the group of adult rats, the corn diet, the N-free diet and the period of starvation produced a significant increase in the DNA content of the homogenates. The increase produced by the zein diet did not reach statistical significance owing to the high variance resulting.

In the group of weanling rats, both diets studied produced a significant increase in the DNA content of the liver homogenates.

The comparison between the DNA concentration in the livers of both groups studied shows that the adult rat has a higher concentration of DNA than the weanling rat. This is in accord with previous observations (8, 10).

Changes in total liver content of DNA in relation to liver weight changes. One of the outstanding effects of a protein-deficient diet is a retarded rate of growth; therefore, animals of a given age fed an appropriate diet will be heavier than animals of the same age fed a protein-deficient diet. If it is postulated that the difference between the 2 types of animals is only a matter of size and that the relative composition of the organs has remained unchanged, the ratio, total organ weight (deficient)/total organ weight (normal), should be the same as the ratio, total organ constituent (deficient)/total organ constituent (normal). If the latter ratio is found to be greater or smaller, it would be necessary to conclude that the content of the constituent in question in the organ from the deficient animal is larger or smaller than that in the organ from the normal animal. This type of calculation reveals changes in the composition of the organ with respect to the total mass regardless of any changes that might have occurred in other constituents.

The data from our experiments were calculated in this form and are presented in table 3. It is evident from these calculations that both groups of animals re-

TABLE 2

Changes in the DNA concentration in homogenates made from the livers of rats fed various rations or starved

Diet	Weanling rats		Adult rats	
	No.[1]		No.[1]	
		mg DNA/g dry tissue		*mg DNA/g dry tissue*
20% Casein	13	8.24 ±1.0[2]	15	14.60 ±2.0
5% Zein	6	11.92[3] ±1.9	13	17.75 ±2.7
5% Corn	11	9.99[4] ±2.8	12	18.95[3] ±2.0
N-free		—	12	17.00[4] ±1.0
Starvation (8 days)		—	6	23.15[4] ±2.5

[1] The number of rats in each group is cumulative from various independent replicate trials designed to be of 2 rats each.
[2] Mean ± SD.
[3] $P < 0.01$.
[4] $P < 0.05$, when compared with the 20% casein group.

TABLE 3

DNA changes in the livers of rats fed various diets or starved

Ratios[1]	5% Zein		5% Corn		N-free	Starvation
	Adults	Weanling	Adults	Weanling	Adults	Adults
Liver wt (exp.) / Liver wt (control)	0.66 ±0.03[2]	0.32 ±0.03	0.71 ±0.19	0.62 ±0.10	0.59 ±0.06	0.59 ±0.06
Total DNA (exp.) / Total DNA (control)	0.71[3] ±0.02	0.37[3] ±0.10	0.81[4] ±0.10	0.76[4] ±0.10	0.77[4] ±0.21	0.86[4] ±0.12

[1] The total number of animals studied in each group was 6.
[2] Mean ± SD.
[3] $P = 0.01$.
[4] $P \leqq 0.05$, when compared with the liver ratio.

sponded to the dietary treatment with a significant increase in the DNA content of the liver.

DISCUSSION

The values found for the average DNA content of nuclei isolated from normal adult rats (10.8 ± 2.5 μμg/nucleus) agree with values reported previously in the literature (3, 5, 9, 11, 14, 18). The average DNA content of nuclei isolated from the liver of the weanling control group was 12.1 ± 0.73 μμg/nucleus, in contrast with the values of 6.7 to 7.2 μμg/nucleus reported by Thomson et al. (3). This discrepancy might be explained by the fact that during the 3-week experimental period the weanling control group increased from 30 g to about 100 g of body weight, and it has been shown by Fukuda and Sibatani (10) that the average DNA content of the rat liver nucleus increases from 5.9 to 11.2 μμg/nucleus when the body weight increases from 25 to 100 g.

Table 4 summarizes some of the data available in the literature on the effect of protein deficiency on the DNA content of the liver cell nucleus. It is evident from this table that except for the results of Ely and Ross (14), the other investigators who studied isolated nuclei were unable to demonstrate any change in the DNA content of the protein-deficient organelles. In a previous publication (24), it has been shown that the use of the Waring Blendor for homogenization abolishes the difference between the DNA content of normal and of protein-deficient nuclei. Therefore, it can be concluded that the negative results obtained by the investigators cited in table 4 should be ascribed to the type of blender used for homogenization.

The histochemical studies of Ely and Ross (14) and Lecomte and De Smul (15), as well as the DNA determinations of Cooper (16), Muntwyler (17) and Villela (18), showed an increase in the DNA content of the liver cell nuclei and the liver homogenates of protein-deficient rats. These results are in good agreement and are in confirmation of our results.

In the weanling group, it was not possible to show any increase in the average DNA content of the isolated nuclei even

TABLE 4

Summary of the data in the literature on the effect of protein deficiency on the average DNA content of the liver cell nuclei

Reference	Wt of rat	Diets				Method
		Normal	N-free	Protein-deficient	Starvation	
	g	*μμg DNA/nucleus*		*μμg DNA/nucleus*		
(9)	—	8.70			9.27	citric acid, Waring Blendor
(10)	200	11.20			11.20	citric acid, Waring Blendor
(14)	130–160	10.20	12.0	16.70 [1]		citric acid, Waring Blendor
(2)	—	8.0			8.0	citric acid, Waring Blendor
(3)	195–250	9.28	9.90		10.26	citric acid, Waring Blendor
(11)	325	10.8	11.0			citric acid, Waring Blendor
(15)	60–65	582.4 [2]		638.6 [2,3]		histochemical
(14)	130–160	0.723 [4]	1.090 [4]	0.961 [1,4]		histochemical

[1] 12% casein.
[2] Arbitrary units.
[3] 3.2% casein.
[4] Extinction coefficients.

though the DNA concentration of the liver homogenates and the calculated ratios of total DNA content and of liver weight between groups clearly indicated an increase in the DNA content of the livers. The reason for this discrepancy has not been found.

The mechanism of the increase in the DNA content of the livers of rats fed protein-deficient diets cannot be ascertained from the data presented. Nevertheless, if it is accepted that this increase could be ascribed to the presence in the livers of malnourished rats of a greater proportion of tetra- and octaploid-type of nuclei as suggested by Cunningham et al. (25) and Lecomte and De Smul (15), it would be necessary to conclude that under the conditions of protein deficiency used, the biosynthesis of DNA was not affected, whereas general protein synthesis was impaired in such a way that the mitotic process was blocked.

From the practical point of view, the increase in the DNA content of the liver of malnourished rats impedes its use as a reference point to express the liver composition in situations of protein deficiency.

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