BRIEF NOTES

MALNUTRITION: INCORPORATION OF THYMIDINE-3H INTO NUCLEAR AND MITOCHONDRIAL DNA

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INTRODUCTION

Protein-calorie malnutrition or fasting leads to a readily detectable decrease in the rate of body growth and cellular proliferation, particularly in young animals (32, 33). In contrast, disorders in cell and organ function have generally been more difficult to demonstrate, perhaps partly due to limitations in methodology (8, 16, 22). Mitochondria are the primary energy source for the work of differentiated cells. Normally, mitochondria continue to proliferate even in non-dividing cells, presumably under the regulation of their own DNA (2, 17, 23). If energy-requiring cell functions are favored over cell proliferation in undernourished animals, the renewal of mitochondria might continue even if cell division is decreased. Comparative rates of DNA production in the nuclei and in the mitochondria of hepatocytes were studied to test the hypothesis that malnutrition might have different effects on the synthesis of DNA in these two intracellular sites.

MATERIALS AND METHODS

All experiments were performed with female albino rats of the Sprague-Dawley strain fed either a stock pelleted diet (Berkeley diet rat and mouse food, Feedstuffs Processing Company, San Francisco) or a semisynthetic diet containing 3.5% or 26% protein (General Biochemicals, Div. North American Mogul Products Co., Chagrin Falls, Ohio: Protein Test Diet, Low (No. 170580), 3.5% protein; and Protein Test Diet, Normal (No. 170390), 26% protein). Animals were killed by decapitation. Liver mitochondria were isolated as previously described (29) by differential centrifugation, washed four times and

purified on a sucrose density gradient to reduce nuclear contamination as described by Neubert et al. (19). Nuclei were isolated by the method of Blobel and Potter (3). DNA was extracted from nuclear and mitochondrial fractions according to Schneider (27). Samples of the extract were used for DNA determination by the method of Burton (5). Purification of mitochondrial fractions by extensive washing followed by density gradient centrifugation may account for concentrations of mitochondrial DNA somewhat lower than in earlier reports (18, 28), namely between 0.25 and 0.50 μg/mg mitochondrial protein, adult animals being near the lower end of this range. For measurement of radioactivity, samples of extract were mixed with 1.0 ml of NCS solubilizer (Nuclear-Chicago Corporation, Des Plaines, Ill.) and toluene-based scintillation mixture and analyzed for ^{3}H at about 40% efficiency. Essentially no differential quenching among samples was detected either by a channel-ratio method or by addition of an internal standard. Incorporation of thymidine-^{3}H into DNA is essentially complete after 1 hr (14, 19). However, all animals were fasted 16 hr before killing since fasting was found to be essential to ensure a consistent yield of mitochondria. The interval between isotope injection and killing is noted in the table and figure legends.

RESULTS AND DISCUSSION

Data in Table I show that in 30-day old rats fasted for 48 hr before injection of thymidine-^{3}H, nuclear DNA (N-DNA) has less than 4% of the specific activity present in the fed control animals. In contrast, the specific activity of mitochondrial DNA (M-DNA) is somewhat greater in the fasted than in the fed rats. Similar changes in mitochondrial incorporation of thymidine-^{3}H are

TABLE I

Incorporation of Thymidine-³H into DNA of Mitochondrial and Nuclear Fractions of Rat Liver after Fasting or a Low-Protein Diet

Young female rats, 30 days old (50–60 g) were fasted or allowed free access to a complete diet for 2 days before intraperitoneal administration of 1.5 μCi/g thymidine-³H (6 Ci/mmole). Adult female rats, >150 days of age (300–350 g), were given the same dose of isotope after a 3 day period of fasting or access to a complete diet (group 1), and 2 wk of a diet containing either 3.5% or 26% protein (group 2). All animals were fasted for 16 hr after isotope administration and before killing. Number of samples and standard errors of the means are indicated.

| | cpm/μg DNA | | |
	Mitochondrial	Nuclear	Mitochondrial/nuclear
Young:			
Fasted (6)*	61.7 ± 2.3‡	1.9 ± 0.2‡	34.8 ± 3.5
Fed (6)*	46.1 ± 3.1	57.1 ± 9.2	0.9 ± 0.16
Adult: group 1			
Fasted (6)	51.3 ± 4.3§	3.4 ± 0.3‖	15.4 ± 1.9
Fed (5)	31.2 ± 4.8	6.0 ± 1.8	9.0 ± 3.3
group 2			
3.5% Protein (3)	105.8 ± 6.1§	7.2 ± 1.4‖	16.5 ± 4.8
26 % Protein (3)	60.3 ± 8.5	13.8 ± 2.8	4.7 ± 0.9

* Total of 18 rats, 3 livers per sample.
‡ $P < 0.002$.
§ $P < 0.02$.
‖ $P < 0.05$.

present in each of two groups of adult rats. However, nuclear incorporation in control adult rats is much lower than in young animals. Consequently the decrease in ratio of M-DNA/N-DNA specific activity as a result of fasting is more profound in the rapidly growing young rats than in the slowly growing adult.

The studies of Neubert et al. (19) have shown that the ratio of M-DNA to N-DNA production in the liver changes strikingly during normal development. In newborn rats the specific activities of M-DNA and N-DNA after administration of thymidine-³H were nearly equal. In old rats, whose liver cells have essentially ceased to proliferate, the specific activity of M-DNA was found to be 50 times greater than that of N-DNA. The extent to which these results and ours are a function of non-homogeneous subcellular precursor pools remains unknown, primarily because reliable methods for measuring these are not yet available. Neubert's results, however, are consistent with the gradually slowing rate of accumulation of total DNA, which is about 99% nuclear, during liver development (9). The results are also in accord with the rapid

rate of renewal of M-DNA and other mitochondrial components in the adult estimated with radioactive isotopes (1, 12, 13, 19). Mechanisms for independent regulation of M-DNA and N-DNA synthesis are suggested by the existence of a mitochondrial DNA polymerase distinct in properties from the nuclear enzyme (15).

While malnutrition has contrasting effects on incorporation of thymidine-³H into M-DNA and N-DNA, it is uncertain whether the rate of synthesis of M-DNA is greater than in control animals. Significant differences in thymidine pool size might be anticipated as a consequence of diet and can influence incorporation data. If M-DNA were synthesized at an increased rate in the malnourished animals, M-DNA per liver or per gram of tissue would be increased unless the rate of degradation of M-DNA were also accelerated. There was no evidence of increased M-DNA per milligram mitochondrial protein or per liver in the undernourished groups, assuming similar yields of mitochondria isolated in cell fractionation.

The turnover of M-DNA was studied in rats fed 3.5% or 26% protein in their diet. Adult rats
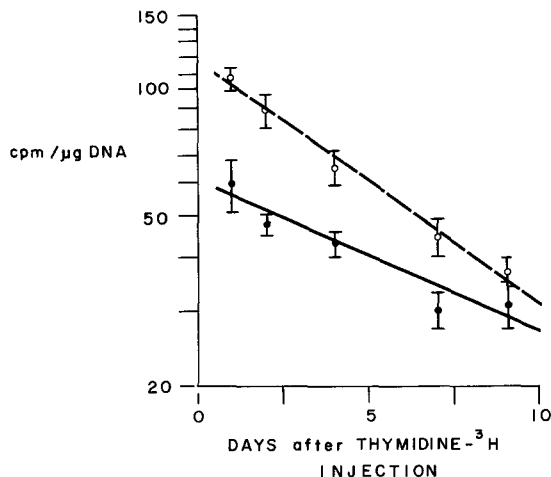
FIGURE 1 Half-life of mitochondrial DNA. Adult female rats, >150 days of age, were given either a 26% protein diet or a 3.5% protein diet for 2 wk. They were then fasted for 3 hr before and after a single intraperitoneal injection of thymidine-^{3}H (6 Ci/mmole), 1.5 μCi/g body weight. Animals were killed after a 16 hr fast 1, 2, 4, 7, and 9 days after isotope administration and resumption of the same diet. At 2 and 9 days, each point represents the mean of six samples with SE of the mean, one liver per sample. Other points represent the mean and SE of three samples. Half-life was 4.9 days in the 3.5% protein group (O) and 7.0 days in the 26% protein group (●).

were used in order to minimize errors introduced by corrections based on estimated changes in liver weight. Adult female rats were given the experimental diets for 2 wk and then fasted for 3 hr before and after a single intraperitoneal injection of thymidine-^{3}H, 1.5 μCi/g body weight. Three to six animals in each group were killed after a 16 hr fast 1, 2, 4, 7, and 9 days after isotope administration (one animal per sample). Between the 1- and 9-day points, body and liver weights changed less than 3%. The half-life of M-DNA was determined from a semilogarithmic plot of counts per minute per microgram M-DNA (Fig. 1). The turnover rate (half-life) in the low-protein group was 4.9 days compared with the control value of 7.0. The value for the 26% protein group is similar to turnover figures for M-DNA in rat liver reported from other laboratories (12, 19). However, the figures must be regarded as tentative since gradient purification of mitochondria does not exclude the possibility of slight nuclear contamination. In addition, overestimation of half-life due to varying degrees of isotope reutiliza-

tion in the two groups is a distinct possibility as in studies of N-DNA (6). Thus, both the incorporation and decay experiments described indicate normal or possibly increased rates of production and destruction of mitochondria in the hepatocytes of protein-deficient rats. The latter possibility has also been proposed by other investigators on the basis of an abundance of autophagic vesicles often containing mitochondria, in ultrastructural studies of hepatocytes, in malnourished animals (7, 10, 20, 30). It is also consistent with the observation of "dividing" mitochondria, particularly in ultra-structural studies of liver in fasting and protein-deficient rats (11). However, turnover studies indicate that the effects of diet on renewal of cellular protein vary with the type and duration of malnutrition and the protein or group of proteins studied (21, 24–26, 31).

The effect of feeding the normal protein diet after a period on the low-protein regimen was studied in an additional group of adult rats. Animals were given the 3.5% protein diet for 2 wk and were then fed the 26% protein diet. After intervals of 1–8 days, they were given thymidine-^{3}H intraperitoneally and then killed after a 16 hr fast for determination of incorporation of thymidine-^{3}H into M-DNA and N-DNA. Fig. 2 shows the results of a representative experiment. After initiation of the 26% protein diet a marked increase in the specific activity of N-DNA is present after 1 day, followed by a rapid drop toward control values. This suggests a brief burst of cell proliferation after the diet change, similar in timing to that described by Bucher et al. (4) after partial hepatectomy. The pattern of incorporation of thymidine-^{3}H into M-DNA is strikingly different from that of thymidine-^{3}H into N-DNA. The specific activity of M-DNA is initially elevated compared with control values. After change to the 26% protein diet, there is a steady decrease until the control range is reached after 2–4 days.

This series of experiments indicates that a change in nutritional conditions can have contrasting effects on the synthesis of N-DNA and M-DNA. The unabated incorporation of thymidine-^{3}H into M-DNA and the loss of label from M-DNA at a normal or greater than normal rate under certain conditions of malnutrition indicate that mitochondria proliferate rapidly even though the rate of cell proliferation is depressed, particularly in the growing animal.
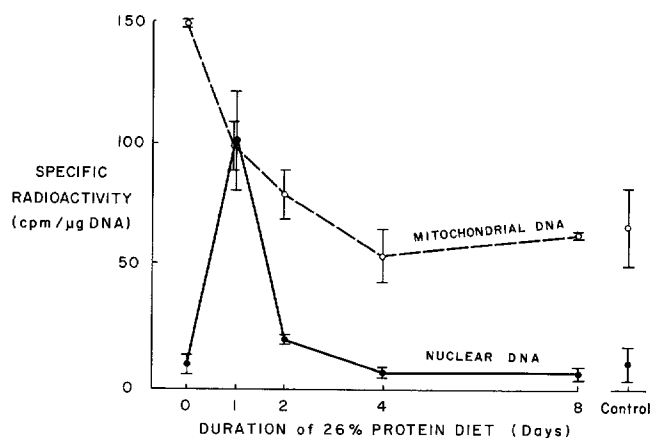
FIGURE 2   Incorporation of thymidine-^{3}H into DNA of mitochondrial and nuclear fractions of rat liver. Adult female rats, >150 days of age, were given a 26% protein diet after 2 wk of a 3.5% protein diet. They were injected intraperitoneally with 1.5 µCi/g thymidine-^{3}H (6 Ci/mmole) after 2 wk of the 3.5% protein diet and 1, 2, 4, and 8 days after change to 26% protein diet. Control animals were injected after 2 weeks of ingestion of the 26% protein diet. All animals were fasted for 16 hr after isotope administration and before killing. Each point represents the mean of two samples whose range is indicated.

The apparently contrasting effects of malnutrition on DNA synthesis in two intracellular sites within the same cell population may favor energy-requiring functions over proliferation when availability of substrates from dietary sources is diminished.

SUMMARY

Rats given a low-protein diet or fasted for 2–3 days incorporate increased thymidine-^{3}H into hepatic mitochondrial DNA compared with animals given a complete diet; in contrast, incorporation into hepatic nuclear DNA is decreased, particularly in young animals. These apparently contrasting effects of undernutrition on DNA synthesis may favor energy-requiring cell functions over cell proliferation.

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