COMPARISON OF INJURY TO SEXUALLY IMMATURE AND MATURE RATS BY HIGH DOSES OF X-RAYS

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Experimental proof has now been obtained that the reaction of the growing animal to ionizing radiation shows a number of fundamentally distinctive features. From a comparatively large number of facts in the literature, it is possible to speak of concrete differences in the reactions to irradiation characteristic of a particular age period [1-9]. The great majority of investigations deal with the study of the influence of doses producing acute radiation sickness [4, 7, 8, 9]. This is due to the necessity for problems in medical practice to be solved in their proper order of urgency. Accordingly, information on the action of small and very large doses of radiation on the growing animal is very scanty. Meanwhile it is obvious that the solution of the problem of age radiosensitivity may be achieved only by the investigation of the reaction of the animal to irradiation with a very wide range of doses. It is especially important to define the threshold doses, beyond the limits of which a qualitative change in the reaction of the animal to irradiation is observed.

In the present research an attempt is made to study the distinctive manifestations of the pathological processes arising in response to the action of large doses of x-rays, causing, on the one hand, the so-called intestinal death, and on the other hand, the development of the pathological condition termed by the majority of researchers "the acutest form of radiation sickness."

METHOD

We studied the action of large doses of x-rays on young rats aged 25-30 days (weight 50 g) and compared it with the action of the same doses on sexually mature rats aged 3-4 months (180-250 g).

Altogether we investigated 311 animals, of which 131 were sexually immature and 180 mature. As an additional control of the postmortem findings 21 unirradiated rats were used.

Irradiation was given by means of a RUM-3 apparatus, with voltage 180 kv and current 20 ma. No filters were used. The distance from the anode of the tube to the animal's spine of 24 cm was used rarely and only for the smallest rats. The experimental rats were placed in pairs at the moment of the experiment in specially made plywood boxes. We investigated the reaction to irradiation with x-rays in doses of 10,000, 15,000, 25,000, and 50,000 r. The dose rate varied from 249 to 475 r/min.

RESULTS

The results of the observations on the times of death of the animals are shown in Fig. 1, from which it may be seen, firstly, that the proportion of rats dying at a given period is determined by the age of the animals as well as by the size of the dose. If the course of the curves for each dose is compared for the sexually immature and

TABLE 1

Mean Duration of Survival of Rats Depending of Age and Dose of Irradiation (the figures in parentheses indicate the mean square deviation)

Dose of irradiation (in r)	Mean duration of survival of dying rats (in days)	
	sexually mature	sexually immature
10 000	3.5 (± 0.6)	3.0 (± 0.42)
15 000	3.5 (± 0.44)	2.8 (± 0.9)
25 000	1.6 (± 0.58)	1.3 (± 0.57)
50 000	1.0 (± 0.25)	1.0 (± 0.01)

TABLE 2

Figures Summarizing the Changes in Weight of the Rats at the Time of Death, Depending on their Age and the Dose of Irradiation

Dose of irradiation (in r)	Sexually mature rats		Sexually immature rats	
	wt of cadavers (as % of initial wt, taken as 100 %)		wt of cadavers (as % of initial wt, taken as 100 %)	
	limiting variations	mean value	limiting variations	mean value
10 000	73–94	83	58–86	72
15 000	69–89	79	64–95	79
25 000	80–97	88	87–90	88
50 000	91–97	94	76–96	86

mature rats separately, these differences can be detected, and as the dose increases they become obliterated. The differences were most marked at a dose of 10,000 r and least at 50,000 r. For example, after irradiation with a dose of 10,000 r, at the end of the first 24hours 2% of the rats had died. At the end of the second day there were no further deaths, at the end of the third day the mortality rate reached 90%, and at the end of the fourth – 100%. Consequently, the bulk of the young rats died in the course of the third day (90%). Meanwhile there were no deaths among the immature rats during the first day after irradiation with a dose of 10,000 r, at the end of the second day 3% had died, and at the end of the third day only 38% of the rats, i.e., the bulk of the rats died after the third day.

By analyzing in this way the course of the curves showing the mortality among the immature and mature rats after the action of various doses of x-rays, the greater vulnerability of the immature rats can be convincingly shown.

Although there is no doubt about the existence of differences on the basis of the results described, it must nevertheless be recognized that these findings are insufficient for the formation of a final conclusion on the importance of age as a factor capable of influencing the character of the radiation injury in animals when irradiated with such large doses of ionizing radiation. Other indices must also be taken into consideration. In Table 1 we show the figures giving the mean duration of survival of the animals depending on their age and the dose of irradiation.

It can be seen from Table 1 that the results in respect of the mean duration of survival of the rats after irradiation confirm the previous conclusion. In this case, moreover, the differences are of moderate degree.

The data summarizing the decrease in weight of the rats at the time of their death, shown in Table 2, are noteworthy.

The figures given in Table 2 show that as the dose increased the loss of weight of the young rats was less pronounced. This relationship also held good after irradiation of the sexually mature rats, although it was less obvious. These features are probably based on differences in the times of death of the animals, which also predetermine the degree of loss of weight: usually, the later the animal dies the more weight it loses. Comparison of the figures of loss of weight by the young and the mature rats shows that the young rats lost considerably more weight. It must be pointed out, however, that the difference was not significant, and in some cases (at doses of 15,000 and 25,000 r) it was absent altogether.

The clinical picture of the lesion and the postmortem findings were particularly interesting. In both the young and mature rats, after irradiation with a dose of 10,000 r, a severe form of radiation sickness developed, accompanied by extreme inhibition, adynamia, severe or total loss of appetite, and indifference to the surroundings. A leading symptom was severe diarrhea. The rats looked dirty, their hair was untidy, and their eyes inflamed and half-closed. At postmortem the typical picture of acute intestinal death was found. This took the form of severe hyperemia of all the internal organs, a considerable decrease in the size of the spleen and thymus, and gross dilatation of the stomach and of part of the intestine. It should be mentioned that the stomach, as a rule, was tightly distended with liquid food contents. In the lumen of the intestine a yellowish-pink liquid could

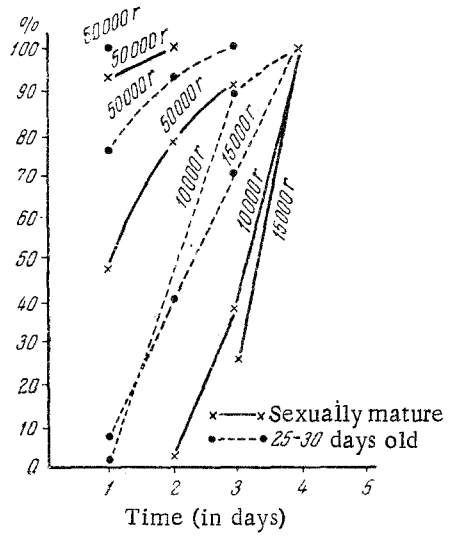

Fig. 1. Curves showing the percentage of rats dying at corresponding times, depending on their age and the dose of irradiation. Along the axis of ordinates—percentage of dying rats; along the axis of abscissas—time of death (in days).

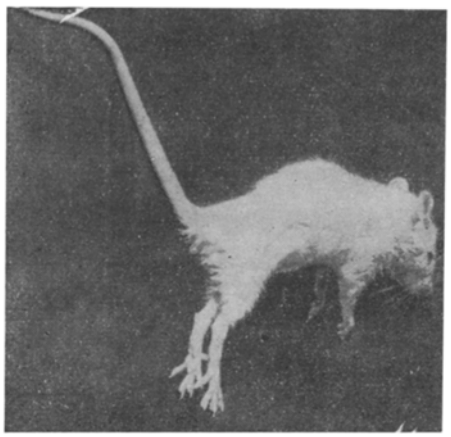

Fig. 2. Convulsions in a young rat 26 hr after irradiation with a dose of 25,000 r (experiment no. 566).

be seen through the thinned walls. In the lower divisions of the large intestine no formed fecal masses whatever were present.

Often in association with grossly hyperemic lung fields large effusions of blood without clearly defined borders could be seen. The brain was severely hyperemic but there were no hemorrhages.

After irradiation in a dose of 15,000 r, the sexually mature rats showed the same form of radiation sickness as after a dose of 10,000 r. In the young rats the course of the disease was much more severe. There was a high mortality among the animals on the second and, partly, on the first day, whereas among the sexually mature rats no deaths took place. Thus when a dose of 15,000 r was used, an obvious intensification of the pathological manifestations of the disease was found in the young rats. This dose apparently was the threshold dose, causing a lesion with signs of a qualitatively new form of reaction to irradiation, which was shown more clearly after irradiation with a dose of 25,000 r.

In the young rats, as a result of irradiation with a dose of 25,000 r, an extremely severe form of radiation sickness developed. Death took place mainly on the first and partly on the second day. Very rarely death occurred at the beginning of the third day. In the affected animals a new symptom—convulsions—appeared for the first time. The development of convulsions was preceded by the characteristic picture of severe radiation sickness. Usually immediately after irradiation the young rat became unable to orientate itself properly. Its movements were uncertain and incomprehensible. It usually moved around in a circle, inclined slightly on one side. In the course of time some weakness and folding in of the limbs could be observed. Shaking of the head and sometimes of the whole body was noted. The hair was untidy. In many animals there was involuntary micturition and defecation. Some animals gave out a squeak, probably on account of a sensation of pain.

In a number of animals severe excitation and restlessness were observed. Roughly ten hours after irradiation attacks of convulsions developed, and these became particularly severe in character at the end of the first and beginning of the second day. Signs of disturbance of the activity of the gastrointestinal tract increased considerably in intensity. The young rats were dissheveled and dirty from the severe diarrhea, and completely refused to take food. The fits gave way to periods of severe adynamia and inhibition. Some of the typical states observed in this period of development of radiation sickness are shown in Figs. 2 and 3.

Regarding the symptomatology of the radiation sickness in the mature rats, mention must be made firstly of the fact that a smaller proportion of them died on the first and second days. In some cases (in 8 %) the rats even died on the fourth day. The picture of the condition bore the same character in the majority of animals. In some rats the typical convulsions described above did not develop. The character of the response reaction to irradiation with this particular dose thus was different in some rats. It must also be pointed out that convulsions developed on the average slightly later in the sexually mature rats. It is most probable that this explains the discovery at postmortem examination of some of the animals of typical signs of acute intestinal death.

The pathologoanatomical signs of the radiation injury consisted above all of vascular disorders of a very severe degree, expressed as severe dilatation of the vessels. Signs of stasis were present in the thymus, heart, intestine and adrenals and, in particular, in the lungs, where massive effusions of blood were found very often. The

Fig. 3. A shock-like state in a young rat 5½ hr after irradiation with a dose of 25,000 r (experiment no. 587).

spleen, which was considerably shrunken, was lighter in color in approximately one half of cases than usual. Because of the vascular disorders the liver appeared unevenly stained. The brain showed severe hyperemia and edema. No hemorrhages were present. Food contents were found, as a rule, in the stomach. Distension was present comparatively rarely, and was moderate in character.

The decrease in the size of the thymus, so typical of the response to lower doses, in these cases was moderate in character and sometimes was ill defined.

As a result of irradiation of the animals with a dose of 50,000 r, the picture of the condition was considerably aggravated. As a rule the animals died during the first 24 hr. Only in the group of the sexually mature rats was death of part of the rats (7%) after 24 hr observed. The earliest deaths among the young rats took place 5 hr after irradiation. Because of the great severity of the lesion, a clinical evaluation of the state of the animals from a comparative aspect was very difficult and often impossible, and accordingly we did not attempt to make one.

Most rats immediately after irradiation showed a characteristically rapid and heavy respiration, loss of orientation, circus movement, involuntary micturition and diarrhea. During these movements the animals suddenly fell on their side, got up again with difficulty and often crawled on their stomach, trailing their hind-limbs. This picture quickly changed into one of complete adynamia and severe inhibition. The animals lay on one side in uncomfortable positions and curled up or else, stretched themselves out in a weakened condition. The picture that developed could be described as that of a shock-like state. In these cases, often immediately after irradiation, the animals completely failed to react to the environment. Strong mechanical stimulation (pricking, pinching, pulling out of the hair) caused no response reaction. The animals did not, however, die at this period. In a number of cases immediately after irradiation excitation took place in the presence of the symptoms described previously. It was more severe in character than that described earlier. Convulsions and paralyses of the limbs appeared sooner, after 2-3 hours. They were very pronounced. Mechanical stimulation likewise caused a sharp response reaction. Even light contact in some cases caused a fit of convulsions to start or to intensify. Periods of convulsions were replaced by a state of total adynamia. In some cases the interchange of these phases was quite rhythmic in character over a definite interval of time.

The pathologoanatomical picture of the lesion may be summarized as a moderate decrease in the size of the thymus and a more marked decrease in that of the spleen, coupled with severe vascular disorders. Severe hemorrhages were also seen in the lungs but were completely absent in the brain.

SUMMARY

The effect of large x-rays doses upon young rats, aged from 25 to 30 days, was compared with that provoked in 3–4-month-old animals (sexually mature). Age was an important factor predetermining the extent of radiation injury. The role of this factor is manifested in the wide range of doses—from 10,000 to 50,000 r. Immature rats are more vulnerable than the sexually mature animals. The development of the so-called acutest form of radiation sickness deserves special attention. A shock-like condition, paralyses, convulsions and other symptoms occurred earlier and were more severe in the sexually immature rats. The age differences diminish with the increase of the dose. The threshold dose is probably about 50,000 r.

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