RELATIONSHIP OF THE DEVELOPMENT OF RADIATION DAMAGE AND REPAIR ON THE REGENERATIVE ACTIVITY OF TISSUES OF Rana temporaria TADPOLES

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Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 55, No. 2,
pp. 108-112, February, 1963
Original article submitted April 24, 1962

It has been shown that the development of radiation damage and repair processes are related to regenerative activity, and particularly to processes of physiological regeneration as indicated by mitotic activity. Comparisons have been made between the different tissues having different rates of physiological regeneration [6, 7]. Very interesting work has been done in this direction where it has been shown that an artificial increase or decrease of regenerative activity caused by some kind of interference leads to changes in the response to irradiation. There are indications that mechanical damage or the influence of certain chemical agents causing an increased proliferative activity of the tissues leads to a more rapid and sometimes to a better defined morphological picture of the radiation damage.

At the same time, this experimentally induced rise in regenerative activity facilitates the repair process [1, 5, 7, 9]. Naturally the opposite effect occurs when regenerative power is reduced. Then not only does the damage induced by radiation develop more rapidly, but its extent may be less apparent; at the same time there is also a reduction of the recovery processes. Thus a fall in the rate of physiological regeneration of the intestinal epithelium in mice induced by starving leads to a reduction of radiation damage, as well as a delay in repair [2]. Glücksmann and Spear [11] showed that in the scarcely differentiated nervous tissue of frog tadpoles, radiation results in the destruction of fewer cells than in control animals. They attribute this effect to an increased sensitivity of the cells in the premitotic stage.

It seemed to us interesting to observe and compare the development of radiation damage and repair in various epithelia differing in their initial rate of cell division.

EXPERIMENTAL METHOD

Our observations were made on Rana temporaria tadpoles. Previous information showed that mitotic activity in the corneal epithelium was always approximately five times as intense as in the epithelium of the tail (Fig. 1). By the end of metamorphosis (37-40th day after the start of hatching) when the forelimbs appear and the tail begins to be reduced, there is a marked increase of mitotic activity in the corneal epithelium, whereas in the tail it almost ceases.

In one set of experiments we stimulated mitosis by the infliction of trauma. We amputated the tail of 8-day-old tadpoles. We found that for a considerable time the mitotic activity in the epithelium of the regenerating part of the tail was approximately twice as intense as in the basal portion (see Fig. 1). By the end of metamorphosis, in both the basal and regenerating portions, figures of cell division were entirely absent.

Total preparations of the cornea and tail of the tadpoles were stained in hematoxylin. As a measure of the changes we counted the mitotic figures and calculated the mitotic index (number of dividing cells as a percentage of the cells of the basal layer of the epithelium). In each tadpole mitoses were counted in the epithelium of both corneas, and in 50 fields of vision of the epithelium of the base of the tail (not concerned in regeneration), and in the regenerating portions. Observations were made with a 90 × immersion objective, and a 7 × ocular.

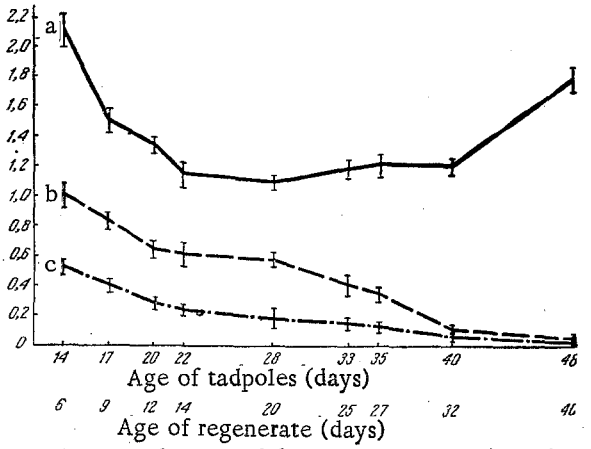

Fig. 1. Age changes of the mitotic index: a) in the corneal epithelium, b) in the epithelium of the regenerating portion of the tail, and (c) in the epithelium of the basal portion of the tail. I – mean error.

We used tadpoles taken directly from their natural habitat. During the experiment they were kept in crystallizing dishes in water at 18-20° from which the sediment had settled out; they were fed with Protococcus, raw kidney, and boiled frog muscle [3]. The 20-day tadpoles with a 12-day regenerating portion were irradiated by an RUM-3 apparatus adjusted as follows: potential 190 kv, current 15 ma, 5 mm Al filter without a tube. Distance from anode to object 30 cm. Dose rate 270 r/min. Doses of 300 and 600 r were given.

This amount of radiation caused no deaths at any time during the experiment. The tadpoles which received 300 r completed metamorphosis, as did the control tadpoles also; irradiation with 600 r extended metamorphosis by several days.

Material for histological study was fixed 2, 4, and 6 hr and 1, 3, 6, 10, 12, 15, 20, 27, 30, and 35 days after irradiation. However, we made no observations after the 20th day, because on account of metamorphosis at later periods there is a disturbance of the ratio of the mitotic activity of tissues which makes it difficult to compare the reactive changes.

We used 5-10 animals for observations at each period of the experiment.

EXPERIMENTAL RESULTS

The results of the first set of experiments are shown in Fig. 2. In the corneal epithelium where the mitotic activity was highest, two hours after irradiation the mitotic index fell from 1.35 to 0.143, i.e., by 90%. In the epithelium of the regenerating portion it fell by 70%, and at the base of the tail, where the cell division was slower still, mitotic activity was reduced by 57%. In all tissues the greatest depression of cell division occurred four hours after irradiation. At this time, in the corneal epithelium mitotic figures disappeared almost entirely, in the epithelium of the regenerating portion the mitotic index was reduced by 86%, while in the epithelium of the base of the tail it fell only by 75%. In the corneal epithelium, after irradiation the rate of cell division fell more rapidly than it did in the cutaneous epithelium of the tail. Therefore in tissue with a relatively large number of mitoses the suppression of mitotic activity was more manifest and more extensive.

Six hours after irradiation repair commenced, as shown by an increase in the number of figures of cellular division. In the first hours after irradiation, the recovery of mitotic activity, just like its reduction, was related to the initial rates of cell division. In the corneal epithelium having the highest mitotic index, mitotic activity was increased the most rapidly, in the epithelium of the regenerating portion it was slower, and in the epithelium of the base of the tail, where the number of cell divisions was the smallest, the activity was the lowest (Fig. 2, A).

At the same time, despite the rapid recovery, in the tissue with the highest mitotic activity (corneal epithelium) the repair of the radiation damage was incomplete (Fig. 2, B). Thus by the third day after irradiation, in the epithelium of the base of the tail the mitotic activity recovered to the control figure; in the epithelium of the regenerating portion of the tail, where the initial level of cell division was higher, the index was only 89%, and in the corneal epithelium, where normally the proliferative activity is highest, it was only 70%. The attained rate of cell division was maintained at later periods. As mitotic activity increased, there were abnormalities in the figures of cell division; we found no other morphological changes of the epithelial cells.

From the results we may conclude that on the one hand tissues having a comparatively high proliferative activity show the effect of radiation damage more clearly and more extensively than do others, and on the other hand there is a tendency for them to produce a more rapid repair, although the process is not complete.

As can be seen from Fig. 3, the results obtained by irradiation of the tadpoles with 600 r do not differ essentially from those of the previous series. The greatest suppression of cell division in all tissues occurred four hours after irradiation. In the corneal epithelium where the rate of cell division was relatively high, just as when a dose of 300 r was given, the reduction of the mitotic index was more marked than it was in the epithelium of the tail. The damage

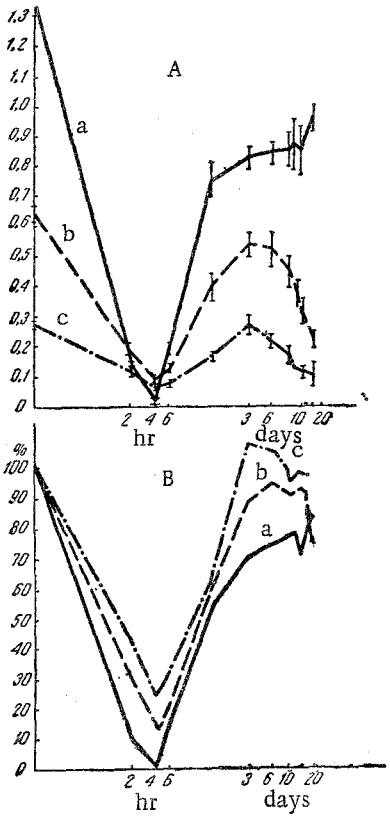

Fig. 2

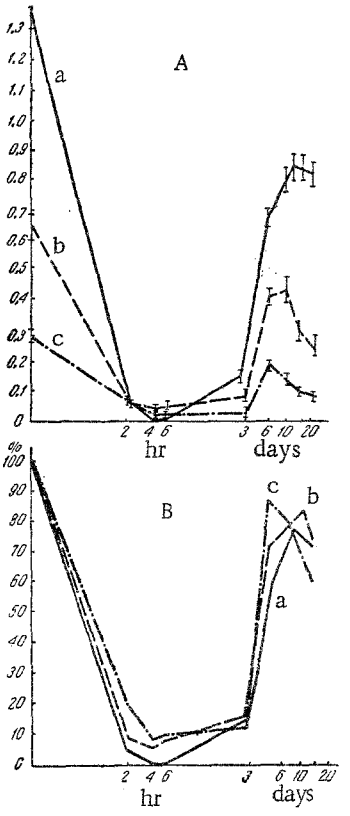

Fig. 3

Fig. 2. Change in the actual value of the mitotic index (A), and its change expressed as a percentage of the control (B) as caused by irradiation of tadpoles with a dose of 300 r. Indications as in Fig. 1.

Fig. 3. Change of the absolute value of the mitotic index (A), and its change as a percentage of the control value (B), induced by irradiation of tadpoles with a dose of 600 r. Indications as in Fig. 1.

from radiation in the tissues was more extensive than in the first series. This was shown by the greater and more prolonged suppression of mitotic activity. No noticeable repair process could be observed before three days. In the corneal epithelium mitotic activity increased more rapidly than in the epithelium of the regenerating portion; in that of the tail basal region it rose still more rapidly. Despite the great rapidity of the recovery processes, in agreement with the results of the first experiment, the mitotic index did not reach the control figures. Under these conditions of radiation in the epithelium at the base of the tail there was no complete recovery of mitotic activity. As mitotic activity recovered abnormal figures of cell division appeared later after the irradiation (16th-20th day), and in the corneal epithelium binucleate cells were quite commonly found. No other morphological changes in the epithelial cells were found.

From the results obtained we may conclude that the manifestation of radiation damage, its extent, and the extent of the repair processes were determined (for a given dose of irradiation) by the initial level of proliferative activity. Thus, in tissue having a comparatively high mitotic activity (corneal epithelium), recovery was more rapid even though the radiation damage was greater and more manifest. Nevertheless the repair was not complete. In general the results conformed with experiments on the development of radiation damage in the corneal epithelium in frogs and mice, where a comparison was made of the reaction of tissues differing in their rate of physiological regeneration [6, 10]. However, in these works, principal attention was paid to the time relationships of the development of changes due to radiation, and the extent of the damage was not considered.

The degree of proliferative activity determines the response of the tissue to the action not only of X-rays but also of other factors. This dependence of the tissue response on the initial level of mitotic activity was established from an investigation of the inhibition of mitoses in the cornea of the mouse, by treatment with adrenalin [8]. Similar results

were obtained by a comparison of the reactive changes of mitotic activity in the epithelium of the upper and lower surfaces of the tongue of the rat [4]. In experiments on fasting tadpoles and mice we found a direct relationship between the normal mitotic activity and the extent of its suppression. Thus in fasting animals, in the tissue with the highest mitotic activity suppression occurred comparatively rapidly and intensely. When the animals were fed after a short fasting period the recovery processes occurred most rapidly in tissue having a relatively high initial mitotic activity, although this level of activity both before and after radiation was reached earliest in tissues normally showing a low mitotic index [3].

There is therefore a considerable resemblance in the tissue reactions induced by a variety of factors. However, many of the morphological manifestations and time relationships were very characteristic of radiation changes.

SUMMARY

Rana temporaria tadpoles aged 20 days were exposed to 300 or 600 r of X-rays. The degrees of damage and recovery in the epithelium of the cornea, tail, and regenerating portion of the tail were studied, and differences were found which were related to the initial rate of cell division. The change of mitotic activity was used as a criterion of the extent of the injury and of the rate of the subsequent repair. The fall in mitotic index was more abrupt and pronounced in the cornea and epithelium where the rate of mitosis was relatively high. After radiation repair processes were more rapid in the tissues in which mitotic activity is normally high; nevertheless the recovery was incomplete. Regenerative activity was more distinctly shown after irradiation with a dose of 300 r; differences between the reactions of the various tissues to irradiation was less well shown with a dose of 600 r.

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