DETECTION OF A STATE OF AUTOSENSITIZATION OF AN IRRADIATED ANIMAL BY MEANS OF LOCAL SKIN TESTS

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We know that allergic reactions play an important part in the development of phenomena which follow the action of ionizing radiation, and the desensitizing perparations are used with success in the treatment of radiation sickness [4]. At present, however, there is very little information about the nature of the allergens which may be responsible for these allergic processes. The possible roles of bacteria, foreign proteins from food and finally, of autosensitization to decomposition products of the body tissues have been suggested.

One of the generally accepted methods of detection of a state of allergy is the intradermal injection of the corresponding allergen to obtain a local hyperemic reaction. So far as we know this method has not yet been used in the study of radiation sickness.

In the present paper we describe the results of an investigation of the reaction of irradiated and healthy animals to the intradermal injection of various allergens.

EXPERIMENTAL METHOD

We used the method of labial injection of allergens, as described by Freund and Stone (into the skin of the upper lip on one side), and recommended by them [7] in experiments on mice and rats when studying the state of active and passive sensitization to foreign protein. Experiments were carried out on 532 white mice and 10 guinea pigs. Identical material was always injected simultaneously into control animals and animals irradiated (on the 1st - 3rd - 5th days) with lethal doses of x-rays (RUM 3 apparatus, 180 kv, 15mA, filter 1 mm copper + 0.5 mm aluminum, distance 50 cm, dose rate 20-21 r/ min). The mice were irradiated with a dose of 600 r, causing death of all the animals on the 7th-9th day, and guinea pigs with a dose of 500 r (death on the 8th-10th day). Every day for 7 days the site of injection was examined and some of the animals used for a histological study of the tissues of the upper lip where the injection had been given and also of the symmetrically opposite area of the same animal.

The allergens used were: 1) sterile milk; 2) sterile horse serum; 3) a suspension of a 24-hour agar culture of Escherichia coli with a density of 100 million organisms per 1 ml of physiological saline;* 4) extracts of homologous tissues of healthy and irradiated animals – small intestine, liver, spleen and bone marrow.

The extracts were prepared from freshly removed tissues (of an animal of the same species), cut up finely with scissors and placed in physiological saline to give a concentration of 20%. The supension was filtered through two layers of sterile gauze and injected at once. The volume of material injected varied from 0.05-0.1 ml (usually) to 0.15 ml.

EXPERIMENTAL RESULTS

Injection of the above mentioned substances into healthy animals caused no perceptible external changes

* The injection of large numbers of bacteria is unsuitable, since in consequence of the action of the endotoxin necrosis of the tissue cells takes place, and then in addition to the protein of the bacteria, the products of tissue destruction also become an active factor.

in the skin of the upper lip during inspection for 16-24 hours and later up to 5 days. Histological examination revealed microscopic cellular infiltrations but without necrosis, edema or other changes (Fig. 1). The mice remained alive and active, looked well and had good appetite. The same results were obtained after injection of the animals with these substances 24 hours after irradiation (28 mice, 3 guinea pigs). In spite of the fact that lethal doses of radiation were given, no clinical features of radiation sickness had developed at this period and the animals did not differ in their outward appearance from controls, although a slight loss of weight (of 1-2 g) could be registered, and in a number of cases there were signs of leucopenia.

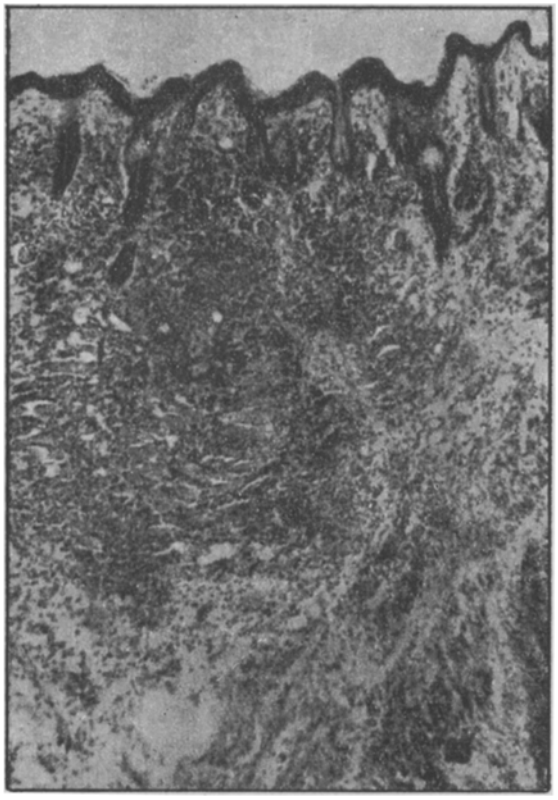

Fig. 1. A microabscess in the skin of the lip of a healthy mouse after injection of an extract of the intestine of an irradiated mouse. Stained with hematoxylin-eosin. Magnification 4 × 10.

Fig. 2. Local hemorrhagic reaction in mice in a positive labial test.

Absolutely different results were obtained after injection of these allergens into mice on the 3rd day, or guinea pigs on the 7th day after irradiation. As can be seen in the Table, it was only in the irradiated animals that a well marked reaction of hyperemic type could be observed after injections of a particular tissue, namely extracts of the small intestine. Under these circumstances acute edema was observed to develop, often involving the whole half of the lip and extending into the neck (Fig. 2). Necrosis of the central part of the edematous tissues very rapidly appeared, sometimes terminating in sloughing of the necrotic tissues. Histological specimens showed total necrosis of the tissues on a background of well marked edema and hemorrhages. No cell reaction on the part of the blood cells (neutrophilic leucocytes) nor in the form of proliferation of the local tissue cells at the periphery of the necrosis could be observed (Fig. 3).

Not only were the hemorrhagic features visible in microscopic preparations, but they could also be clearly seen by external inspection of the animals.

Where the labial reactions were positive, besides the local reaction changes were also observed in the general condition of the animals, which became sluggish, dirty and and unwilling to eat. The body weight fell considerably and the degree of leucopenia increased. Many of the mice and guinea pigs with a strongly positive reaction did not survive for 48 hours after the moment of injection and died on the 4th-5th day after irradiation, although the other irradiated animals from the same batch which were not injected with an extract of homologous intestinal tissue died from radiation sickness only on the 8th-9th day.

We observed the strongest positive reaction in mice after irradiation with a dose of 600 r and injection of extracts of tissue of the small intestine on the 3rd day after irradiation. After the action of larger (750 r) and smaller (400 r) doses this reaction was also observed, but it could be detected only by histological examination. External changes in the form of edema and visible hemorrhages were absent from these animals (52 mice). The reaction in mice irradiated with 600 r and injected with tissue extracts after more than 3 days was also less pronounced. Presumably for the most obvious manifestation of this reaction, not only the dose of radiation would be important, but also the presence of specific reactive properties in the

TABLE

Results of Macroscopic Reading of the Labial Reaction in Mice Irradiated with 600 r, after Injection of Allergens on the 3rd Day after Irradiation, and in Healthy Animals

Materials used for labial injection	Mice											
	irradiated						healthy					
	total No. of mice	in which the reaction was					total No. of mice	in which the reaction was				
		–	+	++	+++	++++		–	+	++	+++	++++
Extract of small intestine of healthy mice	34	24	3	5	2	—	25	22	3	—	—	—
Extract of the small intestine of mice irradiated with 600 r (on 3rd day)	58	15	14	5	11	13	28	27	1	—	—	—
Liver, spleen, bone marrow of healthy mice	32	29	3	—	—	—	25	22	3	—	—	—
Liver, spleen, bone marrow of mice irradiated with 600 r (on the 3rd day)	28	27	1	—	—	—	25	24	1	—	—	—
Physiological saline	30	30	—	—	—	—	15	15	—	—	—	—
Suspension of a 24-hour culture of Escherichia coli	28	23	5	—	—	—	20	16	4	—	—	—
Sterile milk	15	13	2	—	—	—	10	10	—	—	—	—
Horse serum	17	14	3	—	—	—	8	8	—	—	—	—
Total . . .	242	175	31	10	13	13	156	144	12	—	—	—

Note: – no reaction; + slight edema; ++ considerable edema; +++ extensive edema and hemorrhage; ++++ edema speading into the neck and head and intensive hemorrhage.

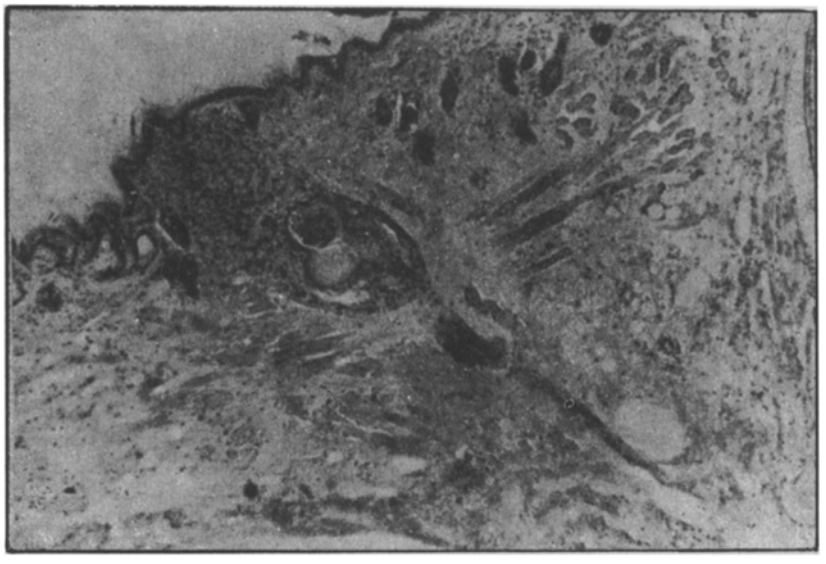

Fig. 3. Section from the site of injection of an extract of the intestine of an irradiated mouse on the 3rd day after the action of x-rays in a dose of 600 r. Necrosis, hemorrhages, absence of infiltration. Stained with hematoxylin-eosin. Magnification 4 × 10.

irradiated animal. Usually we injected 0.1 ml of an extract of intestinal tissue; an increase in this volume to 0.15 ml increased the number of positive reactions.

Thus, of these three different types of allergens which we have mentioned – soluble foreign proteins from mammals, bacterial proteins and homologous tissue extracts – it was only after injection of the last that a local labial reaction, in our opinion hyperergic in character, could be obtained at certain times after irradiation. By the use of this method it is possible to detect in an irradiated animal a state of autosensitization to tissue products of the same species of animal. As can be seen from the results in the Table, a positive labial reaction can be obtained (by this method andiin the conditions of our investigation) only to tissue from the mucosa of the intestine of both healthy and irradiated animals of the same species. Extracts of liver, bone marrow ans spleen failed to give a positive reaction. Tissue from irradiated animals is a more active agent, which may be due, for example, to changes in the antigenic properties of the tissues in an irradiated animal [5]. The fact that intestinal extract shows the greatest activity has a definite relation to its high degree of trauma after exposure to radiation [1, 2, 3, 6, 8].

It is interesting that the injection of living bacteria in the doses given not once caused such a reaction, although cultures taken from the site of injection demonstrated the presence of proliferation of the organisms in the tissue of the lip. These findings are in conformity with the indications of a lowering of the reactivity of the irradiated animal towards bacteria and shed doubt on a leading role for the latter as a source of sensitization in radiation sickness. It may be assumed that in association with the lowered reaction of the irradiated animal, the proliferation and abundant accumulation of bacteria are not the cause of radiation sickness but its consequence, complicating the later course of the disease.

In all the variants of the experiments we injected into the skin of the upper lip of the animals,as extracts, ready prepared products of destruction of cells from different organs, and as seen from the results given the activity of the extracts in the test studied was not identical. This could depend both on variations in the content of allergens in the different organs, and on their partial or total destruction in the process of preparation of the extracts. Finally, all the allergens might not pass into the extracting fluid from the cells and be active in the dose which we used.

We attempted to discover the role of the products of cell destruction formed in the tissue of the lip itself under the influence of indifferent cell-destroying agents. For this purpose we injected into the skin of the lip of animals 0.1 ml of distilled water (49 mice). A few hours later edema developed at the site of injection in both healthy and irradiated animals, and disappeared after 24 hours. In healthy mice, and in mice taken within the first 24 hours after irradiation with a dose of 600 r, no perceptible changes appeared subsequently at the site of injection. In histological preparations the presence of only slight cellular infiltration could be observed. If the distilled water was injected on the 3rd day or later, then the mice irradiated with 600 r developed a typical positive labial reaction, with edema, tissue necrosis and hemorrhages. However, it appeared somewhat later than after injection of intestinal extracts (not 18-24 hours, but 3-4 days after the test injection).

Thus, by the labial injection of extracts of certain homolgous tissues and by the creation at the site of injection of conditions in which products of their destruction are formed and partially extracted in the tissues themselves, a local reaction of a hyperergic type can be produced in an autosensitized animal.

The study of these local manifestations of the general state of autoallergy is of theoretical and practical importance not only in radiation sickness but also in other diseases associated with the action of products of tissue destruction on the body. It is essential to point out the importance of injecting the allergens into definite areas of the body, most sensitive to allergic reactions, for example the skin of the upper lip, since we have satisfied ourselves that negative results are obtained if these tests are done on the skin of the thigh or the abdomen.

SUMMARY

The authors studied the possiblity of detecting the state of sensitization of the irradiated body to the products of disintegration of its own tissues and of those obtained from animals belonging to the same species (labial administration by Freund-Stone's method). It was established that in definite doses of irradiation a hyperergic reaction with edema, necrosis and hemorrhages occurs at the site of administration at a definite period of the radiation sickness. Such a reaction is caused only by the extracts of homologous tissues and not by the introduction of bacteria or foreign proteins. The reaction is negative in the nonirradiated animals and during the first 48 hours after the irradiation.

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* In Russian.

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