THE INFLUENCE OF IONIZING RADIATION ON THE DIURNAL RHYTHM OF MITOTIC ACTIVITY IN THE MOUSE CORNEAL EPITHELIUM

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A study of the diurnal variation of mitotic activity in relation to various influences have been the concern of numerous investigators in recent times [1-15]. Some of the works [3, 9, 10, 13, 14] are devoted to an analysis of diurnal variations of mitotic activity in tissues of animals subjected to ionizing radiation. However the results obtained are quite insufficient for any complete apprasial of these phenomenon. Nevertheless a study of the mode of action of radiation on diurnal variations in mitotic activity would throw light on the still unsolved problem, of the nature of the variations themselves. Furthermore, a study of the time of application and dosage of the radiation in relation to the effect is needed in order to determine the rate of physiological regeneration in irradiated tissues.

In the present work we have made a more detailed analysis of the influence of ionizing radiation on the extent of the diurnal rhythm of mitotic activity, and have worked on the corneal epithelium of the mouse.

EXPERIMENTAL METHOD

In two sets of experiments we used 200 white laboratory-bred male rats weighing 15-20 g. In I set of experiments we used a Dermamobil apparatus, and doses of 200 and of 700 r of soft x-irradiation applied to the cornea (voltage 30 kv, current 15 ma, filter 0.1 mm Al, dose rate 1533 r/min). The hardness of the radiation was selected so that practically the whole of it was absorbed in the corneal epithelium. In the II set of experiments the rats were subjected to total x-irradiation on a RUM-3 apparatus, and the same amounts—200 and 700 r were given (voltage 180 kv, current 15 ma, filter 0.5 mm Cu + 1 mm Al, dose rate 20 r/min).

The irradiation was always given in the morning. Animals were killed in batches of five or more simultaneously at 8.00 h and at 20.00 h 1, 1½, 4, 4½, 7, 7½, 10 and 10½ h after irradiation. The material was fixed in Bouin's fluid and total corneal preparations were made. They were stained in Karachi's hematoxylin, and the mitoses were counted in 10,000 cells.

EXPERIMENTAL RESULTS

The mitotic index (number of mitoses per 1,000 cells) of the corneal epithelium of the control animals varied during the day from 15.75 ±0.69 at 8.00 h to 4.18 ±0.33 at 20.00 h, i.e., almost fourfold. The results of the investigations into diurnal variations of mitotic activity in the corneas of the irradiated animals are given in the table.

From the result given in the table it can be seen that one day after local irradiation with 200 r there was no longer any diurnal variations in mitotic activity. By the fourth day after irradiation there was some recovery, and finally, by the tenth day the normal rhythm had been reestablished. A similar state of affairs was observed when the dose was 700r.

The effect of total irradiation with 200 r resembled that of local irradiation with the same dose, but in the former case the diurnal variation one day after irradiation was only slightly reduced. The diurnal variation was suppressed to a still smaller extent one day after irradiation when the dose was 700 r. However in the previous cases we have considered the amplitude of the daily variations of mitotic activity increased with time elapsing after irradiation, but in this case the variation was reduced.

Mitotic Index of Corneal Epithelium at Various Times after Irradiation

Time between irradiation and decapitation (in h)	Time of decapitation (in h)	Local irradiation		Total irradiation	
		200 r	700 r	200 r	700 r
1	8	5,03±0,48	1,83±0,16	7,84±0,96	2,23±0,16
1½	20	4,78±0,80	2,46±0,36	5,39±0,81	0,88±0,16
4	8	11,65±0,40	7,30±0,48	9,92±0,50	4,78±0,72
4½	20	4,98±0,57	2,68±0,23	4,56±0,35	2,04±0,32
7	8	—	—	—	9,60±0,96
7½	20	—	—	—	6,80±1,66
10	8	11,50±1,46	13,99±0,63	11,78±0,81	—
10½	20	3,81±0,67	5,96±0,51	3,55±0,30	—

It is known that there is a relationship between the mitotic activity of a tissue and the content of the so-called "determining" metabolites necessary for cellular division. In turn, the content of the latter depends upon the ratio of their rate of entry into the tissue to their utilization by the dividing cells.

At the present time the opinion is widely held that the diurnal rhythm of mitotic activity reflects variations in the tissue content of "determining" metabolites, and that the variations develop as a result of diurnal fluctuations in metabolic rate [15]. If this is so, then to explain our experimental results we must put forward the following working hypothesis.

First of all in the phenomenon under consideration we must distinguish the consequences on the one hand of the direct radiation damage to mitotic activity of the tissue examined, and on the other of the radiation damage to the general metabolism and to the associated synthesis of "determining" metabolites.

In the case of local irradiation, when changes in general metabolism are very small and the rate of entry of the "determining" metabolites into the irradiated tissue remains practically unchanged, and while the rate of utilization of these substances by the dividing cells is greatly reduced as a consequence of the radiation damage to mitotic activity, an excess of "determining" metabolites will accumulate in the irradiated tissue. As a result, after a certain interval of time the tissue becomes independent of the rate of synthesis of the "determining" metabolites and diurnal variations of mitotic rate cease to occur. The recovery of the diurnal rhythm occurs together with restoration of mitotic activity, when the content of the "determining" metabolites in the tissue returns to normal on account of an increase in the rate of utilization of these substances by the dividing cells.

Because with total irradiation the general metabolism of the animal is impaired the rate of entry of the "determining" metabolites falls, and during the period of suppression of mitotic activity far fewer of these substances accumulates in the tissue than in the case of local irradiation. It is precisely in this way that we can explain the incomplete disappearance of the diurnal variations one day after total irradiation. Because total irradiation with 200 r does not elicit acute radiation sickness, changes in the general metabolism are quickly restored, and the rate of entry of the "determining" metabolites in the tissue under study returns to normal. Subsequent events proceed as in the case of local irradiation with 200 r. With 700 r of total irradiation, which is near to the minimum lethal amount, disturbance of general metabolism increases in proportion to the time elapsed after action of the radiation, and the diurnal variations in mitotic activity do not die away.

We may use these ideas in the case of total irradiation to make certain prognostications concerning the relationship between the nature of diurnal variations of mitotic activity and the dose. Let us note first of all that cellular division is one of the processes most sensitive to radiation, and is almost entirely suppressed by even small doses, whereas the general metabolism is but slightly disturbed. Hence it follows that the response to small doses of radiation which do not result in changes of the general metabolism should produce results akin to those following local irradiation; then diurnal variation is suppressed together with mitotic activity, and recovers together with the latter. Results [10] obtained in an investigation of the diurnal rhythm of the mitotic activity of corneal epithelium of mice irradiated with 200 rads of high-energy protons (corresponding approximately to x-irradiation with 120-140 r) confirm this supposition.

With increase of dose the extent of suppression of the diurnal variations of mitotic activity is reduced, and when a certain critical limit is reached, when the rates of entry and of utilization of the "determining" metabolites

are once more in equilibrium the diurnal rhythm is once more normal. Such a dose can be found in practice, and is approximately 400 r [9, 10, 13].

It has been shown [3] that after irradiation of rats with an absolute lethal dose there is a progressive reduction of the diurnal rhythm of mitotic activity. However, this effect is not present if the same animals are injected with protective substances [14], which greatly increase the mean duration of life of the irradiated animals, a fact which indicates once more the existence of a connection between the diurnal mitotic rhythm and the general metabolism.

SUMMARY

We describe the effect produced by local irradiation with 200 and 700 r on the diurnal rhythm of mitotic activity in the rat corneal epithelium. The effect of total irradiation with 200 r was similar to that of local irradiation with the same dose. However, variation of mitotic activity was not eliminated in this case. After total irradiation with 700 r there was a progressive decline of the diurnal variation. An explanation of these and of published experimental results is put forward.

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All abbreviations of periodicals in the above bibliography are letter-by-letter transliterations of the abbreviations as given in the original Russian journal. *Some or all of this periodical literature may well be available in English translation.* A complete list of the cover-to-cover English translations appears at the back of this issue.
