# THE ACTION OF LOW TEMPERATURE AND IONIZING RADIATION ON THE SERUM PROTEINS AND PROTEIN FRACTIONS

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Research by many authors has shown that irradiation of animals leads to changes in the normal proportions of the protein fractions of the blood serum [1, 2, 4]. As a result of irradiation of animals by ionizing radiation from different sources, a fall in the albumin, and a considerable increase in the globulin content take place, as a rule. Changes in the serum proteins of an animal subjected to irradiation during the local action of a low temperature, however, have received little attention in the literature. There are merely indications that the application of general cooling stimulates the protective properties of the animal against ionizing radiation [6, 7]. So far as the clinical features and the biochemical changes which accompany the combined action of low temperature and ionizing radiation are concerned, apart from the work of O. V. Rudenko [3] we have been unable to find any relevant data in the accessible literature.

The aim of the present research was to study, in experiments on rats, the changes in the total protein and the protein fractions of the blood serum in response to the action of local cooling against a background of marked radiation sickness.

# EXPERIMENTAL METHOD

Experiments were conducted on male white rats weighing 120-160 g. The total number of animals was divided into four groups of 10 rats each: a control group, a group of animals exposed to the local action of low temperature only, a group of irradiated animals and a group exposed to the combined action of low temperature and irradiation.

The local action of low temperature was effected by placing the animals in an open glass chamber, the bottom of which was immersed in a cooling mixture (ice, salt, carbon dioxide snow) at a temperature of -30° for 4 hours. In view of the fact that the cooling chamber was open and the temperature of the air within was kept at +14°, a purely local effect of cold on the limbs supporting the animals on the floor of the chamber was observed. The body temperature of the rats was measured by means of a thermocouple and did not depart from the range 34-36°, while the temperature of the muscles of the cooled extremities fell to +15° and +20°. After 24 hours the action of the low temperature was shown by the appearance of 2nd-3rd degree frostbite of the hindlimbs and tail. Hyperemia of the skin appeared, and this soon acquired a cyanotic hue. Marked edema of the skin and subcutaneous cellular tissue, cyanosis, and small areas of darkening were later observed.

The animals were irradiated with an RUM-3 apparatus, with a filter of 0.5 mm Cu and 1 mm Al, a current strength of 10 ma, and voltage of 190 kv. The total dose of irradiation was 700 r.

The total serum protein was estimated refractometrically. The protein fractions of the blood were estimated by the method of electrophoresis on filter paper. Electrophoresis was carried out in a veronal-medinal buffer (pH = 8.6) for 20 hours. The electrophoregrams were stained with bromphenol blue, cut into strips 5 mm wide, and after extraction of the dye the protein fractions were estimated on the electrophotocolorimeter.

Changes in the Total Protein Content and the Protein Fractions of the Serum of Rats (in %) after the Action of a Low Temperature and Irradiation

Protein fraction	Control animals	Freezing	Irradiation	Irradiation + freezing
Total protein	7.4	7.3	7.0	5.8
Albumins	51.8	42.5	42,5	30.5
Globulins .	48.2	<b>57.</b> 5	57.5	69.6
α₁-globulins	6.0	8.0	6,3	13.9
α <sub>2</sub> -globulins	7.5	12.7	12.0	13.5
β÷globulins	20.7	27.7	21.8	26.8
γ-globulins	13.5	9.0	17.1	13.8
A/G ratio	1.07	0.7	0.7	0.4

The rats exposed to the local action of cold were sacrificed by decapitation 24 hours after cooling, and the total protein content of the serum and the proportions of the protein fractions were estimated.

#### EXPERIMENTAL RESULTS

From the figures in the table it can be seen that, in the group of animals exposed to the local action of cold, obvious changes took place in the proportions of the serum protein fractions, namely a decrease in the concentration of albumins and an increase in the globulins, mainly affecting the  $\beta$ - and  $\alpha_2$ -globulin fractions. These changes in the serum protein fractions led to a fall in the A/G ratio to 0.7.

In animals exposed to a single dose of total irradiation with x-rays of absolutely lethal magnitude, the investigation was carried out on the 7th day after irradiation when radiation sickness was well developed. At this time the rats showed considerable leucopenia (1200-1500 leucocytes/mm<sup>3</sup>), severe adynamia, refusal to take food, liquid stools, hemorrhages from the ears and lips, and loss of weight. These signs were accompanied by a fall in the total serum protein level and also by characteristic changes in the proportions of the individual protein fractions, namely, a fall in the albumin and an increase in the  $\alpha$ - and  $\beta$ -globulin fractions.

In the next series of experiments, animals previously irradiated were exposed to the action of a low temperature (on the 7th day after irradiation). The course of the frostbite in these rats showed certain distinctive features: the hyperemia of the skin at the points of application of the cold was less intense, the frostbitten limbs were less cyanotic, and formation of blisters took place over a much larger area and their contents were often blood-stained. The tendency towards necrosis was more marked and healing took place more slowly. In this group of animals the most significant changes were observed in the total serum protein concentration and in the proportions of the protein fractions. The total proteins fell to 5.8% as against 7.4% in the control group. The very considerable fall in the albumins (control = 51.8%, experiment = 30.5%) and the increase in the  $\alpha_1$ ,  $\alpha_2$  and  $\beta$ -globulin fractions were noteworthy. The A/G ratio fell from 1.07 to 0.4.

The literature of recent years has contained a number of papers devoted to the investigation of the blood proteins in radiation sickness [1, 2, 5]. The authors have concluded that the fall in the albumin fraction of the serum proteins is connected with disturbances of synthesis of this fraction. V. M. Rodionov and his co-workers [2], for instance, consider that in radiation sickness the new formation of albumins is depressed or even completely arrested. There is still a possibility that albumins may leave the blood stream and enter the tissues as a result of the increased permeability of the vessel walls, which is especially likely when it is remembered that considerable morphological changes occur in the vessel walls in radiation sickness. The combination of the action of low temperature with that of radiation sickness led to a severer course of the disease and to correspondingly more pronounced disturbances of protein metabolism.

The mechanisms underlying the changes of a fall in the total protein concent and a sharp fall in the albumins with an increase in the  $\alpha_1$ -,  $\alpha_2$ - and  $\beta$ -globulins in response to the combined action of irradiation and local cooling have not yet been explained and further research in this direction is required.

# SUMMARY

Experiments were performed on rats. The authors determined the changes in the total protein content of the blood serum and the correlation of the protein fractions in response to the local action of low temperature and irradiation of animals with a lethal dose of X-rays. The combined effect of these factors brings about hypoproteinemia and a change in the correlation of the protein fractions towards a reduction of albumin fractions and an increase of the  $\alpha_1$ -,  $\alpha_2$ - and  $\beta$ -globulins.

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