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ABSTRACT

This paper describes a method for observing the uptake of Fe by rat bone marrow cells in vitro. Results of experiments on effects of anemic serum and human erythropoietin are presented with a brief discussion. It is concluded that the differences in uptake of Fe^{59} are the result of isotope dilution.

BONE MARROW ACTIVITY IN VITRO UNDER THE INFLUENCE OF ANEMIC SERUM AND HUMAN ERYTHROPOIETIN*

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A usable method of studying bone marrow activity directly would present important advantages over whole-animal studies in elucidating the mechanisms of erythropoiesis. Such a method was suggested by Schroeder, Gurney, and Wackman.[1] By use of a modification of that method an attempt was made to demonstrate direct action of erythropoiesis-stimulating human material on bone marrow, and to develop a more rapid and efficient assay technique for this material.

PROCEDURE

Male Sprague-Dawley rats weighing 350 to 500 g are bled by cardiac puncture to the extent of 1/4 blood volume every 2 or 3 days. An amount of Fe^{59} appropriate for counting is put into siliconized 15×85-mm test tubes by a standarized procedure for forming liquid drops. Experimental and control sera are obtained by cardiac puncture and centrifugation. One ml of serum is pipetted into the tubes and mixed with the approximately 0.01 to 0.02 ml of active iron solution already in the tubes. The cell suspension is prepared in Eagle's basal medium.[2] A normal or anemic rat is sacrificed and the humeri, femora, and tibiae removed and stripped. The marrow is removed by perfusion of marrow spaces with the medium. The cells are separated from stroma and dispersed by repeated passage through a wire screen in a syringe. The resulting preparation is poured through a nylon mesh bag. The final product is a suspension of 2 to 4×10^7 single nucleated cells per ml in 27 ml volume. Aliquots of 2 ml of this cell suspension are pipetted into the tubes containing the Fe^{59} and sera, and mixed. These tubes are placed in a 37° water bath. In the later experiments warmed and humidified 0_2 is fed continuously to the atmosphere in the tubes over the cell suspensions. The standard period of incubation is 3 hours. The number of counts per minute added to each tube as active iron is determined by averaging counts of aliquots of 3 to 5 separate dilutions of standard drops. At the end of the incubation period, the cell suspensions are centrifuged, the supernate poured off, and the cells resuspended in 0.9% NaCl; the centrifugation and washing are repeated and after the third centrifugation and decantation the cells are left packed. These preparations are then counted in a well counter(NaI crystal).

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RESULTS

The effects of oxygen, temperature, anemic serum, active human urinary erythropoietin (erythropoiesis-stimulating material from urine of a patient with a hematological disease), and serum from erythropoietin-injected rats have been determined. In four separate experiments the iron uptake was roughly tripled by introducing a continuous flow of warmed and humidified oxygen into the tubes over the surface of the liquid. The determinations of radioactivities of cells incubated at different temperatures were as follows: At 5 to 10°C, 156 cpm; at 24°, 637 cpm; at 37°, 2278 cpm. All are averages of three values from separate suspensions. Only one such experiment was done.

In eight separate experiments large differences in uptake were shown between cells treated with normal sera and cells treated with sera from anemic rats. The data from one such experiment are shown in Tables I and II.

Table I

Uptake of Fe^{59} by cells treated with sera from normal and anemic rats

Tube number	Serum	cpm minus background	Mean
1	normal	5586	
2	normal	4811	
3	normal	5201	
4	normal	5220	5207
5	anemic	12825	
6	anemic	13992	
7	anemic	13270	
8	anemic	11847	12983

Table II

Comparison of Fe^{59} uptake by cells treated with sera from normal and anemic rats

		Normal	Anemic
% uptake		8.2	20.4
Fe added	(μg)	0.015	0.015
Serum Fe	(μg)	3.0	1.2
Total Fe	(μg)	3.0	1.2
Uptake	(μg)	0.25	0.24

The average increase over the uptake of the control suspensions was by a factor of 1.9. These differences were demonstrated both with and without oxygenation; apparently oxygenation did not increase the differences. In one experiment, the effects of sera from repeatedly bled rats and from rats bled but once before the experiment were compared. The serum from once-bled rats effected a 1.2-fold increase in iron uptake, the other a 1.6-fold increase. In another experiment, serum from rats bled only twice, 2 and 12 days before the experiment, was compared to serum from rats bled every 3 days for 2 weeks. Three suspensions were treated with serum pooled from the first group of rats, three treated with a 1:1 mixture of the two pools, and four treated with serum from the second, more anemic group. The cells treated with the mixture took up 1.5 times as much iron as the cells treated with the twice-bled rat serum. The last group of four suspensions took up 1.8 times as much. Serum Fe concentrations were determined in four separate experiments. With this additional information, and on the assumption of complete mixing of Fe^{59} with the Fe present, the absolute amounts of iron taken up by the various samples were computed. In all four cases the absolute amounts were the same. The results of these computations for one experiment are included in Table II.

Human urinary material shown by a routine whole-animal assay[3] to be active in stimulating erythropoiesis in rats was tested for effect upon marrow cells in suspension. The material was taken from known active pools and prepared in the same manner as for the successful whole-animal assay. It was added to the marrow suspensions in doses comparable to what the marrow might experience in the whole-animal assay and in much greater doses. No effect was demonstrated. The same sort of experiment was done by making the cell suspension in rat serum rather than in artificial medium, with the same negative result. An experiment was done comparing serum from a rat injected with a relatively large amount of this active material to serum from another normal rat. The material was injected by tail vein, with no apparent ill effect on the animal, 1 hour before drawing blood for the experiment. No effect was demonstrated.

DISCUSSION

Since the demonstration of increased erythropoiesis in the parabiotic partners of hypoxic rats,[4] the presence of a humoral stimulant of erythropoiesis has been confirmed repeatedly. One of the methods used to demonstrate such a substance has come into use as an assay for human erythropoiesis-stimulating material. This method involves measurement of the rate of incorporation of Fe^{59} into the red blood cells of living rats.[3] It might be expected that rat marrow cells in vitro would take up Fe at a more rapid rate under the influence of anemic rat serum or human erythropoiesis-stimulating material. Some experiments[1] suggested that this might be the case for anemic serum. However, the data presented in this paper lead to the conclusion that the results of such experiments follow from a simple isotope dilution. In the case of human erythropoietin, no stimulation was found. This does not rule out an indirect action. However, such an indirect action did not show itself in the single experiment designed to detect it.

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