PECULIARITIES IN THE REGENERATION OF THE HEMATOPOIETIC TISSUE IN CHICK EMBRYOS EXPOSED TO EXTERNAL γ-RADIATION

A. V. Bursian

Laboratory of Ontogenesis of Animal Nervous Activity (Director, Candidate of Biological Sciences A. V. Voino-Yasenetskii)
I. M. Sechenov Institute of Evolutional Physiology, Academy of Sciences USSR (Director, Corresponding Member of the Academy of Sciences, USSR, E. M. Kreps), Leningrad
(Presented by Academician V. N. Chernigovskii)
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The lesions of the hematopoietic organs developing under the influence of ionizing radiation are regarded as a hypoplastic or aplastic state of the hematopoietic organs [4-9,12]. After the initial stimulation of the bone marrow, the more rapid maturation of the hematopoietic elements and their entering into the peripheral blood stream a profound inhibition of the hematopoietic function develops. In severe cases panmyelophthisis—an irreversible condition of the bone marrow involving complete cessation of hematopoiesis—develops. In cases of myeloparesis regenerative processes start at a certain stage of radiation sickness which lead to a more or less complete restoration of the disrupted function.

Radiation sickness is followed by disorders of both blood cell proliferation and blood cell differentiation. The latter becomes particularly manifest in the restoration period, in the course of which the regeneration may adopt a number of pathological features. Here we have in mind the occurrence of elements characteristic for the embryonal megaloblastic erythropoiesis and of leukemoid conditions.

When speaking of the development of radiation lesions in embryos it must above all be emphasized that although the processes which take place in the hematopoietic organs of embryos exposed to radiation are in principle similar to the same processes in adult animals exposed to radiation, the specific features characterizing embryonal hematopoiesis and the high regenerative capacity of these tissues bring about a number of essential changes.

The literature contains only a few reports concerning the influence of ionizing radiation upon embryonal hematopoiesis [1-3,10,11,13,14]. A. A. Zavarzin and co-workers emphasized that the blood cells of an early chick embryo are very sensitive and that regeneration takes place very rapidly [3]. Others reported a general decrease in the number of circulating elements [13] and an inhibition of blood cell maturation [14]. A general inhibition of hematopoiesis and distorted regeneration accompanied by signs of megaloblastic hematopoiesis have also been reported with regard to mammalian embryos [1,10,11].

There are however no systematic observations concerning the regenerative processes in embryos during the early stages of development. The present paper is devoted to this question.

METHOD

Chick embryos on the fifth day of incubation were exposed to γ-rays emitted by Co^{60} (capacity 1.45-1.2 r per min, dose 1000 r). In the period studied by us (5-12th day of incubation) the only hematopoietic organ present in the embryo consists of the network of hematopoietic capillaries in the yolksac; here the erythropoiesis takes place within the blood vessels and the leukopoiesis in the intervascular reticular tissue. Under normal conditions the products of leukopoiesis appear in the peripheral blood only toward the end of the period studied by us and in small numbers. We investigated sections taken from the walls of the yolksac (fixation in Zenker-formol, staining with iron hematoxylin, according to Yasvoin and with azur-eosin) and films made from the peripheral blood.

RESULTS

If one studies the hematopoietic organs of embryos exposed to radiation the most striking fact is the rapidity with which profound disorders develop in the hematopoiesis. Immediately after exposure to radiation changes can be observed in the original stem cells (Stammzelle) which at that period constitute just as in the control animals the overwhelming majority of cellular elements in the hematopoietic capillary. These cells possess also under normal conditions ameboid mobility. For that reason they frequently show short pseudopodia in the preparations. After exposure to radiation the configuration of the cells undergoes certain changes. Elongated thick protoplasmatic processes of irregular shape develop. In some places they lose their connection with the cell body and form freely lying fragments which give an intensive basophilic stain. The nuclei are of irregular shape and show deep indentations; frequently the constriction is deep enough to divide the nucleus into fragments. It can be observed that the damaged cells are taken up by phagocytic reticulo-endothelial elements. The endothelial layer of the capillaries is thickened and loosened; in some areas perivascular edema can be observed.

Fig. 1. Section through the yolksac wall of a six-day-old embryo exposed to radiation. Perivascular edema. The hematopoietic capillaries are empty. Hematoxylin stain, objective 20×, apochromatic, eye-piece 7×.

On the day following the exposure to radiation (sixth day of incubation) the inhibition of hematopoiesis progresses vehemently. In a number of cases a picture of complete aplasia can be observed in the network of hematopoietic capillaries (Fig. 1). The hematopoietic elements disappear completely (or almost completely). Round the blood vessels massive perivascular edema develops. In the zone of edema the reticular elements appear compressed. Their nuclei are in a state of pycnosis and karyorrhexis.

In a lesser number of embryos the destruction of young hematopoietic elements is not as complete as that described above. Here too however the hematopoietic areas are diminished and contain degenerative forms.

A more or less marked perivascular edema is visible in almost all sections prepared from the wall of the yolksac of 7-,8-, 9-day-old embryos. On the subsequent days edema is found less frequently and is less intensive; even on the 12th day of incubation, however, edema may occur, even in preparations in which the regeneration of hematopoietic areas is well marked. Although the extensive perivascular edema delays the regenerative process to a certain degree in view of the fact that part of the mesenchymal hematopoietic reserves is destroyed, this does not prevent the onset of this process almost immediately after the end of the exposure to radiation.

In the undamaged cells of the reticular syncytium mitoses become more frequent. The endothelial cells take on a round shape and protrude into the lumen of the hematopoietic capillary. The whole course of the subsequent restoration, however, is characterized by disorders in the differentiation and maturation of the blood cells. On sections prepared from the wall of the yolksac of a 7-8-day-old embryo (2nd and 3rd day after exposure to radiation), less frequently at later stages, an increase in the number of reticular stroma cells and an increased frequency of mitoses in these cells can be observed. The total number of early basophilic hematopoietic elements, however, remains very low and they often sustain degenerative changes in the shape of cytoplasmic vacuolization, as well as karyorrhexis. One of the sections prepared from the hematopoietic organs of an 8-day-old embryo is particularly interesting from this point of view: in this case the hyperplasia of the reticuloendothelial apparatus reached a considerable degree. The perivascular spaces were filled with a great number of reticular cells. In the lumen of the

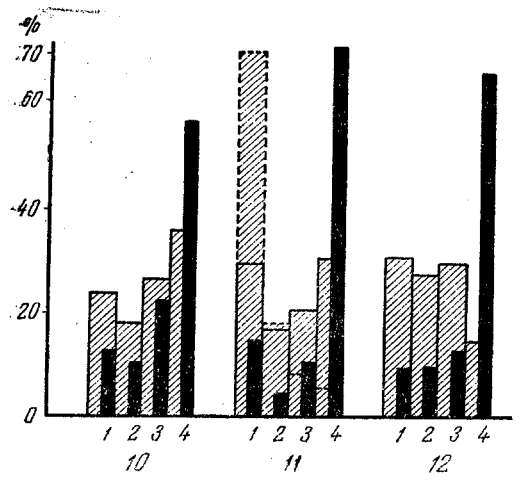

Fig. 2. The percentual composition of hematopoietic cells in a hematopoietic capillary. Black columns: control; hatched columns: after radiation. On the abscissa the stages of maturation, on the ordinate the days of incubation are plotted.

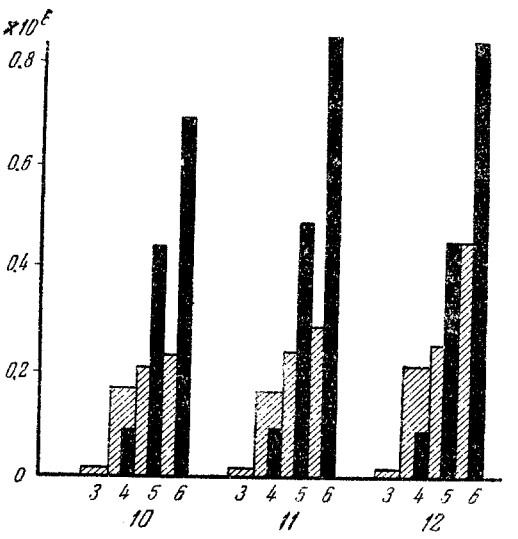

Fig. 3. The number of cells of the erythroid series in 1 mm^3 of peripheral blood. Key to figure same as in Fig. 2.

hematopoietic capillary numerous atypical cells of irregular, elongated shape and with an unevenly basophilic protoplasm could be seen. Many of these cells had protoplasmatic processes with the aid of which part of the cells preserved their connection with the capillary endothelium. No normal hematopoietic cells could be seen. These facts show that the regeneration of the hematopoietic elements encounters some difficulties, notwithstanding the increased proliferation of the reticuloendothelial cells; in some cases the reticuloendothelium produces atypical cells instead of the normal hematopoietic elements.

On the subsequent days the development of the process suggests a permanent inhibition of differentiation, at first in the early basophilic stages, later at the stage of basophilic and polychromatophilic erythroblasts (Fig. 2). Fig. 2 was constructed following the principle of partial myelograms. As it is impossible to draw a perfect analogy between the blood cells of a chick embryo and the generally accepted hematological nomenclature the consecutive stages of erythroblast maturation are referred to by numbers: stages 1-3 correspond to the stages between the hemocytoblast and the basophilic erythroblast; stage 4 corresponds to the polychromatophilic erythroblast. It is quite apparent that 5-7 days after the exposure to radiation the hematopoietic elements of the earlier stages predominate in the hematopoietic capillaries. In one of the embryos exposed to radiation the most immature blood cells constituted 70% of all hematopoietic elements found in the hematopoietic capillaries (columns indicated by a dotted line on Fig. 2). Here, the mitotic activity (3% of the cells were found to be in a stage of mitosis) corresponded to the mitotic activity found in the control sections. It can consequently be assumed that in this case there was no increased proliferation of the cells but an inhibition of differentiation and of the transition into the later stages. This led to an accumulation of undifferentiated immature cells.

In our previous paper [2] we had shown by analyzing the composition of the peripheral blood that exposure of embryos to radiation on the 5th and 8th day of incubation leads, as a rule, to a return to the ontogenetically earlier megaloblastic erythropoiesis. Indeed, elements characteristic for megaloblastic erythropoiesis could be seen in the hematopoietic elements in the shape of large cells containing considerable quantities of hemoglobin, but preserving the structure of the nucleus typical for earlier basophilic elements: a well marked large nucleolus accompanied by a hardly visible chromatin network within the nucleus.

The primitive leucocytes which are formed in the perivascular spaces can be found in unchanged appearance almost in all sections prepared from the hematopoietic organ of the embryos exposed to radiation, even in those cases in which the erythropoiesis suffered considerable damage. This fact is possibly connected with the relatively low activity of leucopoiesis in chick embryos at early stages of embryogenesis.

The peculiarities of hematopoiesis in chick embryos consist particularly in the fact that at early stages of development the hematopoiesis takes place both in the hematopoietic organ and in the peripheral blood. On the

5th and 6th day of incubation the hematopoietic capillaries contain mainly poorly differentiated cells. Cells of the intermediate stages, which have the highest mitotic activity, are mainly found in the peripheral blood; here their maturation is completed. Later the exodus of immature elements into the blood becomes more intensive, but even on the 12th day of incubation the peripheral blood contains 6-7% of polychromatophilic erythroblasts.

Fig. 3 shows the quantitative proportions of various stages of red cell maturation in 1 mm^3 peripheral blood. It can be seen that the number of polychromatophilic and mature red cells (stages 5-6) is much lower than in the control sections, whereas the absolute number of red cells is increased. This fact also suggests an inhibition of red cell maturation with a consequent increase in the number of immature elements.

Radiation damage in chick embryos is thus characterized by an extremely rapid depletion of the hematopoietic areas and the development of perivascular edema, by an early reaction of the reticuloendothelium, inhibition of blood cell maturation and disorders of regeneration, accompanied by the appearance of elements typical for megaloblastic hematopoiesis.

SUMMARY

Chick embryos underwent gamma-irradiation on the 5th day of incubation. Destruction of the endothelium and massive perivascular edema occur in the hematopoietic capillaries of the yolk-sac within the very first hours after irradiation. The hematopoietic elements disappear almost completely. Already on the next day, parallel with the continuing destruction, there appear regeneration elements in the form of proliferation of the reticuloendothelium and formation of new hematopoietic elements. Inhibition of the differentiation processes often leads to the accumulation of elements with low differentiation and prevents the restoration of normal blood composition. Marked affection of hematopoiesis leads in a number of cases to the reestablishment of megaloblastic hematopoiesis.

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