STUDY OF AUTOSENSITIZATION IN RADIATION SICKNESS BY HOIGNÉ'S METHOD

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It is well known that autosensitization develops in many diseases in which cell structures are destroyed and breakdown products are absorbed. There are experimental data indicating the important role of this effect in the pathogenesis of radiation sickness, also [3]. For instance, local hyperergic reactions to tissue breakdown products have been obtained [4, 5], and the appearance of auto-antibodies to denatured protein of tissues and cytolysins has been established [1, 2].

In the search for a method capable of revealing the ability of sera of irradiated animals to react with auto-allergens in experiments in vitro we decided to use Hoigné's method, an account of which was published in 1955 [7-10]. This method was proposed for the demonstration of allergy in humans towards various medicines. The essential feature of this method is the employment of a highly sensitive photoelectric nephelometer to determine the turbidity produced by the reaction occurring when the sensitizer-allergen is added in optimal concentration to the plasma or serum of the patient's blood. Since this concentration is unknown, increasing amounts of antigen are added successively to the same sample of serum. In samples taken from normal people there is a steady decrease in the optical density of the medium.

In the blood plasma of sensitized persons combination with antibodies occurs at a particular range of dilution of the allergen, and this is manifested in a stoppage in the fall of the optical density of the medium (plateau on the curve) or even in an increase in density. When more allergen is added the optimal relationship with the antibody is upset and the reduction in turbidity of the mixture begins again.

We attempted to employ Hoigné's method (which some authors [6, 11] estimate as highly sensitive) for a study of the nature of the interaction of the plasma of normal and irradiated animals with aqueous extracts of intestine, liver, spleen, or bone marrow of the same organism.

METHOD AND RESULTS

To 1.7 ml of freshly prepared citrate blood plasma we added a succession of 0.1 ml batches of centrifuged aqueous extract of the organ in dilution 1 : 5120 to 1 : 80. After each addition we stirred the mixture in the glass with a glass rod. Two minutes later we measured the optical density of the medium on the home-produced photoelectric nephelometer (FEKN-57), and then added the next batch of allergen.

Experiments conducted on normal rabbits [11], guinea pigs and rats [7] showed that the blood plasma of these animals did not react with extracts of internal organs. In every case a steady decrease in the optical density of the mixture was noted. But after the animals had been exposed to lethal X-ray doses* we observed the appearance of a plateau on the curves and a temporary increase in the optical density of the plasma. This was re-

* The guinea pigs (300 r) and rats (600 r) were irradiated on the RUM-3 X-ray apparatus at voltage 180 kv, current 15 ma, focal distance 50 cm for guinea pigs and 40 cm for rats, dose rate 19-20 r/min, filters 0.5 Cu + 1 mm Al. The rabbits were exposed to a dose of 800 r on the RU-12 apparatus at the same voltage and with the same filters, focal distance 60 cm, current 14 ma, dose rate 29.8 r/min, with three tubes operating.

garded as a positive reaction. It occurred most often in the case of interaction with liver extract. Five out of 14 rabbits killed at different times after exposure to 800 r showed a positive reaction on the 3rd or 9th day. The results of one of the tests are shown graphically in Fig. 1.

The tests were positive (on 3rd-5th day) in eight out of 16 guinea pigs exposed to 300 r. The positive reactions occurred most often in the experiment involving the addition of liver extract, less often with bone marrow and spleen, and occasionally with intestine.

In an experiment on eight rats exposed to 600 r we used an extract of a mixture of equal quantities of all the organs mentioned. We observed positive reactions when extracts from normal nonirradiated animals were added to the plasma of irradiated rats.

The animals had to be killed for these experiments, however, and this ruled out the possibility of a study of the dynamics of the investigated effects in the same organism. Hence, we devised a method for obtaining solutions of autoantigens for the repeated conduction of the tests during the life of the animal. As an allergen we used a lysate of formed blood elements (mainly erythrocytes), which are known to possess antigens common to other cells of the organism. To obtain this allergen we took blood from the fasting animal, mixed it with a sodium citrate solution in the usual proportions, centrifuged the mixture, and washed the deposit twice with physiological saline. After this we added distilled water till the volume was equal to the initial volume of blood and again centrifuged the mixture. The clear colored upper layer was used in dilutions 1:15 360 to 1:30 in the experiments.

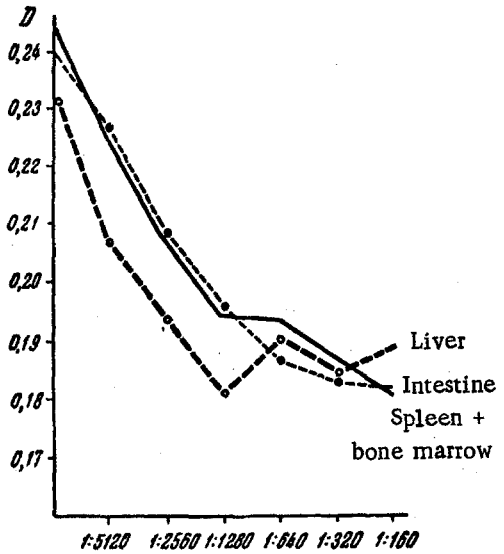

Fig. 1. Curves showing decrease in optical density of plasma on successive addition of increasing concentrations of extracts of organs of same rabbit killed on third day after exposure to X-rays. Distinct positive reaction with liver extract, and positive reaction with extract of mixture of bone marrow and spleen.

Experiments conducted on 12 rabbits and 19 dogs showed that in normal animals the successive addition of batches of erythrocyte lysate caused a progressive reduction in the optical density of the mixture. Four rabbits were subjected to homosensitization (intravenous injection of 5 ml of 20% extract of liver and intestine in equal proportions) and eight were exposed to a dose of 800-1000 r. Both types of treatment resulted in a change in the curves in a number of cases. A plateau appeared on the 3rd-5th day after treatment, thus indicating a reaction between the plasma and the organ extracts.

The main group of experiments by the method employing a lysate of autoerythrocytes was conducted on 19 dogs weighing 9-12 kg, which were exposed to a dose of 300 r, i.e. a lethal dose, of Co^{60} γ-rays* on the EGO-2 apparatus in uniform-field conditions at dose rate 350 r/min. Eight rabbits formed the control group, and 11 were treated with preparations of antibiotics and vitamins. In the experimental group six dogs survived and five died. All the animals showed the clinical picture of acute radiation sickness. The tests were made before exposure and on the 3rd, 10th, 20th, 30th, and 45th day after exposure. On the 3rd day we observed considerable changes in the properties of the plasma of irradiated dogs. There was a considerable reduction in the optical density of the plasma itself. This reduction, as Fig. 2 shows, persisted throughout the period of radiation sickness, both in the treated and in the control animals. Moreover, the blood plasma became capable of interacting with the lysate of its own erythrocytes. This was revealed by the appearance of a plateau on the curve, or even by an increase in optical density (Fig. 3). This last type of reaction had an unfavorable prognostic significance, since all the dogs in which the blood plasma revealed such a reaction died. Positive reactions occurred, both in the control and in the experimental group of animals (see Table). But in the experimental dogs they appeared 7-10 days later and their total number (57.5%) was less than in the untreated animals (84.2%). We noted

*Exposure to 300 r of Co^{60} γ-rays on the EGO-2 apparatus is equivalent in biological effect to exposure to a 600 r dose of X-rays.

Number of Positive and Negative Reactions of Dog Blood Plasma with Lysate of Its Own Erythrocytes

Group of dogs	Reaction	Before exposure	Day of investigation after exposure					Total number of positive reactions during first 20 days of radiation sickness	
			3rd	10th	20th	30th	45th	in absolute figures	in per cent
Control	Negative	8	1	1	1	–	–	3	15.8
	Positive	0	7	6	3	–	–	16	84.2
	Total..	8	8	7	4	Dogs	died	19	–
Experimental (treated animals)	Negative	11	6	4	2	2	3	17	42.5
	Positive	0	5	6	5	4	3	23	57.5
	Total..	11	11	10	7	6	6	40	–

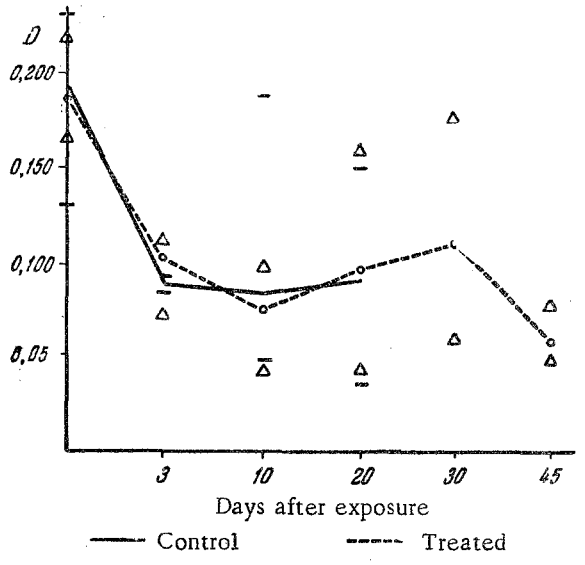

Fig. 2. Fluctuations of optical density of plasma of treated and control animals during acute radiation sickness.

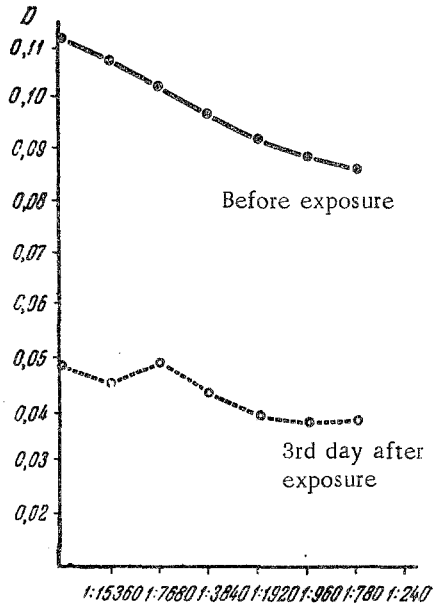

Fig. 3. Positive reaction of blood plasma of irradiated dog with lysate of its own erythrocytes on 3rd day after exposure.

a restoration of the normal form of the curve in the case of the surviving animals, although in some of them positive reactions still occurred on the 45th day.

Thus, in the early period after exposure to a lethal dose of X-rays or Co^{60} γ-rays Hoigné's method can reveal the immunological properties of the blood plasma in its interaction with a lysate of its own erythrocytes. We believe that the manifested ability of the plasma to react with cell breakdown products indicates the existence of a state of autosensitization: The addition of lysates of erythrocytes of animals of another species, foreign serum, or physiological saline to the plasma of irradiated animals caused a decrease in optical density of the mixture, i.e. a negative reaction. The diagnostic and prognostic significance of this reaction in radiation sickness produced by the action of various doses and kinds of ionizing radiation requires further study.

The immunological nature of the investigated reaction was confirmed by experiments with the plasma of rabbits immunized by foreign protein (of guinea pig). The successive addition of heterologous antigen resulted

in a steady decrease in optical density, while the addition of homologous antigen caused an increase in turbidity after a certain concentration of antigen was reached. We suggest that the speed with which the increase in optical density is manifested can be used to assess the avidity of the serum, since in this experiment we used sera with an equal titer of precipitins, but the times of appearance of the positive reaction were different. Thus, Hoigné's method can also be used in serum production. We should note that the intensity of the reaction to foreign antigen is much higher than to autoantigens, since the initiated increase in optical density becomes steadily greater and does not give way to a decrease, as is the case in interaction with tissue products.

SUMMARY

A method for preparing autoallergen solutions was developed (the liver, spleen, bone marrow, and intestine water extracts and lysates of erythrocytes) to stage the reaction according to the Hoigné method with the blood plasma of irradiated animals. As established, when tissue allergen solutions were added in increasing concentrations to the blood plasma of nonirradiated animals a constant and uninterrupted reduction of the optic density, i.e. negative reaction was seen. Beginning from the 3rd day after irradiation with lethal doses of γ-rays (Co^{60}) or X-rays, blood plasma acquires the ability to become bound with tissue antigens; this is manifested in the delayed optic density reduction of the medium, or even in its increase, which, according to Hoigné is evaluated as a positive reaction. The authors hold that the data obtained point to an early development of autosensitization in the irradiated organism.

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