

EFFECT OF PROTEIN DEPRIVATION AND PARENTERAL NITROGEN FEEDING ON TISSUE NUCLEIC ACIDS IN ALBINO RATS WITH TOXIC HEPATITIS

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The concentration of nucleic acids was studied in the tissues (skeletal and heart muscles, liver) of rats with toxic liver damage caused by CCl_4 , associated with protein deprivation and parenteral administration of the amino acid mixture moriamines S-2 and improved casein hydrolysate. In protein-deprived albino rats with toxic hepatitis the RNA and DNA concentration in skeletal and heart muscle was considerably increased while, at the same time, their concentration in the liver was reduced. The RNA/DNA ratio under these circumstances was altered. The RNA content in the hepatocytes was reduced through a decrease in the nuclear fraction of RNA firmly bound with chromatin. Parenteral nitrogen feeding restored the normal nucleic acid concentration in all the tissues studied. KEY WORDS: parenteral feeding; protein hydrolysates; amino acid mixtures; nucleic acids; toxic hepatitis; nuclei of hepatocytes.

The writers showed previously that the nucleic acid concentrations in the tissues are substantially modified in experimental thyrotoxicosis and alloxan diabetes. Parenteral feeding in these pathological states largely restores normal nucleic acid metabolism [3-5]. Experimental toxic hepatitis is accompanied by a marked disturbance of metabolism, which is aggravated by protein deficiency [1, 6, 8].

The important role of nucleic acids in protein synthesis is familiar; it was therefore considered worthwhile to study the concentration of nucleic acids in the tissues in animals with toxic liver damage coupled with protein deprivation and parenteral nitrogen feeding.

EXPERIMENTAL METHOD

Experiments were carried out on 117 sexually mature albino rats weighing 180-250 g. Toxic hepatitis was induced by three subcutaneous injections of a 50% oily solution of CCl_4 in a dose of 0.5 ml/100 g body weight in the course of one week. The presence of hepatitis was confirmed histologically. The animals were divided into five groups. The animals of two groups received the normal animal house diet, whereas the rest received a nonprotein synthetic diet during the experiment [2]. Against the background of protein deprivation the experimental rats received daily subcutaneous injections for eight days of either physiological saline or nitrogen preparations: moriamine S-2 and improved casein hydrolysate at the rate of 0.3 g conventional protein/100 g body weight. As energy-supplying material 40% glucose solution was given (0.5 ml/100 g body weight). The quantity of fluid given to all the animals was 7 ml/100 g body weight.

At the end of the experiment (eight days) the animals of all groups were killed and the concentrations of nucleic acids (RNA and DNA) in the tissues (skeletal muscle, heart, and liver) were investigated spectrophotometrically [10]. The coefficient of molar absorption of these compounds [7] was calculated for skeletal and heart muscles. In three series of experiments the nuclei were isolated from liver tissue [12] and the various RNA fractions in them were determined [15].

EXPERIMENTAL RESULTS

In albino rats with toxic hepatitis kept on an ordinary diet the RNA level in the skeletal and heart muscle rose while their DNA concentration fell, so that the RNA/DNA ratio increased to 2.8 (Table 1). In the liver of

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TABLE 1. Concentrations of Nucleic Acids in Tissues (In mg P/100 g tissue) of Albino Rats with Toxic Hepatitis during Protein Deprivation and Parenteral Feeding (M \pm m)

Group of animals	No. of animals	RNA			DNA		
		skeletal muscle	heart	liver	skeletal muscle	heart	liver
1. Healthy, ordinary diet	29	17.54 \pm 0.92	30.00 \pm 1.22	76.20 \pm 1.60	9.80 \pm 0.48	20.32 \pm 0.61	40.20 \pm 1.24
2. Hepatitis, ordinary diet	14	21.10 \pm 0.59*	33.31 \pm 1.14	70.51 \pm 1.57	7.48 \pm 0.44*	18.45 \pm 0.66	28.11 \pm 1.17*
3. Hepatitis, protein-free diet, physiological saline	8	39.59 \pm 1.85*	40.60 \pm 2.00*	54.24 \pm 4.21*	21.18 \pm 3.43*	22.00 \pm 0.69	31.93 \pm 1.82*
4. Hepatitis, protein-free diet, meriamine S-2	19	13.06 \pm 0.41*	19.38 \pm 1.28*	76.90 \pm 5.56†	8.93 \pm 0.62†	14.61 \pm 1.23*	37.62 \pm 2.06†
5. Hepatitis, protein-free diet, casein hydrolysate	15	13.52 \pm 0.40*	27.03 \pm 1.49†	73.27 \pm 6.04†	8.28 \pm 0.43*	18.56 \pm 1.16	38.77 \pm 2.57†

* P < 0.05 compared with group 1.

† P < 0.05 compared with group 3.

TABLE 2. Concentration of Various RNA Fractions in Hepatocyte Nuclei (in mg per nucleus $\cdot 10^6$) of Albino Rats with Toxic Hepatitis Coupled with Protein Deprivation and Parenteral Feeding (n = 8; $M \pm m$)

Group of animals	rRNA	mRNA	RNA bound with chromatin	Total RNA
Healthy (control)	$0,137 \pm 0,040$	$0,124 \pm 0,005$	$0,990 \pm 0,063$	$1,250 \pm 0,099$
Toxic hepatitis, protein-free diet, physiological saline	$0,210 \pm 0,024$	$0,370 \pm 0,070^*$	$0,440 \pm 0,059^*$	$0,980 \pm 0,102$
Toxic hepatitis, protein-free diet, moriamine S-2	$0,307 \pm 0,011^*$	$0,450 \pm 0,090^*$	$0,620 \pm 0,069^*$	$1,380 \pm 0,154$

* $P < 0.05$ compared with control.

these animals the concentration of nucleic acids was reduced, especially of DNA, so that the RNA/DNA ratio rose to 2.51.

Protein deprivation in conjunction with toxic hepatitis intensified the changes in the nucleic acid concentrations found in the tissues. As a result of the greater decrease in the RNA concentration in the liver, the RNA/DNA ratio fell to 1.7. This ratio also fell in skeletal muscle, to 1.51, but because of a greater increase in the DNA concentration.

According to data in the literature, in hepatitis caused by administration of various toxic substances the number and volume of the nuclei of the liver cells are substantially changed [9]. A significant decrease ($P < 0.05$) in the number of nuclei in the liver tissue also was observed in the present experiments in toxic hepatitis ($32.83 \pm 3.53 \cdot 10^6$) compared with healthy albino rats ($47.46 \pm 3.76 \cdot 10^6$). The mass of each hepatocyte nuclei also was significantly changed.

Under the influence of parenteral nitrogen feeding the concentration of nucleic acids in the skeletal and heart muscles fell compared with that found during protein deprivation, and the RNA level was actually lower than in the healthy albino rats. The RNA/DNA ratio changed significantly following administration of moriamine S-2 (1.46 in skeletal muscle and 1.33 in heart muscle) and the values were a little higher in animals receiving casein hydrolysate (1.64 and 1.46 respectively).

In the liver under the same conditions the normal concentration of nucleic acids was restored and the RNA/DNA ratio corresponded to that in healthy animals. Parenteral nitrogen feeding did not restore the number of hepatocyte nuclei but increased ($P < 0.05$) their mass ($2.71 \pm 0.08 \text{ mg} \cdot 10^{-6}$ compared with $1.02 \pm 0.07 \text{ mg} \cdot 10^{-6}$ in the control).

Administration of a mixture of crystalline amino acids and of casein hydrolysate, which contains a certain quantity of dipeptides as well as amino acids, thus led to parallel changes in the concentrations of the nucleic acids in the various tissues tested.

The decrease observed in the RNA content in the liver tissue and nuclei in toxic hepatitis took place on account of a reduction in the fraction of RNA firmly bound with chromatin, whereas the nuclear ribosomal RNA fraction (rRNA) extractable by salt solution at pH 7 and the transfer form of DNA-like RNA (mRNA) extractable by salt solution at pH 8 were increased (Table 2).

Administration of moriamine S-2 restored the normal RNA content in the liver tissue and hepatocyte nuclei through an increase in all its nuclear fractions.

After administration of moriamine S-2 to healthy albino rats in which protein deprivation was accompanied by a decrease in the concentration of nucleic acids and readily extractable liver proteins, a significant increase ($P < 0.05$) was observed in the RNA level from 55.5 ± 3.2 to $76.5 \pm 9.5 \text{ mg P/100 g tissue}$ and in the level of readily extractable proteins from 110.0 ± 7.5 to $172.0 \pm 6.5 \text{ mg/g tissue}$.

The increase in the nucleic acid content in the liver during parenteral nitrogen feeding observed in these experiments both in animals with toxic hepatitis and in healthy animals agrees with data showing an increase in the RNA content and stimulation of protein biosynthesis in the tissues of healthy albino rats during administration of single amino acids (methionine, lysine, etc.) [13, 14].

On the basis of these results parenteral nitrogen feeding would seem to be indicated in liver pathology.

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