A ROLE FOR AMINO ACIDS IN THE INDUCTION OF DEOXYRIBONUCLEIC ACID SYNTHESIS IN LIVER

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SUMMARY

Nuclear DNA synthesis and mitosis are induced in the parenchymal liver cells of unoperated rats after a shift from a protein-free to a protein- or amino acid-containing diet. Protein or amino acids in the preparatory mash block the responses of the liver to the second diet. The formation of liver DNA after the nutritional shift begins several hours earlier than after partial hepatectomy. A small number of changes in the hepatic levels of amino acids and other ninhydrin-positive compounds result from the nutritional shift and some of these changes occur as well after 70% hepatectomy and the infusion of intact rats with the TAGH solution.

To learn about the stimulus to hepatic nuclear DNA replication and cell multiplication, efforts were made to induce the processes in the intact organ rather than by partial hepatectomy. An infusate (TAGH solution: triiodo-L-thyronine, amino acids, glucagon, and heparin) was devised that raises the levels of fatty and amino acids in blood and that stimulates DNA formation and mitosis in the parenchymal cells of the intact liver (1).

A means has now been found for stimulating hepatic DNA synthesis and mitosis in the unoperated animal without the injection of hormones. Leduc (2) had described a wave of mitotic activity in liver within a day after she shifted intact mice from a low- to a high-protein diet. We find that hepatic DNA replication and nuclear division can be induced in intact rats by shifting them from a protein-free diet to a diet containing protein or amino acids.

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The results suggest that amino acids play a major role in regulating nuclear DNA replication and cell multiplication in liver.

EXPERIMENTAL PROCEDURE

Amino acids, the components of the protein-free diet (cellophane spangles, 12%; glucose, 12%; corn starch, 60%; "Vitamin Fortification Mix", 2%; "Salt Mix, U.S.P. XIII No. 2", 4%; and corn oil, 10%), and proteins were from General Biochemicals. Supplementation of the protein-free diet with protein or amino acids was at the expense of the corn starch. Female albino rats, Fischer 344 (150 g), obtained from Microbiological Associates, were freely given food and water except as indicated. DNA synthesis was measured by the incorporation of (^{3}H) thymidine (New England Nuclear) as previously described (1). Radioautography of histological sections was with Kodak Nuclear Track Emulsion, type NTB3.

RESULTS

Stimulation of Liver DNA Synthesis and Mitosis by a Dietary Manipulation - Hepatic DNA synthesis was induced in intact rats by shifting them from a protein-free diet to a diet containing casein (Fig. 1). As the figure shows, the extent of stimulation was related to the level of protein. In separate experiments, it was found that lactalbumin hydrolysate and egg white were as active as casein and that a mash containing 24% of an equimolar mixture of glycine and L-amino acids (diets with higher concentrations of amino acids were not eaten well) was almost as effective as the 24% casein diet. Zein, lacking tryptophan and lysine, was by itself completely inactive, and it induced little DNA formation when it was supplemented with only one of the amino acids. Supplementation with both amino acids, however, provided a similar response to that with casein.

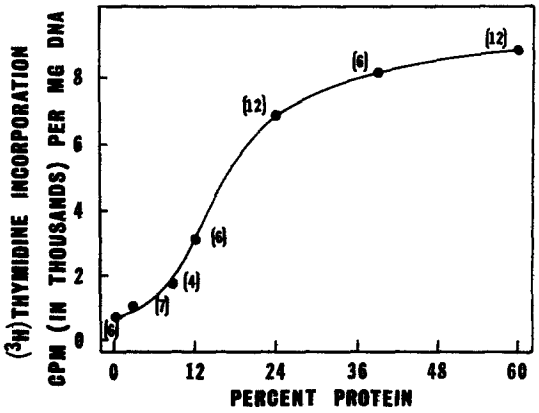

Fig. 1. Stimulation of liver DNA synthesis in intact rats after a shift from a protein-free to a protein-containing diet. Rats that had been freely fed pellets of Purina Laboratory Chow (24% protein) were given a protein-free mash for 3 days. At the end of this time (8 AM), food was removed and, at 4 PM, the animals were given mashes containing the indicated contents of casein. The following morning (8 AM), each rat received 5 μCi of (^{3}H) thymidine in the tail vein and liver samples were taken 1 h later. The specific activity of nuclear DNA was estimated as previously described (1). Each point represents the average of the results obtained with 4 to 12 rats as shown. The average specific activity of liver nuclear DNA of animals that were maintained on the Purina Chow was 570 cpm/mg DNA.

At least 3 days on the protein-free diet were required for a maximal response to a 50% casein diet. Thus, the specific activities of hepatic nuclear DNA (5 μCi of (^{3}H) thymidine at 17 h after the nutritional shift) as a function of time spent on the protein-free diet were as follows: 1 day, 3700; 2 days, 5400; 3 days, 10,500; 5 days, 8900; 8 days, 9100; and 15 days, 9300 cpm/mg DNA.

The possibility that the uptake of thymidine after the nutritional shift was for the repair of DNA that had been damaged during the period of protein deficiency was ruled out by counting labeled liver nuclei and mitotic cells. The incorporation of (^{3}H) thymidine after the nutritional shift, just as after partial hepatectomy, was proportional to the number of parenchymal nuclei that were forming DNA. Thus, about 15- and 50-fold rises in DNA synthesis

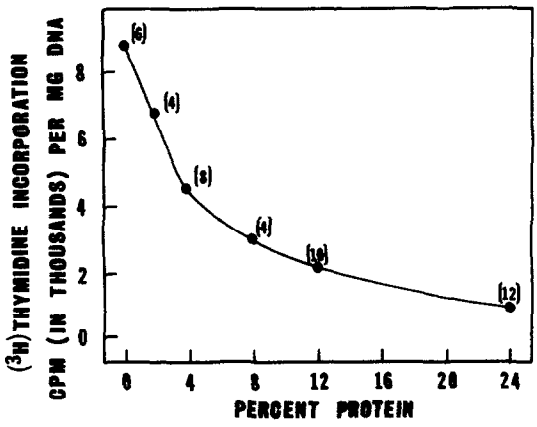

Fig. 2. Effect of adding protein to the preparatory mash on the stimulation of liver DNA synthesis by a high-protein diet. The animals were freely fed mashes containing the indicated concentrations of casein for 3 days. At the end of this time (8 AM), food was removed and, at 4 PM, all the rats were given a mash with 50% casein. The following morning (8 AM), each rat received 5 μCi (^{3}H) thymidine in the tail vein and liver samples were taken 1 h later and assayed as for Fig. 1. Each point represents the average of the results obtained with 4 to 12 rats as shown.

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were found after the change in diet and partial hepatectomy, respectively, as measured both by the labeling of DNA and by radioautography of histological preparations. In addition, with tissue that was exposed for a short time (6 days), the average number of grains over the labeled nuclei were the same for the nutritionally shifted and partially hepatectomized animals.

The nutritional shift caused a large increase in the number of mitotic hepatocytes in the intact liver (no mitotic trapping agent was used). With animals kept on Purina Chow or on the protein-free diet (4 days), less than 0.01% of the parenchymal liver cells were in mitosis whereas the switch from the protein-free mash to the 50% casein mash produced 0.3 to 0.4% of mitotic cells (19 h after the switch). The fact that more than 45% of the mitotic cells were labeled at 3 h after the injection of (^{3}H) thymidine was taken to mean that the entire nuclear genome had been replicated.

Liver DNA synthesis after the nutritional shift began several hours sooner than after 70% hepatectomy. The specific activities of nuclear DNA (5 μCi of (^{3}H) thymidine for 1 h beginning at the times indicated) as a function of time after the animals were shifted to a 50% casein mash were as follows: 6 h, 930; 8 h, 2200; 10 h, 5100; 12 h, 7800; 16 h, 9400; 20 h, 8200; and 28 h, 7100 cpm/mg DNA. After 70% hepatectomy, on the other hand, no increase in thymidine incorporation was detected until 14 h after the operation and the peak of incorporation was reached at about 24 h.

Effect of Adding Protein or Amino Acids to the Preparatory Mash - Addition of casein to the first diet reduced the later response of the liver to the high-protein mash (Fig. 2). The figure shows that animals fed a preparatory diet with 24% casein gave little stimulation of (^{3}H) thymidine incorporation upon being shifted to a 50% protein diet. The figure does not show that 24% of a mixture of 16 amino acids (aspartate, glutamate, asparagine, and glutamine were omitted) was just as effective as 24% casein in suppressing the later response of the liver to the 50% protein diet. To learn whether all amino acids were equally inhibitory, the 16 amino acids were divided into various groups of 4 compounds and the mixtures were tested at a level of 6% of the preparatory diet. One of the mixtures (L-methionine, L-phenylalanine, L-serine, and L-tryptophan) was as inhibitory as 24% casein whereas others (for example, L-cystine, L-methionine, L-serine, and L-threonine) were without effect.

Hepatic Levels of Amino Acids and Derivatives - To compare some of the pool sizes of free amino acids and other ninhydrin-positive compounds in liver after 70% hepatectomy with those in unoperated animals shifted from a protein-free to a protein-rich diet or infused with the TAGH solution (1),

Table I

Hepatic levels of some amino acids and derivatives

For the treatment designated as "50% protein diet", the animals were fed a protein-free mash for 2 days and were then shifted to a 50% casein mash for 15h. The TAGH solution was infused into the tail vein as previously described (1). Liver samples were removed at the indicated times after surgery or the beginning of treatment. The animals were lightly anesthetized with ether and, to minimize postmortem changes in liver pools, the livers were frozen in situ with liquid nitrogen. The frozen livers were weighed and then powdered in liquid nitrogen, the powders were homogenized in 5% $HClO_4$, and norleucine was added to the homogenates to serve as an internal standard. After insoluble material was removed, the supernatant fluids were neutralized with KOH, and $KClO_4$ was discarded by centrifugation. Finally, the neutralized samples were lyophilized to dryness. Analyses were with a Technicon autoanalyzer and each value is the average of the results with two rats. The color developed by the compounds under the unknown peaks was arbitrarily taken to be equivalent to that by leucine. No corrections were made for the contributions of extracellular hepatic fluids.

Treatment	taurine	X3	asp	glu	X8	orn	his
	μ moles/g wet liver						
None	3.2	1.5	0.8	0.85	<0.005	0.1	0.4
Partial hepatectomy, 1 1/2 hours	2.8	1.4	1.2	0.9	0.01	0.15	0.4
Partial hepatectomy, 6 hours	1.8	2.2	1.6	1.4	0.04	0.3	0.4
Partial hepatectomy, 12 hours	1.3	2.9	1.8	1.9	0.085	0.4	0.4
Partial hepatectomy, 20 hours	3.0	2.1	1.5	1.6	0.04	0.3	0.5
Protein-free diet, 2 days*	0.4	0.1	0.9	1.0	<0.005	0.1	0.4
50% protein diet	2.0	4.0	0.9	1.5	0.08	0.3	0.5
TAGH solution, 12 hours	1.5	2.4	2.4	2.1	0.04	0.04	0.5
T_3 (100 μg), 12 hours	3.6	1.0	0.6	1.9	0.04	0.1	0.4

* The rats kept on a protein-free diet showed no large changes from the normal in the hepatic levels of any of the free amino acids (tryptophan was not measured).

$HClO_4$ extracts were examined by Stein-Moore chromatography. Table I shows some of the results that were obtained. The pool of taurine was lowered by partial hepatectomy and by the TAGH solution whereas it was already much reduced in the animals that had been kept on a protein-free diet. Glutamic acid and an unknown peak (X3) that was eluted just before aspartic acid were increased by the three manipulations of the rat. Perhaps

the most interesting change was in an unknown peak, designated as X8, that appeared between alanine and valine. This material, only barely detectable in extracts of normal liver, was increased after the three inductive treatments as well as by the injection of triiodothyronine alone.

DISCUSSION

Some of the observations made with rats kept on a protein-free diet are relevant here. No loss of liver cells is detectable over a 28 day period, (3, 4) and survival time of the animal is measured in months. Liver protein is decreased by about one-fourth during the first 2 days and little further reduction occurs thereafter (5). Breakdown of muscle protein provides the liver with amino acids and the ability of the liver to make protein is not depressed (6-8). The rate of apoferritin synthesis, for example, is the same as in control animals (9). The stimulation of nuclear DNA formation after the nutritional shift would not seem, therefore, to have a trivial explanation related to liver damage or to the correction of a defect in overall hepatic protein synthesis.

Several hypotheses can be offered to explain how a shift from a protein-free diet to one with protein or amino acids might cause the replication of nuclear DNA in parenchymal liver cells. The ability of some, but not other, amino acid mixtures in the preparatory diet to block the response of the liver to the second diet is consistent with the idea that reductions in the levels of certain amino acid degrading enzymes are essential changes of the preparatory period. The activities of several hepatic enzymes involved in the catabolism of amino acids are known to fall in rats kept on a protein-deficient diet (10, 11). Particularly with these points in mind, the hypothesis that we favor is that the dietary shift results in the hepatic accumulation of specific

products of amino acid metabolism that are at least partly responsible for inducing the critical changes of the DNA prereplicative period, perhaps by altering gene function. In this connection, it is encouraging that the residual cells after partial hepatectomy and the intact liver of the TAGH-treated rat appear to have similar problems in handling amino acids as do the hepatocytes of the nutritionally shifted animals. It is also of interest that thyroid hormone influences the catabolism of amino acids in liver (12-14).

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