THE EFFECT OF SUBLETHAL ROENTGEN-RAY IRRADIATION ON THE INDUCTION PROCESS CAUSED BY TRANSITIONAL EPITHELIUM IN CONNECTIVE TISSUE

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The transitional epithelium of the urinary bladder and renal pelvis may, in certain conditions, cause osteogenesis and hemopoiesis in connective tissue [1-4]; in particular, in guinea pigs homografts of the mucous membrane of the urinary bladder may induce osteogenesis in the surrounding connective tissue [1, 2]. We showed in a previous communication [5] that preliminary irradiation of the recipients with sublethal doses of roentgen rays does not suppress the ability of connective tissue to undergo this induction. At the same time, in contrast to normal recipients, in irradiated animals foci of extramedullary hemopoiesis develop near the transitional epithelium in addition to bone tissue on the 17th-20th day.

The interest caused by this phenomenon is of obvious interest in connection with the problem of the compensation of radiation injury to the hemopoietic system. We may ask whether there is any possibility of bringing the time of appearance of hemopoietic tissue in the zone of induction nearer to the time of irradiation. In this connection it is important to know how the time of appearance of extramedullary hemopoiesis by induction in irradiated animals is related to the time of irradiation and to the time of transplantation, and to have information regarding the radiosensitivity of the various stages of the induction process. Such information may also be of value for the analysis of the sources and means of formation of hemopoietic tissue under the influence of transitional epithelium.

In the present communication we describe the results obtained after irradiation of animals at different intervals after the onset of induction caused by the transplantation of transitional epithelium.

EXPERIMENTAL METHOD

A mixture of minced mucous membrane from the urinary bladder of 4 or 5 guinea pigs was transplanted homoplastically into the anterior abdominal wall of 140 guinea pigs, weighing 150-200 g, by means of a tube. The dose given to each recipient was approximately half the mucous membrane from one bladder. Irradiation was given by a cobalt source EGO-2. An irradiation dose of 250-300 r caused the death of 50-60% of the control guinea pigs in 30 days. The animals were divided into 6 groups according to the times of transplantation and irradiation.

Grafts with bone tissue were fixed in alcohol–formalin. Series of paraffin-wax sections were stained with hematoxylin-eosin, by the periodic acid – Schiff technique with counterstaining with hematoxylin, and by the Dominici–Kedrovskii method.

EXPERIMENTAL RESULTS

The grafts in the animals of the first group, fixed on the 14th day, showed the picture of intensive induction of osteogenesis in the connective tissue at sites of atypical infiltration of epithelium (Fig. 1,a). The ectopic bone was well differentiated, although the layer of outer osteoblasts was relatively thin and was interrupted here and there. In many places large basophilic cells with large nuclei were situated among the bony trabeculae. Collections of these cells also surrounded certain epithelial cysts, around which there was no sign of osteogenesis.

Group	No. of guinea pigs	Time after transplantation (in days)	
		irradiation	fixation
First	20	2	14, 20
Second	20	5	13.20
Third	20	8	14, 20
Fourth	20	11	15, 21
Fifth	10	16	20
Sixth	50	20	25, 30, 35

In animals fixed after 20 days active induction of bone was taking place in the cyst walls. Among the osteoblasts, which formed large groups, mitoses were numerous; the layer of osteoblasts was larger than the layer of mature bone tissue (Fig. 1, b). In many places the epithelial cysts were surrounded by a layer of large cells resembling hemocytoblasts, among which young cells of the myeloid series could be found.

The grafts in the animals of the second group, fixed on the 13th day after transplantation, showed the development of bone tissue, although in many places it was atypical in appearance: the layer of ground substance was thin, the individual trabeculae were not joined together, and the ordinary compact structures were not formed. The osteoblasts often appeared degenerated or were almost indistinguishable from reticulum cells and fibroblasts (Fig. 1, c). At the same time areas of normal osteogenesis were also encountered. In animals fixed on the 20th day fragments of bone tissue were arranged around the areas of proliferation of the transplanted transitional epithelium, and osteogenesis was continuing in some places. In most cases, however, the bone tissue was undergoing absorption and the osteoblastic layer was ill-defined. Nevertheless, around some epithelial cysts a layer of hemocytoblasts was present, proliferating and forming young cells of the myeloid series.

In the animals of the third group, sacrificed after 14 days, a considerable amount of bone tissue was observed in the grafts, but its structure differed significantly from normal. The ground substance was irregularly calcified, the bony trabeculae were thin and irregularly shaped (Fig. 1, d). Cartilage tissue and structures occupying an intermediate position between cartilage and bone were frequently found. The ground substance in the bone tissue was generally underdeveloped, and it enveloped an unusually large number of hypertrophied osteocytes, most of which showed obvious signs of degeneration. Marked changes were found in the osteoblastic layer. In some places this was completely absent, and even where it was preserved the osteoblasts appeared to be degenerating. Absorption of bone was observed in many places (Fig. 1, e), sometimes with the participation of typical osteoclasts.

In the animals of the same group, sacrificed after 20 days, the grafts were practically completely absorbed.

In the animals of the fourth group, fixed on the 15th day, the grafts contained bone tissue with well developed ground substance, but in most bone fragments the osteoblastic layer was almost entirely absent and the bone appeared to have polished edges (Fig. 2, a) with clear patterns of resorption. The osteoblasts that were seen here and there were in a state of necrosis.

In most animals sacrificed on the 21st day the grafts were completely absorbed. Signs of fibrosis were observed in the grafts that remained.

In the animals of the fifth group, the grafts fixed after 20 days showed small fragments of absorbing bone tissue. Typical osteoblasts could hardly be found. The bone fragments were surrounded by elongated cells resembling fibroblasts, and the ground substance of the bone was gradually changing into masses of compact collagen with unmasked fibers, among which individual areas could be seen which had not yet completely lost their picture of differentiation of bone (Fig. 2, b).

In the animals of the sixth group, fixed on the 25th day, the grafts contained a large amount of well differentiated adult bone tissue. The osteoblastic layer in some places was well marked, but elsewhere it was almost entirely absent (polished bone) or showed signs of degeneration. In the animals fixed after 1 month the grafts contained much bone as before, and in most cases the osteoblastic layer was well defined. Large, free cells resembling hemocytoblasts were situated between the bone trabeculae, among the reticulum cells. In the animals sacrificed on the 35th-37th day, the grafts in some cases showed absorbing bone tissue, with absence of the typical osteoblastic layer, and fibrous tissue between the bony trabeculae. In other cases the bone preserved its osteoblastic layer, and the space between the bony trabeculae contained a large amount of marrow, characterized by active hemopoiesis with quite typical megakaryocytes (Fig. 2, c, d).

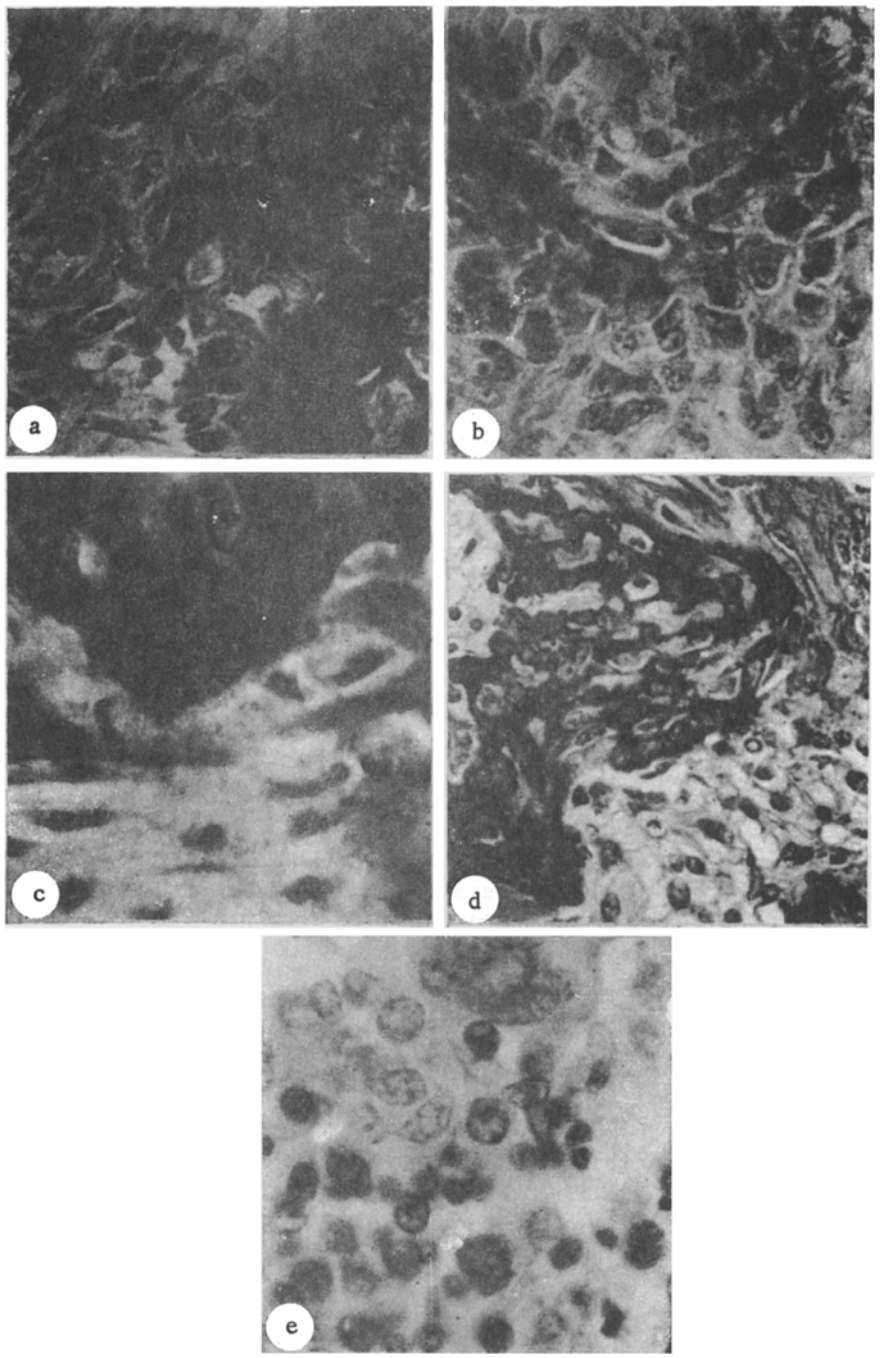

Fig. 1. Homografts of the mucous membrane of the urinary bladder. Fixed in alcohol and formalin and stained with PAS-hematoxylin. a) First group, fixation on the 14th day, objective 10 ×; b) first group, fixation on the 20th day, objective 24 ×; c) second group, fixation on the 13th day, objective 24 ×; d) third group, fixation on the 14th day, objective 10 ×; e) the same, objective 24 ×.

The doses of roentgen-ray irradiation which were used had a relatively insignificant effect on the transplanted transitional epithelium, and in any case did not abolish its inductive power. The considerable differences observed after irradiation in the various stages of induction must evidently be interpreted from the point of view of the effect of irradiation (direct and distant) on the system to be induced, principally at the precise moment of irradiation, for in all the cases under discussion the subsequent stages of induced histogenesis took place in the identical conditions of moderately severe radiation sickness.

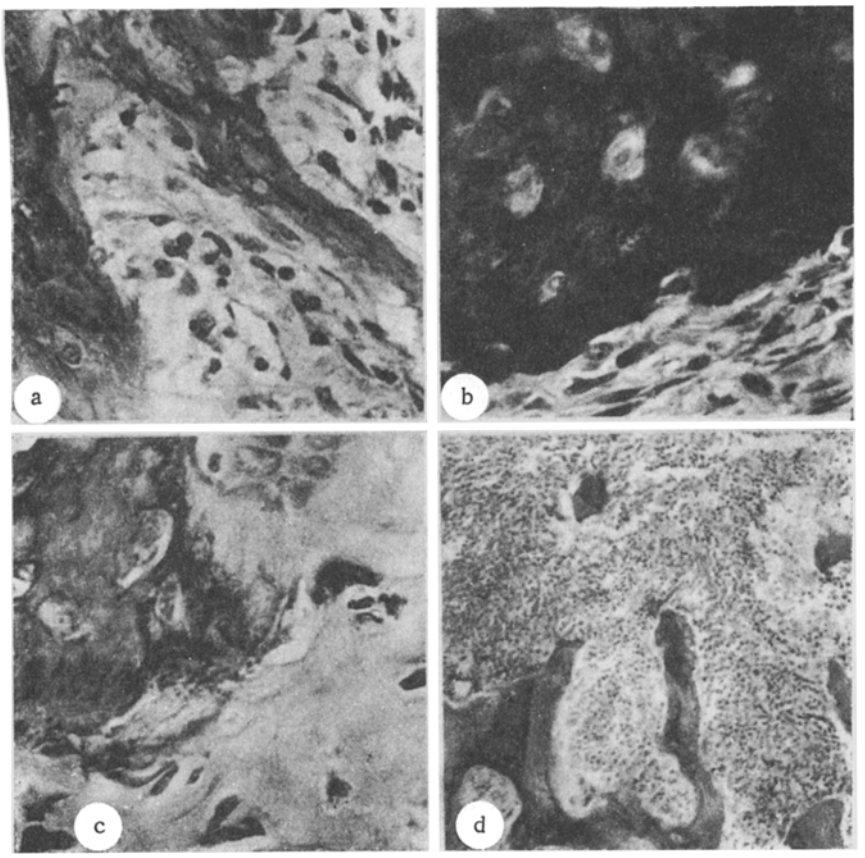

Fig. 2. The same as in Fig. 1. a) Fourth group, fixation on the 15th day, objective 24 ×; b) fifth group, fixation on the 20th day, objective 24 ×; c) sixth group, fixation on the 35th day, objective 10 ×; d) the same, objective 45 ×.

These results show that irradiation on the 8th-11th day after transplantation, when the zone of induction consists of young osteogenic tissue, leads to a severe disturbance of osteogenesis and to the total abolition of the induction effect (absorption of bone) 10 days after irradiation. Under these circumstances the osteoblasts are most severely affected, as a result of which the character of the osteogenesis is modified, and this is followed by total dedifferentiation of the osteogenic tissue.

The intermediate forms of transformation of the connective-tissue elements into osteogenic cells, which were observed in the grafts on the 5th day, are evidently less sensitive, for irradiation at this stage leads only to a slight disturbance of the process of osteogenesis and to insignificant injury to the osteoblasts (on the 9th day after irradiation), with recovery on the 15th day. Still earlier forms (irradiation on the 2nd day after transplantation) were generally indistinguishable in their radiosensitivity from ordinary connective-tissue cells. Irradiation at this time does not alter the course of induction by comparison with irradiation before transplantation.

During the irradiation of animals in which induction had started 20 days previously, and in which the grafts showed well developed bone tissue at the moment of irradiation, the osteoblastic layer was injured while the bone tissue formed before irradiation was preserved. Between 10 and 15 days after irradiation, the injured layer was largely restored. It is evident that mature bone possesses relatively low radiosensitivity and is capable of regenerating its injured osteoblastic layer. In contrast to this, after irradiation at a stage when no fully formed bone is present in the grafts (8th-11th day), regeneration of the osteogenic tissue, which might proceed as a result of repeated induction, does not take place. This may indicate a limited reserve of elements capable of osteogenesis among the population of connective-tissue cells.

The formation of foci of extramedullary hemopoiesis in the zone of induction of the irradiated recipients is of the greatest interest.

The results described above show that the time of appearance of foci of extramedullary hemopoiesis is closely related to moment of transplantation of the epithelium and not to the time of irradiation. By carrying out induction before irradiation, it is therefore possible to shorten the time taken for development of extramedullary hemopoietic tissue appeared in the grafts on about the 20th day after transplantation and irradiation; in the second group of animals on the 20th day after transplantation and the 15th day after irradiation; in the animals of the sixth group between the 25th and 30th days after transplantation, corresponding to the 5th-10th day after irradiation.

It must be emphasized that in unirradiated guinea pigs homografts of mucous membrane of the bladder do not induce development of hemopoietic tissue during the 30 days of their existence [1, 2]. Consequently, conditions are maintained in the irradiated organism, at least for a few weeks, which are essential for the development of hemopoiesis in addition to the customary osteogenesis in the foci of induction. These conditions are evidently related to the insufficiency of the hemopoietic function in irradiated animals and to the mechanism of regeneration of their hemopoietic tissue proper.

These results show that a definite stage of differentiation is essential for the formation of hemopoietic tissue, and that this is reached in the zone of induction from 2 to 3 weeks after transplantation of transitional epithelium. Further investigations will clarify the relationship between the regeneration of the marrow after injury by irradiation and its formation in the zones of induction.

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