PROTEIN AND RNA SYNTHESIS IN THE RABBIT LIVER AFTER BLOOD LOSS

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After acute and chronic blood loss, synthesis of plasma proteins and liver proteins is increased to twice or three times the normal level. After acute blood loss synthesis of cytoplasmic RNA fractions in the liver is increased by 50%, and synthesis of nuclear RNA fractions by 50-100% compared with normal.

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Restoration of the liquid part of the blood after blood loss is largely dependent on regeneration of the plasma proteins. The liver plays the principal role in this process. However, existing data on the effect of blood loss on synthesis of plasma and liver proteins are contradictory. The rate of incorporation of labeled amino acids into plasma and liver proteins of animals after blood loss remained unchanged according to some workers [4], while according to others it increased [2] or, conversely, decreased [3].

To shed light on this problem we decided to study synthesis of plasma and liver proteins of animals after blood loss and at the same time to investigate the rate of synthesis of RNA, playing an important role in the regulation of protein synthesis, in the liver.

EXPERIMENTAL METHOD

Protein synthesis was judged from the rate of incorporation of methionine-S^{35} into liver and plasma proteins.

Experiments were carried out on six groups of rabbits (altogether 30 animals): group 1, normal animals; group 2, 6 h after acute blood loss; group 3, 12 h; group 4, 24 h; and group 5, 48 h after acute blood loss; and group 6, animals after chronic blood losses.

Acute blood loss was carried out as a single procedure to the extent of 20 ml/kg body weight. Chronic blood losses were carried out on alternate days, 5 times altogether, in volumes of 10 ml/kg body weight each time. Methionine-S^{35} was injected intravenously in a dose of 200 μCi/kg body weight. The animals were sacrificed by total exsanguination 6 h after injection of the isotope. Proteins were precipitated from the blood plasma and liver homogenates with 10% TCA, and washed several times with 5% TCA, alcohol, an alcohol–ether mixture, and ether. The residues were dried at 60°. Radioactivity of the dry protein was calculated in thick-layer preparations by means of a BFL-T-25 end-type counter and "Volna" radiometer. Activity was expressed in pulses/min/100 mg protein.

TABLE 1. Incorporation of Methionine-S^{35} (in percent of initial level) into Plasma and Liver Proteins

Time after blood loss (in h)	Plasma proteins		Liver proteins	
	M±m	P	M±m	P
6	189±17	0,01	127±7	0,02
12	302±15	0,001	182±13	0,001
24	272±23	0,001	141±15	0,05
48	265±19	0,001	186±10	0,001
Chronic blood loss	224±29	0,01	174±16	0,01

RNA synthesis in the liver was studied by the Georgiev–Kirby thermal phenolic fractionation method using P^{32} as radioactive label. The investigations were carried out on three groups of rabbits: group 1, normal animals; group 2, 12 h after acute blood loss (20 ml/kg body weight); and group 3, 24 h after acute blood loss (20 ml/kg body weight).

$Na_2HP^{32}O_4$ was injected intravenously in dose of 540 μCi/kg body weight. The animals were sacrificed 1 h later. Ten volumes of 0.14 M NaCl solution was added to the tissue and a homogenate prepared. An equal volume of phenol

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TABLE 2. Incorporation of P^{32} into Various Rabbit Liver RNA Fractions 12 and 24 h after Blood Loss (specific activity in pulses/min/min mg RNA)

Fraction		sRNA	rRNA	R-RNA	mRNA
Normal	Specific activity	537	133	1,016	2,974
	Relative activity	4	1	8	22
12 h after blood loss	Specific activity	805	387	2,457	3,607
	Relative activity	2	1	6	9
24 h after blood loss	Specific activity	810	318	2,736	4,140
	Relative activity	2	1	8	13

Note. Upper figure in column denotes specific activity, lower figures relative activity.

(pH 6.0) was added, the mixture was shaken for 30 min, and then centrifuged at 2500 rpm. Two cytoplasmic fractions were obtained from the upper layer (supernatant) after the second treatment with phenol: transfer (sRNA) and ribosomal (rRNA). These fractions were separated by precipitation of the rRNA with 2 M NaCl solution. Two nuclear RNA fractions were obtained from the intermediate layer (phenolic nuclei) at 45 and 65°: ribosomal RNA precursor (R-RNA) and messenger RNA (mRNA). The nuclear fractions were also treated a second time with phenol and all RNA fractions were then repeatedly reprecipitated with alcohol. All operations on isolation of RNA fractions were carried out in the cold. The RNA fractions obtained were determined quantitatively on the SF-4 spectrophotometer and then precipitated with 50% TCA. The residues thus obtained were passed through "Rufs" millipore filters and repeatedly washed with 5% TCA and alcohol. Their activity was then measured with the "Volna" radiometer and expressed as pulses/min/mg RNA.

EXPERIMENTAL RESULTS

Under normal conditions the rate of methionine-S^{35} incorporation into plasma proteins is rather higher than into liver proteins (Table 1). After acute blood loss we found faster than normal incorporation of radioactive label into both liver and plasma proteins. Incorporation of the amino acid into plasma proteins began to increase 6 h after acute blood loss, reaching a maximum after 12 h. Later (24 and 48 h after blood loss) the rate of incorporation fell slightly, although it still remained above its initial level.

Maximal incorporation of label into liver proteins also was observed 12 h after blood loss, falling slightly 24 h, and again increasing 48 h after blood loss. After chronic blood loss a faster rate of incorporation of methionine-S^{35} into the plasma and liver proteins was also observed. It is clear from Table 1 that after repeated chronic blood losses and after a single acute blood loss the incorporation of label into the proteins increased by roughly the same amount. The fact that our results differ from those obtained by other authors [3, 4] may perhaps be explained by differences in the methods of calculating radioactivity of the dry protein preparations.

Investigation of RNA synthesis showed that the rate of P^{32} incorporation into all liver RNA fractions was increased 12 and 24 h after blood loss (Table 2). Incorporation of P^{32} into sRNA was increased by 40-41% 12 and 24 h after blood loss, and into rRNA by 190% 12 h after blood loss and by 133% 24 h after. So far as the nuclear fractions are concerned, incorporation of P^{32} into R-RNA was particularly increased: by 141% after 12 h and by 169% after 24 h. The increase in incorporation of radioactive label into mRNA was slight: 21% 12 h after blood loss and 39% 24 h after.

It is interesting to examine the ratio between activities of the four isolated RNA fractions with each other under normal conditions and after blood loss. Normally the ratio between specific activities of s-, r-, R-, and mRNA fractions was 4:1:8:22. This ratio 12 h after blood loss was 2:1:6:9, and 24 h after blood loss it was 2:1:8:13 (Table 2). After blood loss, the activity of rRNA thus increased much more than the

activity of the other RNA fractions. This was perhaps due not only to stimulation of ribosomal function and of synthesis of endogenous rRNA after blood loss, but also with a faster transfer and attachment of mRNA molecules to the ribosomes. That was evidently why only a very slight increase in mRNA activity was found after blood loss.

LITERATURE CITED

1. G. P. Georgiev and V. L. Mant'eva, Biokhimiya, No. 5, 949 (1962).
2. N. A. Messineva, The Use of Radioactive Isotopes in Medicine [in Russian], Moscow (1955), p. 171.
3. N. R. Oster, Incorporation of Labeled Amino Acids into Organ Proteins and Blood Serum under Different Conditions [in Russian], Author's abstract of candidate dissertation, Tashkent (1959).
4. I. M. Khlebnikova, Vopr. Med. Khimii, No. 3, 256 (1964).
5. G. P. Georgiev, O. P. Samarina, M. J. S. Lerman, et al., Nature, 200, 1291 (1963).