CHANGES IN THE SERUM PROTEINS OF RATS WITH RADIATION SICKNESS

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Radiation sickness in animals is accompanied by regular quantitative and qualitative changes in the composition of the serum proteins. These changes, studied by electrophoresis of the serum proteins, may take the form either of an increase in the quantity or a redistribution during electrophoresis of the normal components of the serum proteins, or of the appearance of new proteins in the blood, and it may be noted that changes in the antigenic properties of certain tissues in irradiated animals have been described by several authors [1, 3, 4].

The object of the present investigation was to study the dynamics of the quantitative and qualitative changes in the composition of the serum proteins in irradiated animals.

EXPERIMENTAL METHOD

Experiments were carried out on male rats of the August line weighing 165-180 g. Some of the animals (30) received whole-body irradiation with γ-rays from Co^{60} in a dose of 630 R, and 10 rats acted as controls.

Every day 0.8-1 ml of blood was taken from the venous sinus of the eye of a few rats by puncture, with a minimal interval of 4-5 days between repeated punctures of the same animal. The blood serum of each animal was kept in a frozen state until the experiments, and it was subsequently investigated individually along with the serum of control animals subjected to the same treatment.

The total content of serum protein in the blood of the experimental animals was determined by Lowry's method, and calibration curves were plotted in relation to a solution of crystalline globulin of known concentration.

Electrophoretic separation of the serum proteins was carried out by Grabar's method in a 1% agar gel prepared from Soviet agar after purification. The conditions of electrophoresis and immunoelectrophoresis were identical with those described earlier [2]. The dried electrophoregrams were stained with amido black to detect the protein fractions, and these were estimated by examination in a Zeiss densitometer. Immunological development of the electrophoregrams was carried out with a mixture of 5 or 6 compatible rabbit antisera obtained by immunization of rabbits with blood serum of healthy or irradiated (7th day after irradiation) rats. The scheme of immunization of the animals and the conditions of purification and concentration of the antibodies were published earlier [2]. In special experiments the antisera were partially or totally exhausted by Heidelberger's method with the blood serum of control or irradiated rats and used to identify certain antigens in the immunoelectrophoresis experiments and in Ouchterlony's precipitation reaction. After washing in physiological saline, the immunophoregrams were treated with a 0.065% solution of cadmium sulfate to intensify the precipitation bands and placed in the "Herkules-1" photographic enlarger, where the magnified image of the preparations was projected directly onto photographic paper. The zones of precipitation of haptoglobin and ceruloplasmin in the dired immunophoregrams were identified in accordance with the recommendation of Uriel and Burtin [6]. The stained immunophoregrams and the photographs were subjected to further comparative analysis.

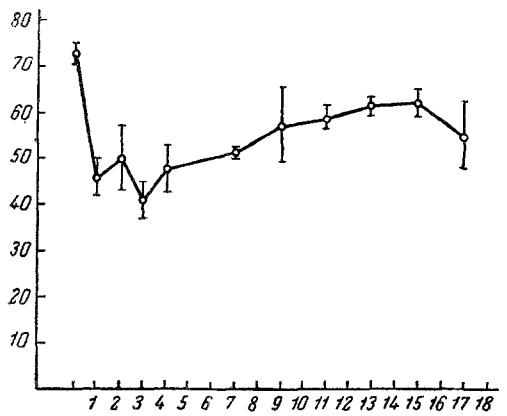

Fig. 1. Protein concentration in serum of rats in the course of radiation sickness. Along the axis of ordinates – protein (in mg/ml); along the axis of abscissas – days after irradiation.

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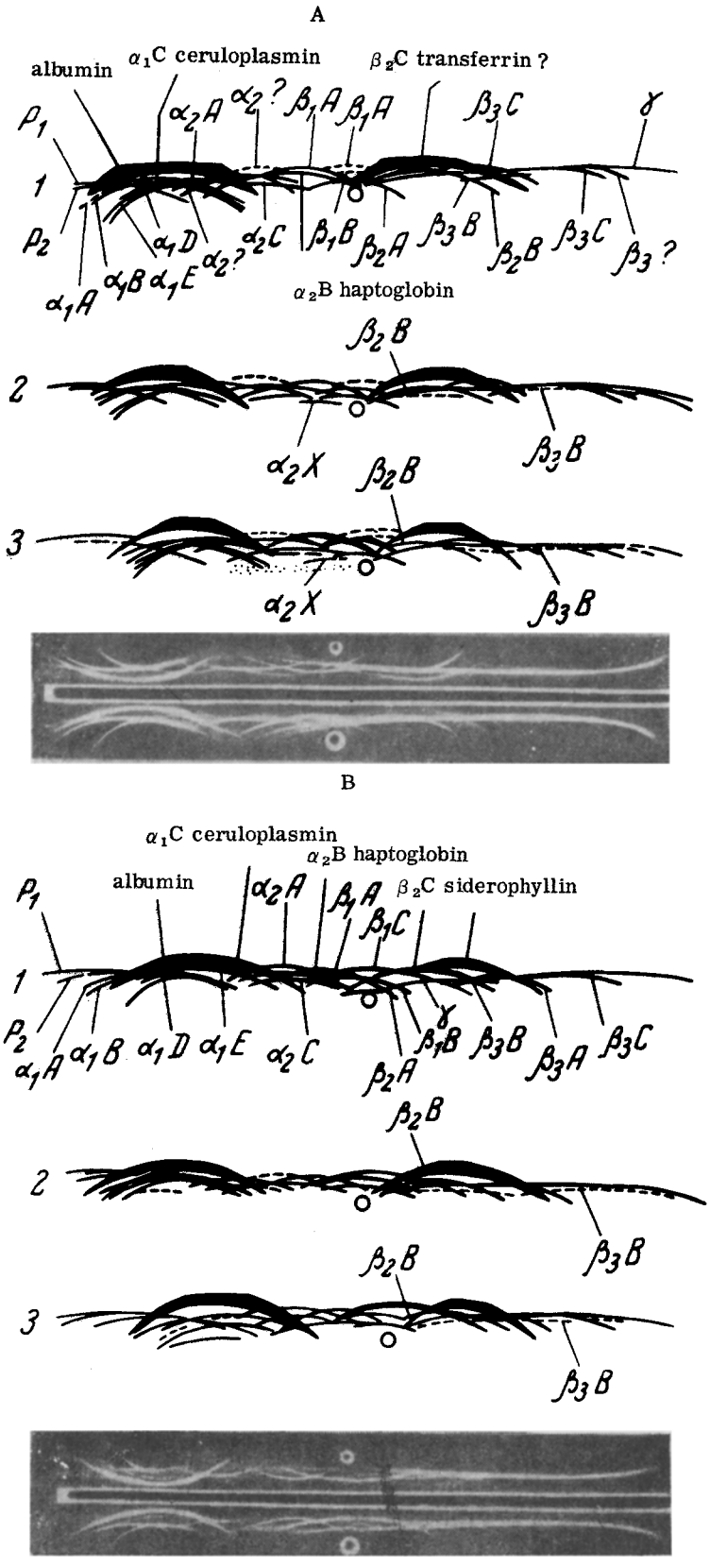

Fig. 2. Immunoelectrophoregrams of sera of control and irradiated rats. The following antisera were used: A) rabbit serum against irradiated rats; B) rabbit serum against unirradiated rats. The following antigens were used: 1) blood serum of unirradiated rats; 2) blood serum of rats on 7th day after irradiation; 3) on 15th day after irradiation; a and b) immunoelectrophoregrams of blood serum of rats on 13th day after irradiation, developed by corresponding antisera.

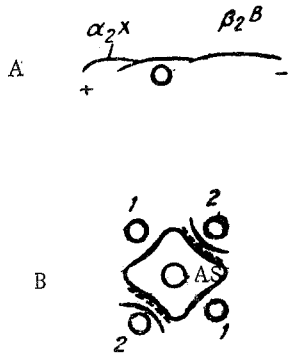

Fig. 3. Changes in antigenic properties of serum proteins in irradiated rats. A) Immunoelectrophoregram of blood serum of irradiated rats on 13th day after irradiation, developed by rabbit antiserum against antigens of irradiated animals, totally exhausted by serum of intact rats; B) precipitation reaction in agar between blood serum of control (1) or irradiated (2) rats and rabbit antiserum (AS) against antigens of irradiated animals, partly exhausted by blood serum of unirradiated rats.

EXPERIMENTAL RESULTS

Taking the blood from the animals of the control group had no significant effect on the quantitative and qualitative composition of the serum proteins and the hemocytogram of the peripheral blood. In this case the serum protein concentration varied very slightly within the range 73 ± 2 mg/ml. However, the analogous procedures with the irradiated animals aggravated the course of the radiation sickness, and all the animals of the experimental group had died by the 18th day after irradiation.

During electrophoresis the serum proteins were separated into four fractions: albumins, and α-, β-, and γ-globulins. The electrophoregrams of all samples of serum of the intact rats coincided completely if the conditions of separation were identical. Irradiation of the animals was accompanied by a rapid fall in the serum protein concentration, so that on the 3rd day the serum of the irradiated rats contained about half as much protein as before irradiation (Fig. 1). These changes primarily were connected with a decrease in the albumins and β-globulins, although the content of proteins with mobilities of the α- and γ-globulins rose slightly at these times. Later the concentration of the serum proteins was gradually restored, but even at the height of restoration (15 day after irradiation) it had not reached the serum protein level of the control animals. Restoration of the protein concentration in the serum of the irradiated animals was accompanied by an increase in the albumins and β-globulins, whereas during the development of radiation sickness the content of α- and γ-globulins gradually diminished.

To discover the character of the changes in the composition of the protein of a given fraction, the serum proteins of the irradiated animals were subjected to immunoelectrophoretic analysis. In the blood serum of the unirradiated rats, each of the antisera used revealed 21-24 antigens, the arrangement of which on the immunophoregram is shown schematically in Fig. 2.*

The immunochemical analysis of the serum proteins of the irradiated animals showed that the serum β-globulins reacted to irradiation before the others (Fig. 2). On the day after irradiation, among the β_3-globulins a fraction appeared which differed from the unchanged proteins in its electrophoretic mobility. The precipitation line of this fraction, determined by both antisera, was no longer bounded by the zone of the β_3-globulins, but continued as a long line parallel to the precipitation arc of γ-globulin. During the development of the radiation sickness this line became clearer and moved farther from the precipitation line of γ-globulin. By the 5th-7th day after irradiation, the physicochemical heterogeneity of this protein became more marked, so that its precipitation zone now took the form of a continuous line representing a double wave.

Similar changes on the 3rd-4th days after irradiation were shown by the β_2 B-globulin, the precipitation arc of which continued as a clearly distinguishable second wave in the zone of the β_1-globulin. Later the amount of fast β_2 B-globulin increased and the double-wave precipitation arc of this protein becam predominant among the proteins with mobility of the β_1-globulins. The physicochemical heterogeneity of the β_2 B-globulins was accompanied by partial changes in their antigenic specificity, and although both components were detected by antiserum against the antigens of the intact rats, the serum proteins of the unirradiated animals did not entirely exhaust all the antibodies against β_2 B-globulin in the serum against the irradiated rats (Fig. 3).

The decrease in the content of β-globulins in the early stage of radiation sickness affected the β_1B- and β_1C-globulin, the precipitation lines of which were detected with difficulty on the 4th day after irradiation or could not be detected at all. Evidently because of this the antiserum against the serum proteins of the irradiated animals contained few antibodies against β_1B- and β_1C-globulins, and revealed these components weakly even in the blood serum of the control rats.

On the 7th day after irradiation, a new protein was found in the serum of the irradiated animals for the first time, antibodies against which were present only in the homonymous antiserum. This protein, called α_2x, migrated during electrophoresis between the α_2- and β-globulins, but diffused badly into the agar: the precipitation line of α_2x-protein, even when developed by its antiserum in different dilutions, always lay close to the axis of migration

* Here and subsequently the proteins are named in accordance with Grabar's nomenclature [6].

of the proteins during electrophoresis. Antiserum against the antigens of the irradiated animals, totally or partly exhausted by the blood serum of the intact rats, during immunoelectrophoresis constantly detected the α_2x-antigen among the serum proteins of all the irradiated animals (Fig. 3). This antigen subsequently remained in the rat's blood until death.

The appearance of the new antigenic property in the blood of the irradiated rats was confirmed by the counter diffusion reaction in agar, using partly exhausted antiserum (Fig. 3). With the blood serum of rats taken on the 8th-18th day after irradiation, the exhausted antiserum gave an additional, well defined precipitation line. In these experiments, in the blood serum of some irradiated animals, starting on the 13th day after irradiation, one further thin precipitation line could be found, and its situation is shown schematically in Fig. 3.

In additional experiments the "radiation antigen" was regularly detected also in the blood serum of animals irradiated with a high dose (850 R) of γ-rays, but it was absent in unirradiated rats infected with a culture of Salmonella paratyphi B.

Radiation sickness in rats was not accompanied by changes in the properties of ceruloplasmin and haptoglobin, which were detected equally well and constantly in the serum of the control and irradiated animals.

The results of these experiments showed that the antigenic spectrum of the serum of rats of the August line (24 antigens) is highly stable and it shows little change in animals with acute radiation sickness: all components of the blood serum of the intact rats were present also in the irradiated animals, although in much smaller quantities. Irradiation of the animals was accompanied by an increase in the physicochemical heterogeneity of some normal protein, and by the appearance of a fraction of "fast" (β_2 B) or "slow" (β_3 B) proteins, retaining or only partially modifying their antigenic specificity. The precipitation line of such proteins became longer and developed into double waves. Similar changes in the properties of human γ-globulin have been described after irradiation of whole blood serum or of the γ-globulin fraction in vitro [7].

Similar changes in the electrophoretic mobility of some normal components of the serum proteins have been described by the authors following irradiation of mice (β_3-I-globulins) and monkeys (transferrin) [2], although the appearance of the fraction of "fast" β_2 B-globulins in rats bears a nonspecific character, because it can be observed in animals infected experimentally with a culture of S. paratyphi B. Following irradiation of the serum of intact rats with large doses in vitro, the electrophoretic mobility of the β_2 B- and β_3 B-globulins remained unchanged. The changes in the electrophoretic mobility of these proteins in irradiated rats, accompanied by the development of new antigenic specificity, may be the result of complex formation between these proteins and the many products of catabolism entering the blood stream of irradiated animals.

The "radiation antigen," discovered by the authors in the blood of the irradiated rats, is not a protein of Cx- or C-reactive type, because it does not appear during the acute phase of the infectious process. The origin of the α_2x-antigen has not yet been established, although its appearance is most probably associated with the entry of certain tissue antigens into the blood stream of lethally irradiated animals.

SUMMARY

Immunoelectrophoretic analysis of serum proteins in rats of the "August" strain irradiated with γ-rays in a dose of 630 R showed an increase in the heterogenity of β_2B- and β_3B-globulins with the appearance of electrophoretically "faster" or "slower" fractions retaining (β_3B) or slightly changing (β_2B) their antigenic properties. Beginning with the 7th day after irradiation the serum of irradiated animals is found to contain a new "radiation antigen" migrating during electrophoresis together with slow α_2-globulins and indicated by α_2x-protein. The protein fails to appear after irradiation of sera in vitro and is absent both in healthy and artificially infected nonirradiated animals.

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