PATHOLOGICAL PHYSIOLOGY AND GENERAL PATHOLOGY

ROLE OF AUTOALLERGY IN PATHOGENESIS OF RADIATION SICKNESS

N. N. Klemparskaya (Moscow)

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In the process of evolution of warm-blooded animals, special forms of reaction to introduction into the tissues and organs of protein irritants developed, consisting in specific changes in reactivity, in the direction of the raising or lowering of their sensitivity to immune bodies formed. This reaction ensures the stability of the organisms with respect to many pathogenic agents, but it may also under certain conditions lead to the death of the organism. As has been shown by I. I. Mechnikov [3], when the irritants are derived from the tissues of the organism itself, cytotoxic antibodies to them are formed, and grave pathological processes may develop as a result of the action of these antibodies on the organs and tissues of the animal.

The role of such autosensitization in the pathogenesis of a number of diseases has been demonstrated; examples are to be found in diseases of the eye, nervous system, kidneys, heart, and in neoplastic disease, toxemia of pregnancy, and trauma [6,8]. In our opinion, autosensitization phenomena bear a close relation to the problem of the pathogenesis of radiation sickness.

There are several possible approaches to the experimental study of the role of autosensitization in morbid conditions due to exposure to ionizing radiation. These are: 1) demonstration of the presence of antibodies acting specifically on the tissues of the given organism, and appearing after irradiation [4]; 2) a study of the tissue allergens of irradiated and control animals; 3) direct experimental proof, by means of introduction of suspensions of tissues into animals, followed by a study of the resulting response. We have been unable to find in the literature any references to work on this aspect of the study of the pathogenesis of radiation sickness. The present paper gives some of our results on this aspect of the problem.

The development of the pathological processes due to irradiation may be presented in the following way. It is known that the action of ionizing radiation involves destruction of cell structures, with considerable enhancement of the permeability of the walls of blood vessels, as a result of which considerable amounts of tissue substances which normally do not enter the circulation now do so. A result of this is the development of a sensitization to tissue products, which leads to production of cytotoxic antibodies. In view of the enhanced vascular permeability of the irradiated organism, these cytotoxins readily reach the tissues, and this may lead to cytolysis and death of the organism.

It is, of course, evident that experimental introduction of tissue suspensions into healthy animals is not fully analogous to the process of autosensitization in radiation sickness, since injuries to the central and peripheral nervous systems and to the vascular system due to direct action of radiation are missing. It should also be taken into consideration that a single introduction of tissue suspension into a single locality does not correspond with the multiple and continuous delivery of tissue substances from many parts of the body of an irradiated organism. In spite of these differences, however, we were able to discern characteristic effects of radiation sickness in animals after experimental introduction of tissue suspensions.

It is known that total irradiation causes damage to all the tissues of an organism, but it may be supposed that the autosensitizing of different tissues may vary, according to the special features of the structure of their proteins, to the extent of breakdown of cells after irradiation, to the vascularity of the given tissue, on which depends the rate and completeness of absorption of breakdown products, and to the amount of each tissue present, since on this depends the amount of breakdown products, and hence the intensity of their sensitizing effect.

In view of our earlier results (1955) on pathological changes in certain functions, which appear first of all in the abdominal region of irradiated animals (considerable lowering of the bactericidal properties of the skin, development of regional leucopenia, elevation of the bactericidal power of the intestinal contents), and of our finding that the most pronounced breakdown of cells is found in the intestinal epithelium, we attach great importance to sensitization of the organism by the intestinal tissues and contents.

As a test of our supposition regarding the prominence of autoallergic processes in the pathogenesis of radiation sickness, we attempted to reproduce the characteristic symptoms of radiation sickness without irradiation, by introduction into healthy animals of tissue suspensions (25-30% suspensions of fresh or preserved tissues in physiological saline), from healthy or irradiated animals of the same species; this amounted to bringing about a state of homoallergy, since induction of autoallergy by tissues from the experimental animal itself cannot be effected without the infliction on it of considerable trauma.

Injection of the suspensions was effected in various ways (into an ear vein, into the skin of the ear and tail, into a thigh muscle, under the skin of the abdomen and thigh), thus permitting of the assessment of the effects of rate of resorption and of irritation of the various reception sites. In some of the experiments the suspensions were mixed with a quarter of their volume of sterilized cream, in order to retard resorption; no pathological effect was found to result from the injection of cream alone.

Since the intestinal mucosa and contents are not sterile, we stored the material with an equal volume of glycerol for 1-2 weeks, in a number of the experiments; this did not affect the results obtained.

The experiments were performed on different animal species (76 rabbits, 462 mice, 43 guinea pigs), differing in sensitivity to experimental immunization and to radiation.

The survival rates of rabbits receiving 3-5 injections of small intestine tissue and contents from irradiated and control animals are given in Table 1 (intervals between injections 5-7 days, volume of suspension injected 1-3-8 ml).

TABLE 1

Mortality of Rabbits After Three Injections of Rabbit Small Intestine Suspensions

How injected	Intestine of rabbits dying of radiation sickness (800-1000 r)			Intestine of healthy rabbits		
	total	survived	died	total	survived	died
Intradermal	20	10	10	7	4	3
Subcutaneous	6	6	—	6	2	4
Intravenous	13	5	8	6	—	6
Intramuscular (with addition of cream)	6	6	—	8	8	—
Cream alone, intramuscular	4	4	—			

It appears from the data presented in Table 1 that mortality varied according to how the suspensions were introduced. The highest mortality resulted from intravenous injections, about half the animals died after intradermal injections, and none died from intramuscular injections (with cream). Death usually followed on the 2-5th day after the 2-3rd injection.

The lower allergenicity and toxicity of tissues of irradiated animals, as compared with those of controls, is clearly shown, particularly with subcutaneous or intravenous injection; this is evidence against the presence of specific "toxins" in the tissues of irradiated animals.

Loss of weight occurred in all the experimental animals, with raised temperature, changing to hypothermia before death, anorexia, and general debility. Leucopenia was frequently encountered, particularly after intravenous injections. Leucopenia resulting from intramuscular injections varied in intensity according to the size of the dose.

At autopsy of the rabbits dying after injections, pulmonary hemorrhages were observed as well as, in some cases, intestinal hemorrhages. Culture of the tissues showed that they, as well as the blood, contained intestinal microorganisms. Histological specimens showed the presence of cytolysis, which was most pronounced in the intestinal epithelium, and also frequently seen in the kidneys, liver, and spleen.

These effects are very similar to those encountered in radiation sickness.

We did very few experiments on suspensions of organs other than the intestine. In 6 experiments on the effect of intravenous injection of suspensions of liver from irradiated and normal rabbits (intravenous injection was found to be the most lethal with suspensions of intestine) we did not observe leucopenia, loss of weight, or death of the animals. Of 3 rabbits given intradermal injections of spleen suspension, one died, with signs of pulmonary hemorrhage and enlargement of the spleen, which weighed 6 g.

The effect of sensitization by tissues of other organs was studied in greater detail in mice and guinea pigs. As is known, the latter are more readily sensitized to various allergens than are other species, and intramuscular injection of intestine and liver tissue suspensions from other guinea pigs caused grave pathological disturbances in these animals.

TABLE 2

Mortality of Guinea Pigs Following Single Intramuscular Injections of Tissue Suspensions Mixed with Cream

Kind of tissue taken from irradiated (600 r) guinea pigs	Amount of 25% suspension (ml)	Number of guinea pigs		
		total	survived	died
Small intestine	7—10	5	—	5
	3—6	12	5	7
	2	2	1	1
	1	3	3	
Liver	6—8	6	1	5
	3	8	7	1
	1	2	2	
Cream alone	5	5	5	—

As appears from Table 2, the results of the injections depend largely on the amount of tissue injected. With more than 6 ml of suspension most of the animals died, whereas with doses smaller than 2 ml they survived, without developing morbid symptoms, other than slight swelling at the site of injection.

Many of the animals showed leucopenia, and all of them lost weight. Cultures of organs taken at autopsy either gave no growth, or revealed the presence of the normal microflora of the animal. Hemorrhages were found in the lungs and at the injection site, as well as less frequently in the intestines. In addition, those animals which survived large and medium doses of suspension developed round or oval ulcers, up to 2 cm in diameter, in the skin over the site of injection, although the material was deposited in a thigh muscle. These observations point to the possibly important role of tissue sensitization in development of trophic ulcers in irradiated animals.

In our experiments with mice we used tissue suspensions from both irradiated and normal animals, with or without cream, injected intramuscularly, subcutaneously, and into the tail.

As appears from Table 3, the differences between mortality following injection of tissue from irradiated and control animals are not significant. The condition of injected mice resembled that of mice suffering from radiation sickness; the animals were limp, unkempt, and had ruffled fur, they refused food, and many had diarrhea, they lost weight, and stopped growing. The viscera were either sterile, or they contained intestinal bacteria. Death usually ensued on the 3-5th day after the 2-3rd injection.

TABLE 3

Mortality in Groups of White Mice After Injection of Suspensions of Tissues From Irradiated and Control Mice (Radiation Dosage 600 r)

Tissue	Animal from which tissue taken	How suspension introduced					
		subcutaneously		into tip of the tail		intramuscular	
		without cream	with cream	without cream	with cream	without cream	with cream
Intestine	Control (not irradiated)	0/13	5/15	19/51	7/15	2/23	21/22
	Irradiated	0/15	19/23	2/26	16/26	8/10	28/32
Liver	Control (not irradiated)	15/35	0/10	5/26	0/10	—	9/12
	Irradiated	0/6	—	0/3	0/3	—	3/9

Our experiments on different species of animals thus show the lethal effect of parenteral introduction of certain amounts of tissues of animals of the same species, as well as the resemblance of the resulting pathological changes to those of radiation sickness.

These resemblances are not only superficial ones. The similarity of the nature of the processes involved in experimental autosensitization and in radiation sickness was confirmed by the results of experiments in which it was found that the condition of the animals was aggravated by combining the two pathogenic factors. Animals which had had injections of tissue suspensions exhibited higher mortality after irradiation than did uninjected animals, and their survival time was shorter. Conversely, injection of even the lowest doses of tissue suspension into irradiated animals was rapidly lethal to them. Thus 4 out of 6 guinea pigs died after injection of 3 ml of intestinal tissue suspension, given 24 hours after irradiation (50 r), as compared with 1 death out of 3 control guinea pigs. Five out of 6 guinea pigs given 3 ml of liver tissue suspension after a radiation does of 50 r died, as compared with only 1 death in a group of 8 control animals. Seven mice out of a group of 20 died within 5 days of receiving a radiation dosage of 600 r, as compared with 10 out of 20 mice previously given intestinal tissue suspension. Of seven rabbits sensitized to rabbit intestinal tissue, six died within 1-4 hours of receiving a dose of 800 r, as compared with only one death in a group of 16 unirradiated rabbits.

The more rapid onset of death may be explained on the following basis. Where a process of autosensitization is in progress, irradiation is equivalent to a "challenging" factor, and the rapidly developing allergic phenomena ensuing lead to the death of the animal.

Introduction of tissue suspensions into animals which have previously been irradiated, i.e. into animals in which autosensitization due to the action of radiation is in progress, similarly plays the part of a "challenging factor," causing death. Thus, when intestinal tissue suspensions are injected into the abdominal skin of rabbits on the 3rd day after irradiation, at a dosage of 100 r (the animals are usually still in good condition), they die within 12-20 hours, i.e. within a time much shorter than would have been the case with irradiation alone.

We consider that these findings are of importance for the understanding of the more serious effects of exposure to radiation in conjunction with processes involving resorption of tissue breakdown products (trauma, infection, pregnancy, etc.).

Thus, in our opinion, the basic process developing after the action of ionizing radiation is the autosensitization of the organism by degradation products of its own tissues, which enter the bloodstream; this takes place as a result of the increased permeability of the walls of the blood vessels of the irradiated animal. In this, the fundamental difference between the degree of autosensitization resulting from local and generalized irradiation is determined by the nature of the tissue products, and by the radiation dosage. The greater gravity of the effects of

generalized irradiation, as compared with the same dosages applied locally, is due, in our opinion, to the involvement in the processes of breakdown and resorption of a large mass of highly vascularized intestinal tissue (normally containing considerable amounts of tissue autolysis products), which is, as has been shown by L. B. Popelsky [5], toxic to animals when introduced intravenously.

The results of our investigation of autosensitization processes in radiation sickness may explain the lack of response of irradiated animals to antigenic and allergenic stimulation, for which no reason has yet been advanced. This lack of reactivity is shown, for example, by the almost complete absence of immunobiological reactions in active immunization of irradiated animals.

P. F. Zdrodovsky [2], and I. E. Alatyrtseva and S. A. Usmanova [1] have demonstrated suppression of reception of antigenic stimuli in sensitized animals. It may hence be supposed that autosensitization developing in an irradiated animal affects the activity of all of its physiological systems, rendering it unreceptive to new antigenic stimulation.

We think that the questions posed by B. N. Tarusov [7] in his review of the theory of the pathogenesis of radiation sickness can be satisfactorily answered on the basis of our autosensitization theory. A theory of pathogenesis of radiation sickness should explain why a small dose of radiation gives a large effect, why there is an incubation period, and why a secondary reaction appears, proceeding at a faster rate. The serious effects of radiation of relatively small energy content can be explained, in the light of our findings and interpretations, as being an effect which initiates the process of autosensitization, which then proceeds further at an accelerated rate, according to the laws of immunobiology. Its clinical manifestations become apparent after an incubation period, necessary for the formation and accumulation of cytotoxic antibodies.

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