THE RELATIONSHIP BETWEEN THE LOCALIZATION OF RADIATION INJURY AND THE FUNCTIONAL STATE OF AN ORGAN

COMMUNICATION 3. MORPHOLOGICAL CHANGES IN THE UTERUS OF ALBINO MICE IRRADIATED IN VARIOUS PHASES OF THE ESTRUS CYCLE

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In previous investigations [2] we showed that the state of functional strain imposed on an organ during exposure to ionizing radiation is determined mainly by the localization of the radiation injury in the particular tissue system concerned. In these investigations the increased functional load on the organs was produced artificially: by administration of drugs (especially diuretics). It thus became necessary to investigate the role of increased effort of an organ when in a state of physiological functional strain during exposure to radiation. The most convenient test object for such experiments appeared to be the uterus of sexually mature animals. In this case the period of estrus must be regarded as a state of high functional activity of the organ, while the period of diestrus is a time of physiological rest.

We found no references in the accessible literature to the study of the morphology of the uterus in radiation sickness in relation to the phase of the estrus cycle. No information on the radiation pathology of the normal uterus is to be found even in the largest treatises on the morphology of radiation sickness [1, 3].

EXPERIMENTAL METHOD

The investigation was conducted on 220 sexually mature pure bred female mice of line C57. Throughout the period that they were kept in our laboratory (3 years) no spontaneous disease of the reproductive organs was observed in mice of this line. The phases of the estrus cycle were determined by the usual method of cytological examination of vaginal smears. We made histological investigations of the ovaries, uterus, and genital passages of a large series of normal animals in order to obtain an accurate idea of their structural features in relation to the phase of the estrus cycle. These preliminary investigations formed a reliable criterion of the possibility of a correct interpretation of the morphology of the radiation injury. The ovaries, fallopian tubes, uterus, and vagina were embedded whole in paraffin wax for microscopic examination. The material was studied in serial sections using different staining methods (eosin, hematoxylin-eosin, picrofuchsin, iron-hematoxylin, mucicarmine, and so on).

The experimental animals were mice in the stage of estrus, and the controls were mice in the stage of diestrus. The experimental and control animals were always irradiated simultaneously in two plywood boxes placed side by side under the tube of the apparatus. The animals received whole-body irradiation from an RUM-3 apparatus under the following technical conditions: voltage 180 kv, current 10 ma, filter 0.5 mm Cu + 1 mm Al, dose rate 45-48 r min, focus distance 30 cm, field of irradiation 10 × 15 cm, dose 1400 r (measured by a GRI dosimeter).

EXPERIMENTAL RESULTS

All the irradiated animals die from an acute form of radiation sickness. When the results of the investigation were evaluated, attention was paid to the duration of survival of the animals, the clinical picture of the disease, and the morphological characteristics of the state of the internal reproductive organs.

The mice irradiated in the period of estrus survived on the average 6.7 ± 0.6 days, while the animals irradiated during diestrus died on the average after 7.7 ± 1.3 days.

The clinical picture in the experimental and control animals was that usually seen in acute radiation sickness. However, during the last days of life of many of the experimental animals a peculiar type of behavior was observed. The mice assumed a flexed position, and as it were "sat" by the wall of the jar. This position probably provided maximal relaxation of the muscles of the abdominal wall, and was evidently due to pain in the region of the pathological uterus.

Macroscopic examination of the dead experimental mice invariably showed marked swelling and congestion of the tubes and the cornua of the uterus, against the general background of radiation sickness. Petechial hemorrhages were often observed on the serous membrane, in the fatty areolar tissue surrounding the tubes and ovaries, and on the surface of the ovaries. Sometimes in the uterine cavity there were blood-stained mucous contents or blood clots. In only a few control animals were occasional small hemorrhages observed on the surface of the ovaries.

The periods of survival of the irradiated animals varied so considerably (from 3 to 14 days) that the progress of development of the radiation injury could be studied. The morphological changes in the ovaries of the experimental and control animals were identical and did not differ from those usually observed in radiation injuries, so that we shall not consider the results of these experiments further.

The most conspicuous changes were observed in the cornua of the uterus. At death of the animals 3-4 days after irradiation, multiple small foci of necrosis were observed in the mucosa, submucosa, and interstitial tissue of the muscular layer of the uterus. Amid the pale, amorphous masses of detritus, shadows of disintegrating leukocytes could sometimes be observed. In the surface epithelium a few cells could be seen which had died in a state of division. Their cytoplasm was homogenized, and highly oxyphilic, and the hemoglobin formed large masses without the usual structural details. Somewhat later (5-6 days), the epithelium began to die, at first the surface epithelium and later the glandular. Over a wide area the outlines of the cells of the surface epithelium were indistinct, and large and small, strongly basophilic granules of chromatin were arranged haphazardly in the pale cytoplasm (Fig. 1). Here and there the surface epithelium was absent or filled the interior of the uterine cornu in the form of an amorphous mass. The cells of the glandular epithelium were reduced in size, with a dense, intensively stained cytoplasm. The nuclei were small, compact, and strongly basophilic, or disintegrating into separate, irregular fragments. Some glands were distended with secretion, and over a wide area were without epithelium. The submucosal and intermuscular areolar tissue was edematous and its blood vessels engorged; small hemorrhages and accumulations of hemosiderin were found here, the latter being free or in the cytoplasm of macrophages. The muscular elements of the uterus preserved their usual structure. In some cases, however, in which pregnant animals were accidentally irradiated, the myometrium was considerably affected. The chromatin in the large cells of the hypertrophied muscles formed large granules, concentrated beneath the nuclear membrane or scattered unevenly throughout the pale cytoplasm, with no sharp outlines (Fig. 2).

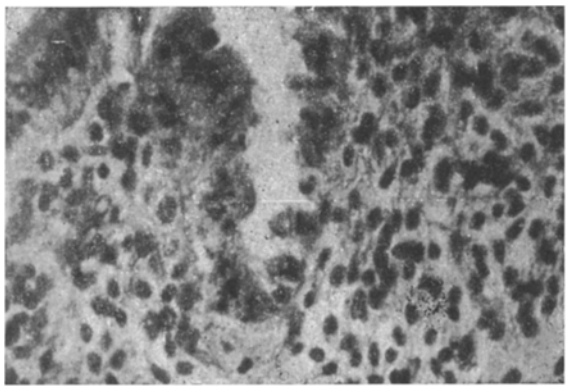

Fig. 1. Mucous membrane of the uterine cornu after whole-body irradiation of a mouse during estrus. Pyknosis and rhexis in the surface epithelium. Stained with hematoxylin-eosin. Magnification: objective 45 ×, eye-piece 12 ×.

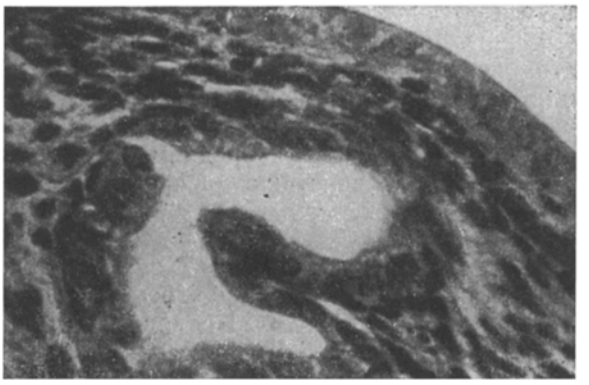

Fig. 2. Mucous membrane of the uterine cornu after whole-body irradiation of a mouse during diestrus. Preservation of the structure of the surface and glandular epithelium. Stained as above. Magnification: objective 45 ×, eye-piece 12 ×.

The walls between the arteries and arterioles were considerably thickened as a result of permeation with a pale mass of protein flocculate. The endothelium, with intensively stained small, compact nuclei, which in transverse sections of the vessels formed a striking, clearly outlined "dotted line," was especially prominent against the background of the pale, swollen vessel walls. The vascular changes were more marked in the fatty areolar tissue around the fallopian tubes and ovaries; here a considerable perivascular edema was observed.

Most neurons in the intramural ganglia and in the ganglia of the parametrial areolar tissue were in a state of necrobiosis. Their cytoplasm was pale, with diffuse borders, and many cells were without nuclei and contained granules of chromatin, or their nuclei were small and compact, and no nucleoli were visible.

In the stratified squamous epithelium of the cervix uteri there were many small areas in which the cells were enlarged, pale, and irregularly shaped, with pyknotic or disintegrating nuclei. Here and there desquamation of the dying cells could be observed, and sometimes they remained in the layer of surface epithelium. Signs of marked dystrophy of the mucous membrane were seen in the glandular epithelium, and only individual cells were in a state of rhexis or pyknosis. No changes affected the submucose and the muscular elements of the cervix uteri. The structure of the fallopian tubes was normal.

The morphological changes in the uterus of the control animals were slight (Fig. 2). The radiation injury was confined to necrobiosis of solitary cells of the localized perivascular edema was sometimes observed. The structure of most neurons was preserved.

The phase of estrus is characterized morphologically by the presence of necrobiotic changes in the uterine mucosa, and it accordingly becomes necessary to find criteria for differentiation between physiological changes and the manifestations of radiation injury. The most reliable and constant sign of radiation pathology is death of leukocytes in areas of infiltration typical of the period of estrus. In the latter phase of the course of radiation sickness, foci of necrosis arose at the sites of these areas of infiltration. Other characteristic signs were the absence or the small number of mitoses in the surface and glandular epithelium and the constant death of the dividing cells. Finally, the picture of necrobiosis itself was extremely typical. In a radiation injury caused by this level of energy signs of rhexis and pyknosis always predominate, whereas in the physiological process lysis is observed. In this respect the changes were particularly demonstrative in the glandular epithelium, where, instead of the usual, typical mucous dystrophy, even when considerable tissue edema was present shrinking and condensation of the cells were observed. The earliest signs of development of radiation injury were observed in the elements of the areas of leukocytic infiltration of all the layers of the walls of the uterus. Necrobiotic changes subsequently developed in the structural elements of the uterus itself, the first cells to die being the dividing cells of the regenerating surface epithelium. At the same period of development of the pathological process morphological changes were detected in the vascular and nervous systems of the organ.

The exceptional contrast between the morphological picture of the radiation injury in the experimental and control animals, and the strict constancy of these changes are proof of the fact that the degree of manifestation of a radiation leision is directly dependent on the state of functional strain of an organ.

It is a noteworthy fact that this principle is also fully applicable to the different parts of an organ, to individual tissue systems, and even to individual tissue cells. All the changes described were most marked in the middle areas of the uterine cornua, and their intensity diminished appreciable in the distal and proximal divisions of the organ. The radiation injury of the cervix uteri, for instance, was limited to the presence of necrotic foci in the surface epithelium. The fallopian tubes in general remained intact after exposure to radiation. In other words, a selective involvement was observed of those areas of the uterus carrying the maximal functional load during estrus and pregnancy.

From this point of view our accidental observations on irradiated pregnant animals are of considerable interest. In these conditions of functional hyperplasia, the muscular elements of the uterus were extremely sensitive to the action of ionizing radiation, and they showed the typical picture of radiation injury. Meanwhile, in resting conditions, in the absence of pregnancy, no changes detectable by the usual morphological methods of investigation could be found in the uterine muscle of the irradiated animals. Finally, the study of the nervous system of the organ gave a demonstrative picture. A definite impression was gained that after irradiation when the uterus was in a state of functional strain, the necrobiotic changes in the neurons were much more marked than when it was in a state of rest.

The phase of the estrous cycle determines not only the character of the local changes in the reproductive organs of the irradiated animal, but also influences the whole course of the disease. Irradiation during estrus worsens the course of the radiation sickness and results in a statistically significant ($t = 2$) shortening of the survival period of the animals.

We believe that these findings show that the localization of the radiation injury in a given organ is largely determined by the level of functional strain on the organ during irradiation. A high functional load on an organ during irradiation results in morphological changes which mainly affect this particular system of the organism.

LITERATURE CITED

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