THE EFFECT OF THE METHOD OF NUCLEI ISOLATION ON THE AVERAGE DNA CONTENT OF THE NUCLEUS[1]

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A difficult problem encountered in tissue analysis is the method of expression of the analytical results. The demonstration of the constancy of the average DNA content of diploid nuclei in interphase in all the cells of the tissues of animals and plants suggested the possibility of using the DNA concentration as a fixed point of reference that could be used to express the composition of a given tissue.

In the field of nutrition there exists the controversy about whether or not

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protein-deficient diets increase the average DNA content of the liver cell nucleus and, therefore, the absolute DNA content of the liver.

A number of the investigators (1–5) who have studied the average DNA content of the liver cell nuclei isolated from the livers of rats fed a protein-deficient diet have been unable to show any significant increase in the DNA content. A common fact about all of these studies is that homogenization of the livers has been carried out with the Waring Blendor. On the other hand, histochemical studies (6, 7) and DNA analysis of whole homogenates (8–10) have shown a significant increase, under conditions of protein deficiency, in the average DNA content of the isolated nuclei and in the DNA concentration of the liver homogenates.

Since the Waring Blendor has been shown to cause much destruction of intracellular organelles, it seemed possible that the failure to demonstrate any increase in the average DNA content of nuclei isolated from the livers of protein-deficient rats might be due to an artifact produced by too drastic a method of homogenization. The purpose of the present study is to show the effect of the Waring Blendor on the average DNA content of nuclei isolated from normal and protein-deficient rats.

Methods

Young adult rats (190–250 g) of the Sprague–Dawley strain were divided into two groups: group 1 was fed a diet containing 20% casein (vitamin-free casein, Nutritional Biochemical Corporation) and group 2 consumed a diet containing 5% zein (Nutritional Biochemical Corporation) as the sole protein source. The other constituents of these diets were the following: cottonseed oil, 10 ml; cod-liver oil, 1 ml; minerals (11), 4 g; and enough cornstarch to complete 100 g of diet. A vitamin solution (4 ml) was added to every 100 g of the diets. The composition of this vitamin solution has been given elsewhere (12).

All the animals were allowed free access to tap water and were kept on the experimental diets for 3 weeks. At the end of this period the animals were decapitated, bled, and the livers removed. Four of the livers obtained from each dietary group were used for the isolation of nuclei in dilute citric acid by the method of Dounce (13), using the Waring Blendor for homogenization. Eight of the livers in each dietary group were used for the isolation of nuclei by the same method, except that the homogenates were made with a ball-type homogenizer (14).

DNA was extracted from the isolated nuclei by the method of Schneider (15) and determined colorimetrically by the diphenylamine reaction of Dische (16). The total number of nuclei in each preparation was determined by direct counting of a very dilute suspension of nuclei in 0.05 M citric acid in a Levy-type hemocytometer chamber. Three freshly loaded chambers were counted for each nuclei preparation. The total number of nuclei counted was about 1800 per preparation and the reproducibility of the countings was within 5% or less.

The results were expressed as $\mu\mu$g of DNA per nucleus. The statistical significance of the difference between means was determined by the *t* test.

Results and Discussion

The average DNA content of the nuclei studied is presented in Table I. It is evident that, when a gentle method of homogenization (ball-type homogenizer) was used, the data obtained indicate that the protein-deficient diet

TABLE I

Effect of the method of homogenization on the average DNA content of the liver nuclei*

Group	Diet	No. animals	Ball-type homogenizer ($\bar{x}\pm$S.D.)	No. animals	Waring Blendor ($\bar{x}\pm$S.D.)
1	20% casein	8	10.78±2.5	4	11.11±1.21
2	5% zein	8	15.50†±1.6	4	9.51±0.8

*Results expressed as $\mu\mu$g/nucleus.
†$P < 0.01$ when compared with the casein group.

caused a significant increase in the average DNA content of the nuclei. No changes in the average DNA content of the nuclei isolated from the livers of the malnourished rats could be demonstrated when the Waring Blendor was used for homogenization.

The results previously presented clearly indicated that protein malnutrition causes a significant increase in the average DNA content of the liver cell nuclei, as was suggested by the studies of Ely and Ross (7) and Lecomte and De Smul (6). The negative results obtained by several other investigators (1–5) who have studied this problem could be due to the use of the Waring Blendor for homogenization.

The histochemical studies of Cunningham *et al.* (17) and Lecomte and De Smul (6) suggested that any increase in the average DNA content of nuclei should be ascribed to an increase in the polyploid population and not to an increase in the individual DNA content of each nucleus. One possible explanation for the low values of the DNA content of nuclei that were isolated with the Waring Blendor from the livers of protein-deficient rats might be found if the following postulates are accepted: (*a*) protein malnutrition causes an increase in the polyploid cell population of the liver, as is suggested by the increase in the average DNA content of the liver cell nuclei of protein-deficient rats according to Cunningham *et al.* (17) and Lecomte and De Smul (6); (*b*) the use of the Waring Blendor for homogenization destroys preferentially the polyploid population of nuclei due to a greater susceptibility of this type of nuclei to mechanical trauma, as is suggested by Rose and Schweigert (18); and (*c*) this selective destruction of a great part of the polyploid population of nuclei will increase the proportions of diploid-type nuclei having a normal amount of DNA.

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