Iron Metabolism in the X-Irradiated Animal

Technical Progress Report

Abraham Mazur

City College, City University of New York
New York City, N.Y.

July 1, 1973 - June 30, 1974

Prepared For The U.S. Atomic Energy Commission Under Contract
No. AT (11-1) - 2385

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Enclosure (A)

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Introduction

In our earlier experiments we reached the conclusion that the six multiple hemoglobins found in the circulating rat red cell were synthesized non-uniformly in marrow erythroid cells. This was based on results of experiments in which rats were injected with ^{59}Fe and circulating red cells sampled at various time intervals in order to determine the specific radioactivities of each of the six hemoglobins. Based on the concept that the "oldest" marrow erythroid cells are the first to be sent into the circulation and that the "youngest" marrow erythroid cell will appear in the circulating red cell only after a period of time equal to its "maturation time", our results indicated that the major site of the biosynthesis of Hb's III and IV were associated with the "oldest" marrow erythroid cell, whereas the major site of synthesis of Hb V was associated with the "youngest" marrow erythroid cell. This followed from our finding that at the earliest time following in vivo labelling of marrow erythroid cells, the specific radioactivity of Hb V was only about one-third that of Hb's III and IV. With time (7-10 days) all specific radioactivities became equal.

Our earlier studies had also shown that the spleen of young adult rats make a contribution of some significance to newly formed circulating red cells. Therefore, splenectomized rats were used in these studies.

I. Effects of x-irradiation on erythroid cell hemoglobin synthesis.

Previous studies in our laboratory confirmed the fact that hemoglobin synthesis in marrow erythroid cells was markedly inhibited as a result of x-irradiation, as measured by the activity of the enzyme ferrochelatase which incorporates iron into protoporphyrin to make heme. It is our intention to determine whether these effects are due to inhibition of all stages of maturation of the erythroid cell or whether its action is selective.

Since the ^{59}Fe experiments summarized earlier can identify "young" from "old" erythroid cells by their synthesis of Hb #V and Hb #IV, respectively, we have started such a series of experiments. Problems have arisen from consideration of the design of such experiments. If the spleen is removed (as in experiments with control animals) the rats will not survive the 11 days required to measure maturation time. If the spleen is lead-shielded, it will serve as a source of nucleated erythroid cells which populate the marrow. In any case, if ^{59}Fe is injected after x-irradiation there is the certainty that this ^{59}Fe will be incorporated into erythroid cell hemoglobin of the shielded spleen or spuriously into erythroid-active cells prematurely sent into the circulation.

In our first series of experiments we have used rats with an intact spleen, injected them with ^{59}Fe and then subjected them to whole body x-irradiation (900r) without shielded spleens, some 12 hrs. after ^{59}Fe administration. Although there is a sharp drop in erythroid activity in such animals, early samples of circulating red cell hemoglobin contain equal specific radioactivities in Hb #V and Hb #IV. These results suggest that whatever ^{59}Fe - labelled cells are present in the bone marrow prior to x-irradiation, these get into the circulation very rapidly (rapid maturation) in response to an impaired overall erythropoietic activity of marrow and spleen. It is likely that marrow erythroid cells labelled at the time of x-irradiation, are still capable of entering the circulation, either as nucleated erythroid cells or reticulocytes.

We are currently studying splenectomized rats subjected to x-irradiation, as well as x-irradiated rats injected with ^{59}Fe immediately following x-irradiation at various time periods.

II. Effects of CoCl_2 and the Walker 256 Carcinosarcoma on marrow synthesis of hemoglobins in the rat.

These experiments were designed to study the effects of various stimulating and inhibiting treatment procedures on the maturation rate of

marrow erythroid cells as measured by the time required to achieve equal specific radioactivities in Hb #V and Hb #IV in circulating red cells.'

The Walker tumor-bearing rat sustains a marked impairment in marrow erythroid cell activity as measured by the extent of incorporation of ^{59}Fe into total marrow hemoglobin. Rats bearing the Walker tumor also sustain a marked stimulation of spleen erythroid activity which almost counteracts the inhibition of marrow activity. In our experiments this is reflected by the fact that the time required for achieving equal specific radioactivities of Hb #V and Hb #IV in tumor-bearing rats is somewhat longer (15-20 days) than in controls, although the 24-hour value for tumor rats yields a ratio of Hb #V: Hb #IV specific radioactivities higher than that of controls; controls = 0.34, tumor rats = 0.55. This suggests that any erythroid cells still functional in the marrow are sent into the circulation prematurely. Indeed, some nucleated erythroid cells can be seen in the circulation.

The results with rats injected with CoCl_2 are of interest. There is a preliminary stimulation, as reflected in a higher ratio of specific radioactivities of Hb's #V: #IV after several days (2-3) and followed by a drop which lasts for several days. This effect continues and results in a prolonged period during which the ratio never equals unity, even after 20 days. Although these experiments need to be continued, the likelihood exists that CoCl_2 has some inhibitory effect, perhaps directed at specific erythroid cells.

III. Separation of Marrow Erythroid cells according to their content of Hb #V and Hb #IV.

Since our in vivo experiments utilizing ^{59}Fe as a label suggests that Hb #V is a "marker" for the youngest erythroid cells, we are presently separating such cells in order to confirm directly the non-uniformity of biosynthesis of the six rat hemoglobins.

We have recently obtained a thin layer counter-current distribution apparatus which makes use of the principle of separating cells in an aqueous medium, partitioning the cells between two immiscible layers of polymers-polyethylene glycol and dextran (Albertsson Technique). This method separates cells according to differences in their surface properties and ^{59}Fe - labelling studies suggest that one can achieve separation of cells according to age. Conditions for such separations have now been achieved and we are accumulating sub-fractions of the various nucleated erythroid cells which will be analyzed for the six rat hemoglobins by the technique of isoelectric focusing.

It is also intended that this technique will be used to study marrow erythroid cell distribution from rats subjected to x-irradiation, with and without spleen shielding.

Note: The principal investigator has spent approximately one-fourth of his research time on this contract; aided by a graduate student working on his research thesis for the PHD. degree. A similar portion of total time will be spent during the next contract year.

Enclosure (A)(cont'd.)