INCORPORATION OF S^{35} - METHIONINE IN THE SERUM PROTEINS OF IRRADIATED DOGS AFTER LOSS OF BLOOD

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The study of the effect of irradiation of the biosynthesis of serum proteins is also of interest to the explanation of the general mechanism of the biological action of ionizing radiation and to the solution of the more specialized problem of the treatment of blood loss combined with radiational trauma. The study of the rate of synthesis of the serum proteins in irradiated animals has been the subject of several investigations [5-7, 10]. Because of the contradictory nature of the results obtained, however, no definite conclusions have emerged from this research.

We accordingly decided to make a further investigation of the rate of incorporation of S^{35}-methionine in the different fractions of the serum proteins in control and irradiated animals. The investigation was carried out on dogs subjected to loss of blood. As shown by V. D. Blokhina [1], irradiation produces in dogs a fall in the albumin concentration and a rise in the concentration of α - and β-globulins in the serum. It has also been established [3] that after blood loss these changes become more pronounced.

EXPERIMENTAL METHOD

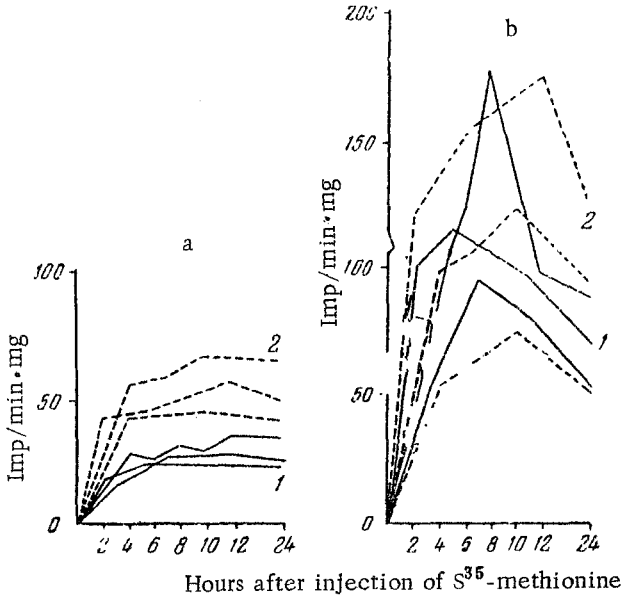
Experiments were carried out on adult male mongrel dogs of various weights and ages.

The technique of bleeding and of subsequent replacement of the lost blood with Ringer's solution, the determination of the volume of the circulating blood by means of the dye T-1824, and the determination of the concentration of the serum proteins with the electrographic separation on paper have been described previously [4].

The volume of blood lost amounted to 5-5.5% of the body weight. Irradiation was carried out by paired RUM-3 apparatus at 195 kv and 15 ma. Filters – 0.5 Al and 1.0 Cu. Skin-focus distance 1 m. Radiation dose 700 r with a dose rate of 9 r/minute.

S^{35}-methionine was injected intramuscularly on the 3rd day after blood loss. The dogs received no food for 18 hours before administration of amino acid and for 10-12 hours afterwards. The time of administration of the label was chosen in accordance with previous findings [4], showing that at this time the appearance of extravascular reserves of serum proteins in the blood stream has virtually ceased. Radioactive methionine was injected in a dose of 0.006-0.01 μC/g body weight. Blood samples were taken at different times – from 1 to 24 hours after injection of the methionine. The intensity of incorporation of the label in the proteins was judged by their relative specific activity, i.e., by the ratio of the activity of 1 g of protein to the activity of S^{35}-methionine injected per 1 g body weight. The activity of the protein was determined with a gas-flow counter. For this purpose 2 mg of protein was placed on the target, made from foil with an area of 2 cm^2.

A preparatory electrophoretic separation of the serum proteins into fractions was carried out in a starch gel,



Changes in the activity of the albumin (a) and α_2-globulin (b) during the first 24 hours after injection of radioactive methionine to dogs. 1) Irradiated animals; 2) control animals.

in a specially constructed apparatus. The albumin obtained by this method contained no electrophoretic contaminants. The α_2-globulin contained a trace of albumin. In order to purify it, after precipitation with trichloroacetic acid, the property of albumin to dissolve in alcohol was utilized. The α_3-globulin fraction contained 14% of an admixture of α_2- and α_4-globulins. The rest were more heterogeneous. The fraction conventionally designated β_1-globulin contained about 40% of β_1 globulin, about 30% of β_2-globulin and 15% each of α_2- and γ-globulins, and the fraction conventionally designated β_2-globulin contained about 50% of β_2-globulin, about 30% of β_1-globulin and about 20% of the α- and γ-fractions, while the γ-globulin fraction contained about 20% of admixed β-globulins. The α_1- and α_4-globulin fractions were not separated. *

EXPERIMENTAL RESULTS

In the figure are illustrated the changes in the activity of the albumin and α_2-globulins in the course of the first 24 hours after injection of S^{35}-methionine to control and irradiated animals. It can be seen from the graph that the activity of the serum albumin in both the control and irradiated animals reached a maximum 10-12 hours after the administration of the labeled amino acid. The same thing was observed in respect to the α_3- and both β-globulins of the serum of these animals. On comparing the peaks of the activity of the α_2-globulins in the same groups of dogs it can be seen that the most active samples of this protein were isolated from the serum of the irradiated dogs 5-8 hours after injection of the methionine, and in the control animals after 10-12 hours. In the table are given the values of the maximum relative specific activity (MSA) of the different fractions of the serum proteins of the control and irradiated dogs. For all proteins except the α_2-globulin of the irradiated animals the results given are of the activity of samples isolated 9-12 hours after the injection of S^{35}-methionine (for the α_2-globulin, after 4-8 hours).

It can be seen from the table that the maximum relative and specific activity of the total proteins of the serum was in all cases higher in the irradiated dogs.

Irradiation affected the activity of the individual fractions of the serum proteins differently, however: the maximum relative and specific activity of the albumin in the control animals was almost twice that of the irradiated animals; the maximum relative and specific activity of the α_3-globulin fraction was higher in the group of irradiated dogs; the same applied also the the β_1-globulin fraction, but on account of its heterogenicity it was difficult to say to what degree the increase in its activity was due to the β_1-globulins themselves; the maximum relative and specific activity of the other fractions differed insignificantly in the control and experimental animals.

Since the reports in the literature suggest that the albumin and the main bulk of the α-globulins are synthesized in the liver [8, 9], the changes in such factors as permeability, dilution of the label and so on must have affected the specific activity of both proteins equally. The reduction in the specific activity of the albumin of the irradiated dogs with, at the same time, an increase in the specific activity of the α_3-globulin, permits the assumption that, comparing the irradiated animals with the controls, changes have occurred in the processes directly determining the rate of incorporation of methionine in the proteins examined, and furthermore that the rate of incorporation into the albumin fell whereas that into the α_3-globulin (and, possibly, also the β_1-globulin) was increased. Such a conclusion is not, however, in agreement with the findings of Kay and Entenman [7], Stevens, Gray and Schwartz [10] and of E. P. Smolichev [5].

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Relative Specific Activity of the Serum Proteins of Control and Irradiated Dogs after Blood Loss

Character of expt.	Expt. No.	Relative specific activity							Rel. spec. activity of α_3 globulin / albumin
		albumin	globulins					total proteins	
			α_2	α_3	β_1	β_2	γ		
Control	1	7.1	22.6	17.3	17.3	20.5	7.1	15.5	2.4
	2	8.3	15.7	15.7	9.6	12.4	7.1	11.3	1.9
	3	11.9	20.5	13.0	12.7	15.1	7.3	—	1.1
	4	8.7	17.8	14.8	14.9	17.3	—	13.8	1.7
Mean	—	9.0	19.1	15.2	13.6	18.3	7.2	13.2	1.8
Irradiation	5	4.5	17.5	20.5	17.2	18.5	7.4	16.2	4.5
	6	5.1	25.6	25.4	23.8	23.8	10.9	22.7	4.9
	7	4.1	—	23.2	22.7	18.3	8.1	20.7	5.7
	8	5.7	29.0	25.2	—	16.6	8.0	—	5.3
Mean	—	4.8	24.0	23.6	21.2	19.3	8.6	19.9	5.1

Kay and Entenman found that during incubation of liver slices from control and irradiated rats in the presence of radioactive glycine, this amino acid was more rapidly incorporated in the proteins of the slices taken from the irradiated animals. In this basis, these authors came to the conclusion that the fall in the albumin content and the increase in the serum globulin content of the irradiated animals could not be explained by disturbance of the synthesis of these proteins in the liver. It is doubtful, however, whether such a conclusion is justified from these findings. S. Ya. Kaplanskii, A. E. Gurvich and L. K. Starosel'tseva [2] showed recently by means of immunochemical method that only an insignificant part of the total quantity of soluble liver proteins are identical with the serum proteins. This makes it very doubtful that the rate of synthesis of the serum proteins can be estimated by the rate of incorporation of a labeled aminocid into the total protein of liver slices.

Stevens et al. [10] studied the incorporation of S^{35} -methionine into the albumin and globulins in rabbits before and after these animals were irradiated. These authors separated the serum proteins into 2 fractions – albumin and globulins – by means of precipitation of the latter with sodium sulfate. In the irradiated animals the specific activity of the proteins of both fractions, just like that of the total serum protein, was increased. These findings thus differed from those described in our paper in respect to the albumin.

As V. D. Uspenskaya has shown, the α_3 -globulins of the dog are no less soluble than the albumin. It is possible that one of the α-globulins of the rabbit is also not precipitated by sodium sulfate in the concentration used by the American workers. In such a case the increased incorporation of the label in this protein might have masked the fall in the incorporation of the amino acid in the albumin. It is interesting that Gabriel and Chang [6], who separated the proteins by the method of paper electrophoresis, showed that in rats, irradiation affects the incorporation of S^{35} -methionine in the different fractions of the serum proteins unequally.

E. M. Smolichev [5] studied in rabbits the action of internal irradiation on the incorporation of S^{35}-methionine in the serum proteins. The internal irradiation of the rabbits was given by injecting the animals with large doses of P^{32}. Just as in response to external irradiation, the animals ahowed a fall in the concentration of serum albumin in the blood and an increase in the concentration of the β-globulins. E. P. Smolichev found that the time interval between the injection of the label and the maximum activity of all fractions of the serum proteins in the irradiated animals was lengthened to several days. On the basis of this observation, the author concluded that there was a fall in the rate of synthesis of all fractions of the serum proteins in rabbits exposed to irradiation.

As has been shown above, we were unable to detect the irradiated dogs any increase in the time during which the activity of the different fractions of the serum proteins reached its maximum. It is obvious that the

activity of the protein synthesized in the body after the administration of the labeled amino acid can only increase while the ratio between the number of its "labeled" and "unlabeled" molecules present in the body in the free state, is greater than this ratio for the molecules of the same amino acid incorporated in the protein. The presence of a high concentration of free labeled amino acid in the body for a period of several days after its injection is unlikely. This suggests that the phenomenon observed by E. P. Smolichev is not connected with a dalay in the incorporation of labeled amino acids in the serum proteins, but is the result of some error in technique. Thus the reports that the rate of incorporation of amino acids in the different fractions of the serum proteins is changed by the action of irradiation in a uniform manner in our opinion requires verification.

The material presented in the present paper demonstrates that the fall in the serum albumin concentration and the increase in the α- and β-globulin concentrations in the blood of irradiated animals are accompanied by an increase in the rate of incorporation of amino acids in the globulins (in dogs, in the α_3 and, possibly, the β_1-globulins) and by a decrease in the rate of incorporation of this amino acid in the serum albumin. This can be regarded as an index of the decrease in the rate of synthesis of the serum albumin in the irradiated animal. In order to have good grounds for postulating an increase in the rate of synthesis of α_3-globulin, it is necessary to be satisfied as to the identity of the amino acid composition of this protein in the unirradiated dogs with that of the α_3-globulin which appears in large quantities in the blood of the irradiated animals.

From the results described and the subsequent discussion it appears that even large doses of ionizing radiation do not interfere with the processes of protein synthesis in the liver, but the normal ratio between the rates of synthesis of the different serum proteins in the irradiated animal may be changed and lead to an alteration in the protein composition of the blood plasma of the irradiated animal.

SUMMARY

The authors studied the S^{35}-methionine incorporation into the electrophoretic fractions of serum proteins in irradiated and control dogs following blood loss. It was established that in irradiated animals the rate of incorporation of the labeled amino acid into the albumin is reduced, but in case of α_3-globulin (and possibly β_1-globulin) it is increased.

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