THE EFFECT OF PROTEIN DEFICIENCY ON THE DISTRIBUTION OF NUCLEAR VOLUMES IN PREPARATIONS OF RAT LIVER NUCLEI[1]

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The effect of a protein-deficient diet and the subsequent recovery period on the distribution of nuclear volumes has been studied. The results support the hypothesis that protein deficiency produces an increase in the proportion of polyploid-type nuclei in the liver as reflected in an increase in the average content of DNA per nucleus. This increase in polyploidy was shown to occur early in the experimental period, suggesting that it represents part of an adaptation mechanism more than a secondary effect produced by the deficiency of amino acids. Throughout the study, it was not possible to demonstrate any relationship between patterns of nuclear volume distribution or the average nuclear volume, and the average DNA content per nucleus. On this basis, it has been suggested that the nuclear volume might not be a unique function of the degree of ploidy, but most probably is an expression of the protein content of the organelle.

Introduction

The studies of Falzone, Barrows, and Yiengst (1), Santen (2), and Umaña (3) have shown that rat liver nuclei can be separated into two different populations when suspended in acid medium (pH 2.5). One population is made up of small stromal, mostly diploid nuclei, and the other of large parenchymal mostly polyploid nuclei. The ratio between these two populations in the normal adult rat (about 250 g body weight) was found to be very close to 1.

Protein deficiency of different types has been shown to produce an increase in the concentration of DNA in the liver of malnourished children and rats (4–8). This increase was shown to be the combined effect of a reduced cytoplasmic content and, most probably, an increased proportion of polyploid-type nuclei. Since the increase in polyploidy under these conditions might be the manifestation of an important adaptive mechanism, it was decided to search for new direct experimental evidence in support of the hypothesis of increased polyploidy in the liver in response to a limited supply of amino acids in the diet. The data presented in this article have been interpreted as indicating that polyploidy can, indeed, be considered as an early adaptive mechanism to the protein-deficient diet.

Methods

Seven groups of five male rats (200 g) of the Sprague–Dawley strain were used for the experiments. One group was killed the first day of study (zero time control), and the rest were fed a diet containing 5% corn protein prepared as

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TABLE I

Effect of a 10-day period on a protein-deficient diet and the subsequent recovery on the volume distribution of rat liver nuclei*

Type of diet	Time (days)	Nuclear diameter (μ)						Mean nuclear volume (μ^3)	Mean DNA content ($\mu\mu$g/ nucleus)
		4	6	8	10	12	14		
		% distribution of nuclear volumes							
20% casein (control)	0	10.1±5.2†	20.9 ±5.0	17.3±8.0	31.5 ±5.5	14.8 ±6.8	5.1±3.7	445 ± 8.4	10.3 ±1.6
5% corn	3	11.0±2.1	19.1 ±5.9	13.9±6.6	33.4 ±8.2	16.8 ±10.0	5.7±3.4	472§±10.0	13.3§±1.7
5% corn	5	5.8±3.1	19.2 ±4.3	21.9±8.5	43.0§±5.9	8.2 ±3.3	1.5±1.7	404§± 4.0	15.2§±1.3
5% corn	10	8.5±0.8	15.3‡±2.2	21.8±9.1	41.1‡±6.3	10.7 ±8.0	2.4±2.0	426§± 6.6	13.5‡±2.9
20% casein	3	7.3±3.2	16.8 ±4.5	15.7±3.8	41.5 ±3.6	13.9 ±2.7	4.5±1.8	471¶± 3.2	13.6
20% casein	6	7.6±1.2	22.7‖±1.7	12.6±5.9	26.3¶±6.0	26.5¶±5.2	4.2±2.0	499¶± 4.4	12.9 ±0.6
20% casein	12	5.6±2.4	17.1 ±4.3	17.2±8.1	34.3 ±5.9	21.6‖±5.1	4.1±1.6	502§¶± 4.5	10.0‖±1.1

*Nuclei isolated in dilute citric acid (pH 3.6) with the ball-type homogenizer.
†Standard deviation.
‡$P \leqslant 0.005$ with respect to the control group.
§$P \leqslant 0.001$ with respect to the control group.
‖$P \leqslant 0.005$ with respect to the group fed the corn diet for 10 days.
¶$P \leqslant 0.001$ with respect to the group fed the corn diet for 10 days.

described elsewhere (4). Three of the groups were killed on the 3rd, 5th, and 10th days on this diet; then, the remaining three groups were changed to a 20% casein diet (4) and killed on the 3rd, 6th, and 12th days after the change.

Nuclei were isolated from the livers of the animals in dilute citric acid (pH 3.6) as described in the previous article (3). The counting of the total number of nuclei, the determination of the DNA content per nucleus, the measurement of the nuclear diameters, and the group distribution of the nuclei were carried out as described in the previous article (3).

The statistical significance of the difference between means was calculated by the *t* test.

Results

The data presented in Table I shows the effect of the corn diet and the subsequent recovery period on the distribution of nuclear volumes, the average nuclear volume, and the average DNA content of the nuclei. After 3 days on the protein-deficient diet, there was a significant increase in the average DNA content per nucleus and in the average nuclear volume, without any changes in the nuclear volume distribution. After 5 days, a significant increase in the 10-μ nuclei was already evident as well as a decrease in the 12- and 14-μ nuclei. This change in volume distribution was reflected in a significant decrease in the average volume of the nuclei. The pattern found after 10 days on the corn diet did not change significantly from the one found for the group studied after 5 days on this diet, indicating that a certain degree of stabilization had been reached.

Three days after the animals were started on the casein diet, the average nuclear volume increased significantly above the control value. After 6 days on the casein diet, the average nuclear volume increased still further and the proportion of 10-μ nuclei decreased drastically to levels below the control values. These changes were accompanied by a significant increase in the proportion of nuclei 6 and 12 μ in diameter, without any significant change in the average DNA content per nucleus. At the end of the recovery period, the volume distribution of the preparation was very similar to that of the control group, except for a significantly higher proportion of the 12-μ nuclei and a nonsignificant decrease in the smaller 4-μ nuclei. These minor differences in volume distribution resulted in a significantly higher average nuclear volume, in the presence of normal DNA values per nucleus.

Discussion

One of the effects of protein malnutrition on the liver is a significant increase in the concentration of DNA (4, 5). The cause of this increase can be traced to the diminished cytoplasmic content of the hepatocytes (6) and, in the case of adult animals, to an increase in the average DNA content per nucleus (4, 5, 7–9). The studies of Falzone *et al.* (1), and of Santen (2) have provided evidence that liver nuclei with a diameter larger than 6.5 μ are of parenchymal origin and are mostly polyploids. Therefore, our results can be interpreted as providing

further experimental support for the hypothesis that the increase in the average DNA content per nucleus is due to an increase in the proportion of polyploid-type nuclei. The fact that this phenomenon can be shown relatively early in the experimental period (3 days) suggests that this is more an adaptation mechanism than a secondary effect. The metabolic significance of this mechanism is difficult to determine, mainly because of the obscure significance of polyploidy in the liver. Nevertheless, it could be speculated that nuclei with higher than the diploid amount of DNA might be able to synthesize more RNA per unit time than nuclei containing a diploid amount and, therefore, that this situation would greatly increase their efficiency in the synthesis of protein. Following this line of thought, it might be possible to interpret the increased proportion of polyploids in the malnourished liver as resulting from a mechanism by which the organ tends to increase its efficiency of synthesizing vital proteins. This idea might find some support in the fact that during the recovery period, in which the cells appear to be replenishing their protein stores after the period of protein deficiency, the average DNA content per nucleus remains elevated for a period of 6 days.

The significant decrease in the average nuclear volume produced by protein deficiency, in the presence of an increased DNA content, had been previously described by Ely and Ross (7), and Umaña and Tejada (6). At the time of these reports, it was not possible to give an appropriate explanation of this phenomenon. In the light of the data presented in Table I, it is now possible to ascribe this change in volume to a variation in the proportion of the different nuclear sizes, which evidently can occur independently of the degree of ploidy of the organelles.

The dependence of the average nuclear volume on the proportion of the different types of nuclei, and the possibility of significant changes in this measure without a concomitant and significant redistribution of the nuclear volumes and (or) changes in DNA content, stands against a direct correlation between this measure and the degree of ploidy of the nuclei. The fact that, without any significant changes in DNA, the proportion of nuclei 12 and 14 μ in diameter, and with it the average nuclear volume, increased significantly during recovery would suggest instead that these changes could be more easily explained in terms of protein gain. This interpretation appears more likely in view of observations that the nuclear volume, without any simultaneous change in the DNA content, has been shown to be affected by different hormones (10–13), the stage in the cell cycle (14, 15), and, in general, the physiological activity of the cell (16–18).

The return of the average amount of DNA per nucleus to normal values at the end of the 12-day recovery period would have to be explained by a conversion of polyploids to diploids, most probably through an immediate mitotic division of the cells carrying this type of nucleus.

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