PATHOLOGICAL PHYSIOLOGY AND GENERAL PATHOLOGY

CIRCULATION IN THE BLOOD OF TISSUE ANTIGENS PRODUCED BY RADIATION SICKNESS

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In our preceding publications [6, 7] we communicated the alterations in the antigenic properties of tissues resulting from irradiating animals with roentgen or gamma rays. We advanced the hypothesis that the ionizing rays alter the tissue albumins which then become antigenic stimulators and cause immunological reorganizations as, for example, a sensitization of the organism playing in this way an important role in the pathogenesis of radiation sickness.

By now we have gathered additional data describing the antigenic alterations induced in tissues by the ionizing radiation. By means of studying the reaction of active anaphylaxis with desensitization we have uncovered new antigenic capacities of the blood, the spleen, in the microsomes and hyaloplasm of the liver, in the mitochondria and microsomes of the mucosa of the small intestine of irradiated rats, as well as similar findings in the bone marrow, mitochondria and hyaloplasm of the mucous membrane of the small intestine of irradiated rabbits. *

EXPERIMENTAL METHODS AND RESULTS

The experiments were preformed on rats (150-180 g) receiving 2000 r from an experimental gamma source, the general activity of Co^{60} having 200 curies the dose strength being 34.1 r/minute. For purposes of examination, tissues were taken as a mixture from 6-8 animals. Rabbits (3-3.5 kg) were irradiated similarly with doses of 1100 r. We do not present now all the reactions of active anaphylaxis with desensitization, these illustrating the alterations in the antigenic properties of the tissues, as part of them [6, 7] has been published and others have given results analogous to those we present below.

In Table 1 are given protocols of examinations of irradiated rats 48 hours after exposure, the antigenic qualities of the tissues being studied. The framework of the experiment and the evaluation of the results follow the work done previously [7]. The table demonstrates that the blood of irradiated rats has antigens not present normally.

The literature [2] reveals the fact that even within the first hours after irradiation of the organism there can be observed the destruction of many tissues: bone marrow, lymphatic glands, mucous layer of the small intestine. It is also known [2] that the blood of the irradiated animals experiences an increase in its albumin content, the reason being the entrance of the albumins from the destroyed tissues. This is made all the more probable by the fact that vessel and tissue permeabilities are markedly increased in the irradiated animals [4].

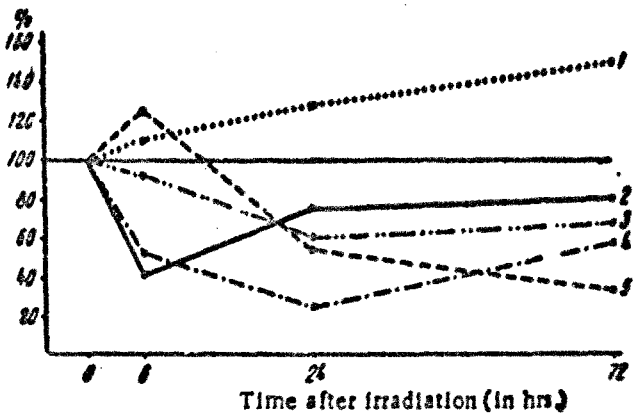
To trace the transfer of tissue albumins into the blood we set up an experiment in which radioactive methionin (S^{35}) was used to label tissue albumins. The methionine solution in an amount of 0.6 μc was given subcutaneously to the white rats. After two days some of the animals were sacrificed and the radioactivity of a whole series of tissues was determined. Half the animals were subjected to an irradiating dose of 800 r.

* The cellular microstructures were separated (mitochondria and microsomes) by the method of differential centrifugation in ASL-1 and ASL-2 separators.

TABLE 1

Differences in the Antigenic Properties of Irradiated and Normal Rats

No. Guinea Pig	sensitization		Desensitization							Challenging injection		
				1st. injection		2nd injection		3d. injection				
	Antigen	dose (in cc)	Antigen	dose (in cc)	reaction	dose (in cc)	reaction	Dose (in cc)	reaction	Antigen	Dose (in cc)	reaction
1	Blood irradiated rats	0.5	Blood irradiated rats	0.1	++++					Serum irradiated rats		
2		0.5		0.025	++	0.1	±	0.1	—		0.15	++
3		0.5		0.012	+	0.1	+	0.15	—		0.15	+++
4		0.5		0.012	+	0.05	+	0 1	—		0.1	++
5		0.5		0.015	+	0.05	±	0.1	—		0.1	++
6	Blood normal rats	0.5	Blood normal rats	0.025	++++					Serum normal rats		
7		0.5		0.012	++	0,1	—				0.1	—
8		0.5		0.012	+	0,1	++	0,1	—		0.15	—
9		0.5		0.012	++	0,1	—				0.1	—
10		0.5		0.01	++	0,1	—		—		0.1	—
11	Not sensitized			0.05	—	0.1	—	0.1	—		0,15	—



Redistribution of Labelled Substance (S^{35}) Methionin in the Organism of White Rats after Irradiation.
1) Liver; 2) bone marrow; 3) testes; 4) mucous intestinal membrane; 5) spleen. The radioactivity of the organs of the irradiated animals at the various stated time intervals is expressed in terms of percentage in relation to the radioactivities found at similar times in similar organs of the control animals sacrificed at the same time. Each point of the curve represents the arithmetic average of four determinations. The radioactivity of the bone marrow is expressed not in terms of weights of the tissue but as a unit of its weight.

TABLE 2
Titers of Complement Binding Antibodies in the Immune Sera Against the Tissues of Irradiated and Normal White Rats

Antigens	Antisera against						
	Mucous membrane irrad. rats		Mucous membrane normal rats		Sera irrad. rats		Sera normal rats
	64	65	62	63	68	69	67
Mucous membrane irrad. rats	1/3200	1/1600	1/1600	1/3200	1/50	1/50	1/200
Mucous membrane normal rats	1/1600	1/400	1/1600	1/3200	1/5	1/10	1/50
Liver irrad. rats	1/20	1/40	1/50	1/100	1/20	1/50	1/100
Liver normal rats	1/5	1/10	1/20	1/20	1/10	1/10	1/50

TABLE 3

Results of the Complement Fixing Reaction by the Antisera Against Liver Tissue, Taken at Various Time Intervals After Irradiation (2000 r)

Sera dilutions	Antisera against													
	5 hrs. after irradiation				one day after irradiation				3 days after irradiation					
	rabbit No. 26		rabbit No. 53		rabbit No. 19		Liver taken		rabbit No. 54		rabbit No. 18			
	Antigens													
	normal liver	liver 5 hrs. after irrad.	normal liver	liver 5 hrs. after irradiation	normal liver	liver 1 day after	normal liver	liver 1 day after irradiation	normal liver	liver 3 days after irradiation	normal liver	liver 3 days after irradiation	liver 1 day after irradiation	liver 5 hrs. after irradiation
1:20	++++	++++	++	++++	++++	++++	+	++++	+	++++	++++	++++	++++	++++
1:40	++++	++++	+	+++	++++	++++	+	++	±	++++	++++	++++	++++	++++
1:80	+++	++++	±	+	++++	++++	±	+	-	+++	++++	++++	++++	++++
1:160	+	+	-	-	++	+++	r	±	-	-	++++	++++	++++	++++
1:320	-	-	-	-	-	-	-	-	-	-	+++	++++	++++	++++
1:640	-	-	-	-	-	-	-	-	-	-	+	++	++	++
1:1280	-	-	-	-	-	-	-	-	-	-	-	++	+	-

TABLE 4

Influence of Intramuscular Injections of Immune Sera (Against the Mucous Membrane of the Intestinal Membranes of Irradiated Rats Receiving 650 r) on the Course of Irradiation Sickness

Material use	No. Animals	Average blood leucocyte count (in thousands)							survived a month	avg. life of rats dying (in days)
		before irradiation	in days after irradiation							
			2	6	9	13	17	20		
Inmune serum against intestinal mucosa of irrad. rats	7	13.0	0.8	0.6	—	—	—	—	0	6.9
Same serum after adsorption	7	14.5	22.0	15.0	13.6	11.0	17.6	17.1	1	14.0
Irradiated controls	7	16.0	4.0	3.0	3.2	4.3	1.8	—	0	11.0

At the expiration of 6, 24 and 72 hours following the irradiation both the irradiated and nonirradiated animals were sacrificed and the radioactivity of their tissues was determined. At each time interval 4 rats were sacrificed. In the irradiated rats even within 6 hours time redistribution (see Fig.) of the marked element takes place: the quantity of marked proteins in the bone marrow, mucous membrane of the small intestine and in the testes diminishes, while in the liver and spleen radioactivity rises. After a day and in 3 days the quantity of labelled substance within the liver rises, diminishing in the other organs.

The observed transfer of the labelled albumin, after irradiation, from one organ to the other and particularly to the liver can occur only by way of the blood stream. This in itself demonstrates that, when antigenic complexes arise within the irradiated animals, these abnormalities are associated with the fact that within the bed of the circulating blood stream are present tissue antigens from the intestine, bone marrow and so on. In so far as the antigenic capabilities of these tissues are altered by irradiation, these changed properties are expressed by the altered antigens now entering the blood stream.

If indeed these altered antigens from the intestine circulate in the blood and can enter the liver, then the antigenic differences between the tissues of the intestine, liver and the blood of the irradiated animals should diminish. To verify this supposition we prepared a liver antiserum and a blood antiserum in both irradiated and normal rats.

The antisera were prepared by 5-step intravenous immunization of rabbits. As antigens there were use aqueous saline extracts (1:10) from the intestinal mucosa and blood sera of rats. 8 days after the last injection of the antigen, the rabbits were exsanguinated the immune sera were obtained and with these the complement fixing reactions (CFR) were set up.

From Table 2 it is evident that the antigens obtained from the tissues of irradiated rats react in much greater titers with antisera against other tissues than do the analogous antigens of normal rats.

Thus the results of these reactions demonstrate that the antigenic differences between a series of tissues (mucous intestinal membrane, liver, sera) are less marked in the irradiated rats than the same differences between these tissues as seen normally.

The reaction alone is insufficient to form a reason for its cause. However, it is permissible to consider that the antigens from the irradiated intestine enter the blood stream and reach the liver, one of whose functions is known to be the interception from the blood of foreign albumins [1, 5]. In other words, the postradiation increase of antigenic similarity between the tissues of the liver, blood sera and mucous membrane of the intestine may be the result of the circulation within the rat organism of antigens which have been altered by the irradiation of the mucous intestinal membrane and the other tissues.

In Table 3 are presented the results of two experiments in which with the aid of the CFR test the degree of antigenic alterations caused in white rats at varying postradiation stages was determined; after 5 hours, one day and after 3 days. The last day is really the terminal stage as 2000 r caused death in the majority of animals by the 4th day. The methods of preparing the antigens, the antisera and the setting up of the CFR test have been detailed above.

From Table 3 it can be seen that antigenic differences between the normal and radiated tissues are in evidence even 5 hours after irradiation. True they are quite weak and can be caught only by observing the degree of hemolysis. Three days subsequent to irradiation the antigenic differences become quite pronounced and are revealed not only by the degree of hemolysis but also by the titer of the complement fixing antibodies.

The facts as presented here seem to be proof that irradiation causes in the organism an accumulation within the liver of circulating antigens produced from tissues, particularly the intestine, damaged by this radiation.

The data at our disposal does not permit us to state the precise role in the pathogenesis of radiation sickness played by the circulation in the blood of the abnormal antigens. However, it seems evident to us that they must act as pathological irritants and therefore inevitably must have some definite role in the pathogenesis of radiation sickness.

If this is true, then before future investigators there is uncovered a new path of influencing in some manner the pathologic process caused by the action of radiation energy. We have in mind the possibility of preparing specific immune sera against the altered antigens and, in this fashion, specifically neutralizing their pathologic action. There are enormous technical difficulties in the way of obtaining these specific antisera against the altered tissue components, as there is no certain method of adsorbing the cytotoxic antibodies against the normal components. For this reason the results of the experiment given in Table 4, can be doubly considered as being only preliminary, serving, however, to call attention to the observation that the adsorbed serum exerted a favorable influence on the course of the radiation sickness of white rats.

In the experiment we took a mixture of sera No. 64 and No. 65 against the mucosa of the intestine of irradiated rats (for titers of the sera see Table 2). One group of animals received the entire serum, the other serum adsorbed by the method of P. N. Kosyakov and others[3], the aim being to remove the antibodies against the normal tissue antigens; the third group serving for a control of the irradiation, all receiving gamma rays in 650 r dosage as described above. The antiserum was given intramuscularly the day before irradiation, on the day, and on the day following. The dose of the entire serum was 0.2, 0.5 and 0.3 cc; the doses of the adsorbed 0.5, 1.5 and 1 cc.

The results are given in Table 4.

From Table 4 it can be seen that the entire serum, containing many cytotoxins against normal tissues, had a markedly negative influence on the course of irradiation sickness in white rats. The same serum, after adsorption of the normal tissues, seemed to furnish a somewhat longer, as compared with the controls, life to the irradiated animals and appeared to prevent the development of leucopenia in them.

SUMMARY

Abnormal tissue antigens have been demonstrated as a product of irradiation of the organism. Proteins labelled with methonin S^{35} have been obtained from the bone marrow, spleen, testes and intestinal mucosa. Their passage via the blood stream to the liver has been demonstrated. Irradiation diminishes antigenic differences. As irradiation sickness progresses, these abnormal antigens must play an important role in the progress of the disease.

Adsorption of the antigens against the normal tissues opens a tentative path into very interesting future research for possible treatment of irradiation sickness.

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* In Russian.