

EXPERIMENTAL BIOLOGY

THE ACTION OF A CHEMICAL IRRITANT (OZOKERITE) ON THE EPIDERMIS AFTER LOCAL X-RAY IRRADIATION

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During the histological study of the reaction of the skin to x-ray irradiation, the classic picture of the changes in the epidermis of the guinea pig was described by Miescher [12], who found the development of polymorphism of the nuclei, the appearance of multinuclear giant cells and degeneration of the cells of the basal layers. Similar changes were also described in the epidermis of the rabbit [1, 4, 6 and others]. Under these circumstances it must be pointed out that in morphological investigations it is customary to use large doses of x-rays: 1800, 3600, 4000 and 10,000 r and over, and the biopsy is taken several weeks or months after irradiation. So far as changes in the epidermis are concerned, after small doses and shorter periods of observation, much less material is available for the morphologist. Miescher [12], for instance, points out that after a dose of 800 r the histological picture of the epidermis of the guinea pig generally speaking remains normal, although isolated multinuclear cells are encountered and disturbances occur in the course of karyokinesis. L. V. Funshtein [10], using rabbits, which are less sensitive to irradiation than guinea pigs, could find no definite changes in the epidermis with a dose of 466 r.

In our investigation we studied the changes in the epidermis resulting from irradiation with x-rays in doses of 500 and 1000 r, and from stimulation of the physiological regeneration after such irradiation. As a stimulator of the processes of regeneration we used an ozokerite paste, whose action on regeneration of the skin we have often studied in various experimental conditions [7, 8].

EXPERIMENTAL METHOD

Experiments were carried out on 30 adult rabbits (weighing about 2 kg). The experiment began with irradiation of the right ear of the rabbit with x-rays in a dose of 500 r (first series) or 1000 r (second series). Conditions of irradiation: voltage 160 kv, current 5 mA, focal distance — 30 cm, dose rate 70 r/min. In one group of animals of each series biopsy specimens were taken from symmetrical areas of both ears 2 days after irradiation, and in the other group — after 2 weeks (14 days). After removal of the biopsy, the inner surface of both ears was smeared with ozokerite paste once daily. The next biopsy specimens were taken from all the animals 4 days after the first application of paste; in addition in some cases additional biopsies were taken 8, 16, 30 and 60 days after the first application of paste. The specimens were fixed in 20% formalin, and frozen sections were cut and stained with hematoxylin-eosin.

During microscopic study of the preparations, by means of an ocular micrometer the total thickness of the stratum germinativum and the stratum granulosum of the epidermis of the inner surface of the ear was measured, and the rows of cells in these layers were counted. The result of the counting and measurement was expressed as a formula in which the first item represents the number of rows of cells in the stratum germinativum, the second the number of rows of cells in the stratum granulosum (if the stratum granulosum is not continuous the figure 1 is placed in brackets), and the sum represents the total thickness of the epidermis (without the stratum kerativum in microns. This enables the reaction of the epidermis to be expressed quantitatively and the changes in reactivity to be shown in cases where there are no qualitative morphological changes in the cutaneous epithelium.

EXPERIMENTAL RESULTS

First series of experiments. There were 15 animals in this series. In 5 rabbits the first biopsies were taken 2 days after irradiation, and in 10 rabbits after 2 weeks. In the unirradiated ear the condition of the epidermis of the inner surface was expressed by the formula $2-3 + (1) = 20 \mu$. This means that in the stratum germinativum there were 2 or 3 rows of cells, and in the stratum granulosum one incomplete row of cells, while the thickness of the epidermis without the stratum kerativum was 20μ . At the same time the average result for the irradiated ears when the biopsies were taken after 2 days was $3-4 + 1 = 26 \mu$, and after 2 weeks, $3 + 1 = 24 \mu$. These figures indicate that as a result of irradiation the epidermis became thicker, and also that the thickening of the epithelium was accompanied by an increase in the number of cells in that tissue.

A characteristic feature of the epidermis of the irradiated ears is the increased dimensions of the nuclei in certain cells. We saw nuclei enlarged to twice their normal size 2 weeks after irradiation in 7 of the 10 animals. However 2 days after irradiation these nuclei were present in only one of the 5 rabbits investigated.

The action of ozokerite on the epidermis of the irradiated and unirradiated ears gave different results. If the smearing with ozokerite began 2 days after irradiation the state of the epidermis after four applications was expressed in the unirradiated ear by the following formula: $6 + 1-2 = 42 \mu$, and for the irradiated ear: $4-5 + 1 = 34 \mu$; in the group of animals in which the smearing with ozokerite began 2 weeks after irradiation, the epidermis of the unirradiated ear corresponded to the formula $5-6 + 1 = 43 \mu$, and of the irradiated ear, $4-5 + 1 = 38 \mu$. These figures show that preliminary irradiation of the ear with a dose of 500 r lowers the reaction to subsequent stimulation; in fact the lowering of the reaction is still greater if the original state of the epidermis before the first application of the paste is considered. The increase in thickness of the epidermis of the irradiated ear in cases where application of ozokerite began 2 days after irradiation was 2.8 times smaller than that of the unirradiated ear; meanwhile where application of paste began 2 weeks after irradiation, this difference was reduced and the increase in the thickness of the irradiated epidermis was only 1.8 times smaller than the increase in the thickness of the epidermis of the unirradiated ear.

Smearing the skin with ozokerite leads to the disappearance of the enlarged nuclei from the epidermis, i.e. to the restoration of the normal structure of its cells. When the application of the paste began 2 days after irradiation, enlarged nuclei were not found after 4 days nor when the biopsy was taken at later periods. Where the application of ozokerite began 2 weeks after irradiation, enlarged nuclei were present in the epidermis of 3 animals after 4 daily applications, but were not encountered later.

When the ozokerite was applied for longer periods of time (8, 16, 30 and 60 days), we found that the fall in reactivity of the irradiated epidermis was a temporary condition. Only 16 days after the onset of application of paste not one case of asymmetry could be found; the reaction of the epidermis was the same in the irradiated and unirradiated ears. The same thing was observed also at later periods.

Second series of experiments. This series comprised 15 animals also. In 5 rabbits the first biopsies were taken 2 days after irradiation, and in 10, after 2 weeks. In contrast to the first series the dose of x-rays was 1000 r. The experimental method was as before.

Comparison of the state of the epidermis of the irradiated and unirradiated ears showed that as a result of irradiation with a dose of 1000 r, the epidermis became thicker than that of the unirradiated ear in 12 of the 15 animals; in the other three cases no difference was found. Under these circumstances, 2 days after irradiation the epidermis corresponded to the formula $3 + 1 = 24 \mu$, and 2 weeks afterwards, $3 + 1 = 22 \mu$. Histological study of the preparations showed the presence of cells with enlarged nuclei in the epidermis of the irradiated ear in 3 of the 10 animals in which biopsies were taken 2 weeks after irradiation.

The rabbits of this series showed a lowering of reactivity towards both x-rays and ozokerite. Furthermore the reaction of the epidermis to the application of ozokerite to the irradiated ear was significantly weaker than to the unirradiated ear. In the group of animals in which the application of paste began 2 days after irradiation, the epidermis of the unirradiated ear corresponded at first to the formula $3 + (1) = 20 \mu$, and after four applications, $3-4 + 1 = 29 \mu$; the epidermis of the irradiated ear was respectively $3-4 + 1 = 24 \mu$ and $3-4 + 1 = 26 \mu$. Consequently, as a result of four applications of ozokerite the thickness of the epidermis of the unirradiated ear increased by 45% and that of the irradiated ear by only 8%, i.e. the increase was almost 6 times smaller.

In the group of animals in which the application of paste began 2 weeks after irradiation, the reaction was

expressed rather more strongly, but here also the increase in the thickness of the epidermis of the irradiated ear as a result of four applications of ozokerite was 3 times smaller than on the unirradiated side (32% against 100%). The epidermis of the unirradiated ear at first corresponded to the formula $2-3 + (1) = 18 \mu$, and after application of the paste it took the form $4 + 1 = 36 \mu$; on the irradiated ear, before application of paste the epidermis corresponded to the formula $3 + 1 = 22 \mu$, and after application, $3-4 + 1 = 29 \mu$.

As a result of 4 daily applications of ozokerite, in some animals the number of enlarged nuclei in the epidermis was increased, or where such nuclei were not seen before the applications were started, they began to appear. After application of ozokerite for 16 days, polymorphism of the nuclei of the epidermis was not yet found, and the reaction to ozokerite was expressed equally in the irradiated and unirradiated ears.

Our results show that the action of x-rays in doses of 500 and 1000 r on the epidermis of the rabbit has a dual character. On the one hand, after irradiation of the epidermis it thickens and there is an increase in the number of cells in its stratum germinativum and stratum granulosum; this earlier x-ray reaction may be regarded as stimulation of physiological regeneration on its part. On the other hand the irradiated epidermis, when acted upon by ozokerite, is found to be less stimulated into proliferation than is the unirradiated; this demonstrates the presence of an inhibitory, retarding action of x-ray irradiation on the course of the regenerative processes in the epidermis. These findings are in agreement with those of A. A. Zavarzin and G. S. Strelin [5], who in another animal and by the use of a different method, concluded that x-ray irradiation has a dual action.

According to our findings, the inhibitory action of x-rays in a dose of 500 r is less pronounced than in a dose of 1000 r. This is in accordance with the generally accepted view that the reaction depends on the size of dose applied, and no special discussion is called for.

Comparison of our results of experiments carried out 2 days and 2 weeks after irradiation shows that the inhibitory action of x-ray irradiation on the epidermis is more marked in the first few days than after 2 weeks.

In G. S. Strelin's laboratory, where the reaction of the corneal epithelium to x-ray irradiation was determined by the mitotic activity, I. B. Bychkovskaya [3] obtained results which showed that after small doses (2-50 r) the suppression of mitotic activity continues for a few hours in all and it is then restored. Workers using irradiation in large doses [2, 9] point out that the regenerative power of the tissues is restored a few months after irradiation. For this reason we consider that our results, obtained with average doses and by the use of a rather different method, are in agreement with these reports in the literature. At the same time we found that the reactivity of the tissues during the latent period is already modified, and this modification can be demonstrated by the application of an additional stimulus, namely ozokerite.

One of the signs of the action of radiation on the epidermis is enlargement of the cell nuclei, which was also observed in our material. At a dose of 500 r, enlargement of nuclei was observed in one case after only 2 days, and in the majority of cases 2 weeks after irradiation; four applications of ozokerite led to the disappearance of the large nuclei from the epidermis. At a dose of 1000 r, enlarged nuclei appeared only 2 weeks after irradiation, and then in only a minority of the animals, but they were most often found 4 days after the start of application of ozokerite; at later periods after the start of the applications (8 or 16 days or over) large nuclei did not appear. These findings show that ozokerite is similar in its action on the irradiated epidermis to yellow paraffin [2] and naphthalanum [11], and brings about very rapid restoration of the normal structure of the epidermis.

SUMMARY

X-ray irradiation of a rabbit's ear with the dose of 500 and 1000 r results in thickening of the epidermis and in increase of the number of cellular layers. In additional irritation with ozokerite the irradiated epidermis gives a weaker reaction than the one which was not subjected to irradiation. X-ray irradiation produces a double effect: at first it intensifies the physiological regeneration of the epidermis, and later it decreases the reactivity of this layer of the skin.

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