THE EFFECT OF ERYTHROPOIETIN UPON UTILIZATION OF GLUCOSAMINE BY MARROW CELLS IN CULTURE

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It is now widely accepted that the hormone erythropoietin is an important regulatory factor in the process of red blood cell formation. Several recent reports on the action of erythropoietin on erythropoietic tissues *in vitro* have been for the main part concerned with the incorporation of precursors into heme or hemoglobin or with the gross uptake of radioiron into cells (Erslev, 1962; Powsner *et al.*, 1962; Korst *et al.*, 1962; Krantz *et al.*, 1963).

We shall report here, briefly, some observations on another, as yet incompletely defined, biochemical process which is affected by erythropoietin. Marrow cells in short term cultures incorporate glucosamine-1-C^{14} into an insoluble product, which we have tentatively assumed to represent material derived from cell membranes and stroma. The biosynthesis of this fraction from glucosamine is significantly stimulated by the addition of erythropoietin to the culture medium.

METHODS AND MATERIALS

Marrow cells from the femora and tibiae of 200 g male Sprague-Dawley rats were suspended in the medium at a final concentration of about 10^7 cells per ml. The medium consisted of equal parts of NCTC 199

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and unfiltered calf serum which had been heated at 56° for 30 minutes. It also contained 40 units of penicillin G per ml, 25 μg of streptomycin per ml, 0.02 μmoles per ml of glucosamine-1-C^{14} HCl (5.1 μC per μmole); and erythropoietin where indicated. Each incubation mixture had a final volume of 2.0 ml. The cultures were gassed with a stream of 5% CO_2, 95% air for one minute before the tubes were closed and incubated at 37°. After incubation the cells were washed free of medium with cold saline, then washed successively with 5% trichloracetic acid, alcohol, and alcohol:ether 1:1. The residue was dissolved in performic acid; aliquots were plated on weighed stainless steel planchets as infinitely thin samples, and counted in a Tracerlab thin window "Omniguard" counter. All results were expressed as cpm per mg of dry residue. The sheep plasma erythropoietin (step III lot 137) was obtained from the U. S. Public Health Service Study Section on Hematology and assayed by the fasted rat method. (Fried et al., 1957). The units of activity are those defined previously (White et al., 1960).

RESULTS AND DISCUSSION

Marrow cell cultures incorporated glucosamine into the insoluble fraction continuously during the first 48 hours of incubation. In the presence of 3.5 units per ml of erythropoietin, this incorporation was significantly increased (Fig. 1). Both the control and stimulated curves are curvilinear with an upward concavity and appear to represent initially an exponential course of incorporation for the control and an increment added to the control curve which represents the stimulated condition. The difference between the two curves is linear with the time and can be extrapolated to zero difference at 2.5 hours. These data suggest that the effect of erythropoietin on glucosamine incorporation is indirect, and that the direct effects are exerted in the first 2.5 hours of incubation of the cultures under these conditions. In each of three other experiments with varying times of incubation, the difference curve extrapolated to zero at about 2.5 hours.

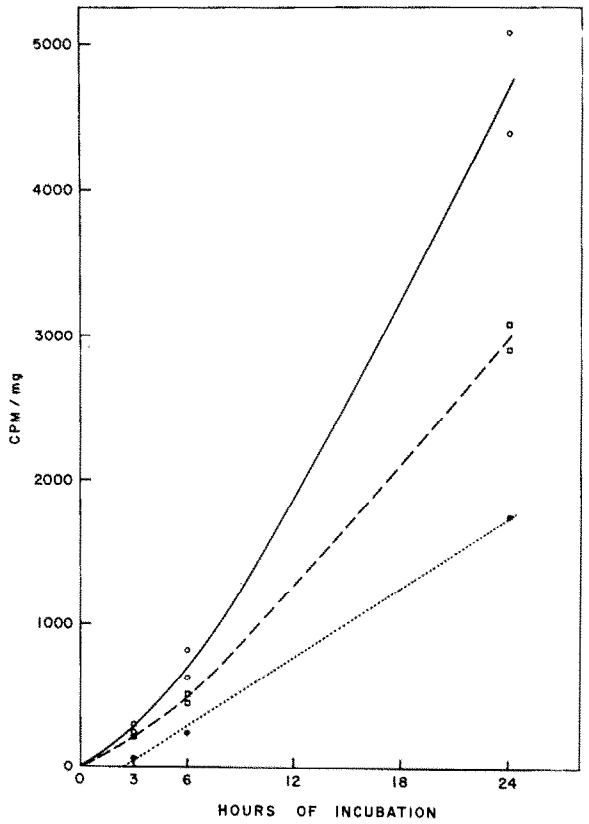

Fig. 1. Time course of erythropoietin effect on glucosamine-1-C^{14} uptake into marrow cells in culture: o ——— o with erythropoietin; □ - - - - - □ without erythropoietin; ..... difference curve.

The effect of amount of erythropoietin upon glucosamine incorporation was studied at 24 hours, with the results shown in Fig. 2. A plot of the response, in cpm per mg, versus the logarithm of the amount of erythropoietin has the sigmoid shape expected for hormone action. This type of curve may represent the increased probability, with increasing amount, of successful encounters between hormone molecules and sensitive cells.

With some preparations of erythropoietin the dose-response curve shows a decline at levels of about 1 unit per ml, suggesting that an inhibitory substance is present in these impure fractions. These observations obviously limit the use of glucosamine incorporation as an assay method for erythropoietin, except in a highly purified state.

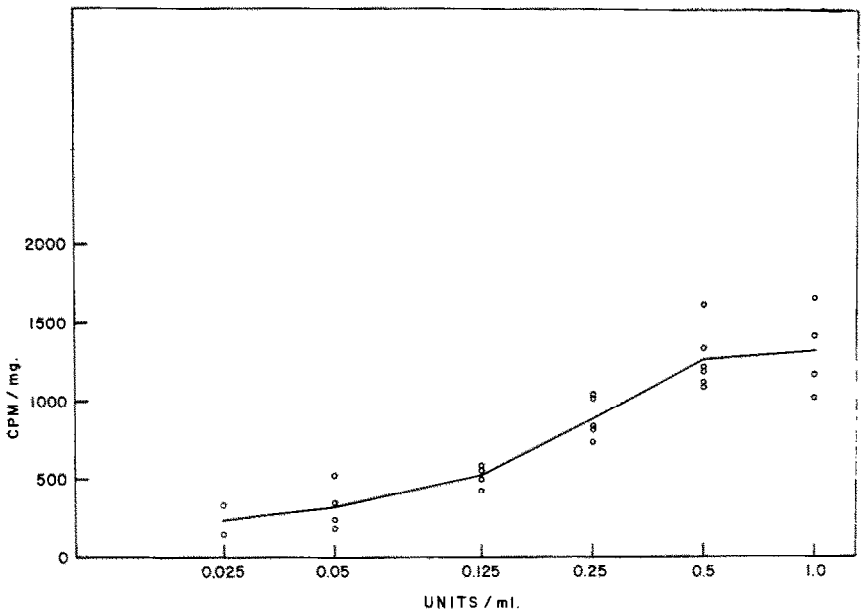

Fig. 2. Relation of glucosamine uptake to erythropoietin concentration.

The identity of the radioactive material synthesized by these cells from glucosamine is not yet established, but we have evidence showing that about 30% of the insoluble radioactivity is in the form of N-acetyl neuraminic acid and/or N-glycolyl neuraminic acid.

More extensive data on the specificity of erythropoietin action on marrow utilization of glucosamine will be published in the near future.

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