BIOCHEMISTRY AND BIOPHYSICS

S^{35}-METHIONINE UPTAKE BY PROTEINS OF THE STRUCTURAL ELEMENTS OF TISSUE CELLS IN IRRADIATED RATS

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Disturbances of protein metabolism in the structural elements of tissue cells are known to occur when the animal organism is exposed to ionizing radiation in doses sufficient to produce acute radiation sickness. These disturbances are characterized by quantitative changes in the protein content of the nucleus and cytoplasm, changes in the properties of desoxyribonucleoprotein and desoxyribonucleic acid [2, 3, 4] and changes in antigenic properties of the tissues [5, 6].

In connection with this it appeared important to study the level of S^{35}-methionine uptake by cell proteins of irradiated animals since such uptake reflects to a certain degree the state of synthetic processes taking place. We were interested not in tissue proteins en masse but in individual proteins of isolated cellular microstructures.

The problem with which the present communication is concerned is the study of radioactive methionine uptake by proteins of cell nuclei (desoxyribonucleoprotein, acid and "residual proteins"), mitochondria and microsomes (water-soluble ribonucleoproteins, lipoproteins) and hyaloplasma in the liver and small intestine mucosa of rats with acute radiation sickness.

EXPERIMENTAL METHODS

Examination of the experimental group of rats for radioactive proteins was carried out three days after irradiation of the animals with roentgen rays and three hours 30 minutes after administration of labelled methionine. The control group of animals was also examined for radioactive proteins 3 hours 30 minutes after administration of S^{35}-methionine. The latter was always given subcutaneously in doses of 0.5 ml calculated on 3 μC per 1 kg body weight.

Experiments were performed on white male rats (total of 150 animals) weighing 190-210 g which were kept fasting for 12 hours preceding the experiment and were only allowed water. Determinations were carried out on homologous tissues from 10-20 rats.

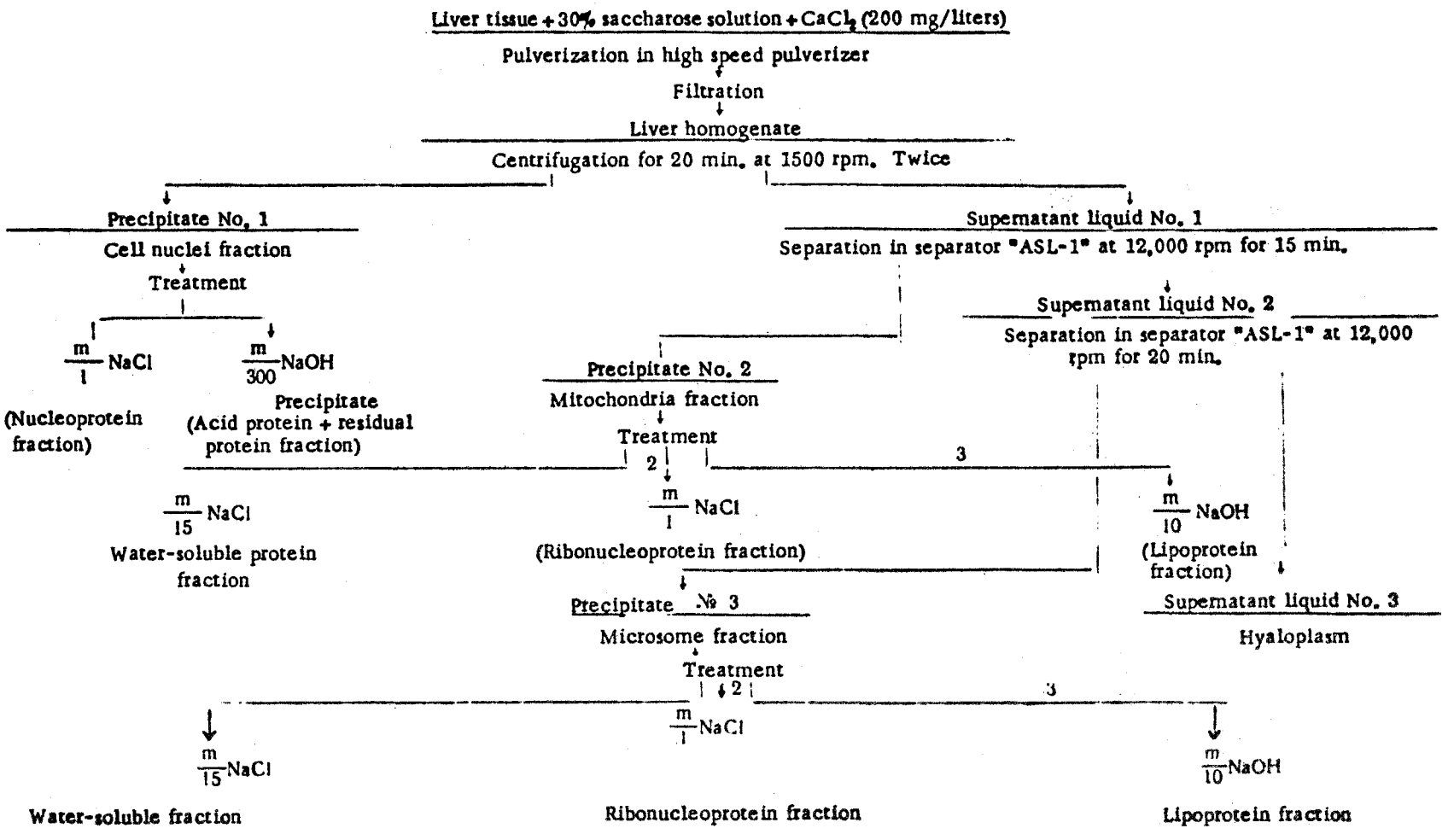
The animals were subjected to a single total irradiation with a voltage of 180 kV, current of 15 ma, focal distance of 30 cm, using copper (0.5 mm) and aluminum (1 mm) filters. The summated dose per rat was equal to 800 r. Under the given conditions of irradiation all the animals died within 7 days. The highest incidence of death was observed on the 5th day (80%).

A Dounce's [7] method was used for isolation of cell nuclei. Fractionation of nuclear proteins was carried out by the method of I. B. Zbarsky and S. S. Debov [1].

The cytoplasmic structures – mitochondria, microsomes and hyaloplasm – were isolated according to the scheme presented below. This diagram also shows the scheme for fractionation of the microstructures into separate proteins.

SCHEME

for isolation of structural elements of the cell and fractionation of proteins from these microstructures



Isolation of solid protein preparations and determination of their radioactivity were performed as follows. The proteins were precipitated from corresponding solutions (see scheme) with 20% trichloracetic acid, washed three times with 5% trichloracetic acid to remove radioactive methionine adsorbed on their surface and rendered lipid-free by thrice repeated successive treatment with 96° alcohol, alcohol-ether mixture (1:3) and ether. The protein preparations so obtained were exposed to the air until no more odor of ether could be detected and were then shifted through a sieve.

Radioactivity was determined on 10 mg dry protein placed on a stainless steel disc with a surface measuring 2 cm^2. A fine, maximally uniform layer of protein was obtained as follows. The weighed protein sample was carefully distributed over the whole surface of the disc by means of a glass rod. Then 5-6 drops of ether was added carefully to the protein from a pipet from the edge of the disc and the whole stirred by rotary movement.

Slow, uniform evaporation of ether took place under these conditions and the protein particles suspended in it were precipitated over the whole surface of the disc in a smooth, fine layer. The advantage of this method of preparation of "targets" as compared to the use of aqueous alcohol lies in the rapidity of the procedure (2-3 minutes) and the formation of a uniform layer of protein. Radioactivity counts were carried out with the use of installation B with a thin-walled counter.

EXPERIMENTAL RESULTS

Data illustrating the effect of roentgen rays on the uptake of radioactive methionine sulfur by the proteins of cell nuclei and cytoplasm are provided by results obtained for normal and irradiated rats and are presented in Tables 1-3.

TABLE 1

S^{35}-Methionine Uptake by Proteins of Cell Nuclei in Liver and Mucosa of Small Intestine in Normal and Irradiated (800 r) Rats (Expressed as Percentage of Introduced Radioactivity) *

Nuclear proteins	Liver		Small intestine mucosa	
	normal	experimental	normal	experimental
Nucleoprotein	2.03	3.22	5.20	5.54
Acid	3.31	4.83	6.93	9.76
"Residual"	1.23	2.45	2.01	1.67

* Average figures from 4-5 experiments.

TABLE 2

S^{35}-Methionine Uptake by Hepatic Cytoplasmic Proteins of Normal and Irradiated (800 r) Rats (Expressed as Percentage of Introduced Radioactivity) *

Cytoplasmic proteins	Mitochondria		Microsomes		Hyaloplasm	
	normal	experimental	normal	experimental	normal	experimental
Total					0.98	0.95
Water-soluble	0.86	0.90	0.80	0.85	—	—
Ribonucleoproteins	1.58	0.95	1.55	0.34	—	—
Lipoproteins	1.48	1.05	1.43	1.15	—	—

* Average figures from 3-5 experiments.

Table 1 shows that radioactivity of nuclear proteins in the liver of irradiated rats is higher than in normal rats. As regards nuclear proteins in cells of the small intestine mucosa, definite increase of labelled methionine uptake is observed only in the acid protein. Radioactivity of nucleoprotein and "residual" protein remains within normal limits in this case.

Data presented in Table 2 indicate that irradiation is associated with substantially decreased S^{35}-methionine uptake by the ribonucleoprotein and lipoprotein fractions of hepatic cytoplasmic proteins; the greatest depression of uptake is seen in ribonucleoproteins of the microsomes (down to 22% of normal). The level of S^{35}-methionine uptake by water-soluble mitochondria and microsome proteins and hyaloplasm proteins remains within normal limits.

Unlike the liver, the cytoplasmic microstructures in the small intestine mucosa of irradiated rats show slower S^{35}-methionine uptake by all the proteins studied, including water-soluble ones. This is most pronounced in the ribonucleoprotein fraction from mitochondria and microsomes (Table 3).

TABLE 3

S^{35}-Methionine Uptake by Cytoplasmic Proteins in Small Intestine Mucosa of Normal and Irradiated (800 r) Rats (Expressed as Percentage of Introduced Radioactivity) *

Cytoplasmic proteins	Mitochondria		Microsomes		Hyaloplasm	
	normal	experimental	normal	experimental	normal	experimental
Total	–	–	–	–	1.80	1.40
Water-soluble	2.55	1.45	2.80	1.30	–	–
Ribonucleoproteins	2.30	0.90	2.00	0.60	–	–
Lipoproteins	2.43	1.35	2.10	1.00	–	–

* Average figures from 3-5 experiments.

Roentgen irradiation thus leads to increased uptake of radioactive methionine sulfur by nuclear proteins of hepatic cells and by acid protein of intestinal mucosa. S^{35}-methionine uptake by desoxyribonucleoprotein and "residual" protein of the nuclei in intestinal mucosa is unchanged as compared to the normal. The uptake of radioactive methionine sulfur by the cytoplasmic proteins of the liver and intestinal mucosa is slower in irradiated rats than in normal ones.

SUMMARY

The rapidity of inclusion of S^{35}-methionine into the cells of various tissues was studied in rats in acute radiation sickness. Inclusion of S^{35}-methionine was studied in the proteins of cellular nuclei (desoxynucleoprotein, acid and "residual"), mitachondria and microsomes (water-soluble ribonucleoproteins, lipoproteins) and hyaloplasm of the liver and of the mucous membrane of the small intestine.

It was established that x-ray irradiation causes increased inclusion of radioactive sulfur methionine into all proteins of the cellular nuclei of the liver, as well as in the acid protein of the mucous membrane of the small intestine. Inclusion of S^{35}-methionine into the desoxyribonucleoprotein and "residual" protein of the nuclei in the mucous membrane of the small intestine does not change in comparison with the same in normal conditions.

Decreased inclusion of radioactive sulfur methionine is noted in cytoplasmic proteins of the liver and in the mucosa of the small intestine. This decrease is especially pronounced in ribonucleoproteins of mitochondria and microsomes.

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* In Russian.

** Original Russian pagination. See C.B. Translation.