THE EFFECT OF HEMIN ON THE SYNTHESIS OF GLOBIN

Gail P. Bruns and Irving M. London

Department of Medicine, Albert Einstein College of Medicine and Bronx Municipal Hospital Center, New York, New York

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Prior studies on the synthesis of heme and globin in rabbit reticulocytes (Kruh and Borsook, 1956 and Morell et al, 1958), rabbit bone marrow (Morell et al, 1958) and dog reticulocytes (Nizet, 1957) have indicated a parallelism in the rates of the two synthetic processes in vitro. The two mechanisms, however, could be differentially affected by various influences, e.g., by irradiation (Richmond et al, 1951) or by the addition of cobalt or lead or purine ribosides or by incubation in a hypotonic medium (Morell et al, 1958 and Kassenaar et al, 1957). Such differential effects indicated that the normal parallelism in rates could be disturbed by various agents, but the question of a mechanism for the normal physiologic coordination of the synthesis of heme and of globin remained to be explored.

The studies which are reported here and in the accompanying paper (Karibian and London) provide evidence for a mechanism which may serve to coordinate the two biosynthetic processes. The findings which are the basis for the proposed mechanism of regulation may be summarized briefly: (1) on the addition of hemin in a concentration of approximately 10^{-4} M to rabbit reticulocytes in vitro, there is a marked inhibition in the utilization of glycine for the synthesis of

heme; the principal site of inhibition is among the reactions which are concerned with the formation of deltaaminolevulinic acid (ALA); (2) under these same conditions, the addition of hemin results in an increase in the incorporation of C^{14}-valine into the newly formed hemoglobin. Heme can inhibit its own formation and can increase the synthesis of globin.

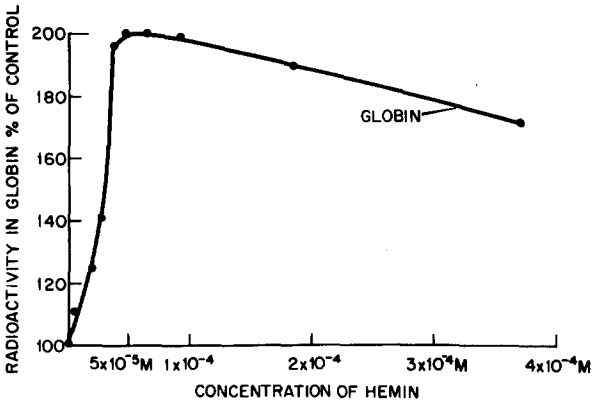
Methods: New Zealand rabbits were maintained on "low iron diet" (Nutritional Biochemical Corporation) and deionized distilled water and were repeatedly bled to maintain a hematocrit of 20-25% and a reticulocyte count of 15-20%. Blood obtained by cardiac puncture was centrifuged at 1500 x g for 15 minutes and the plasma was removed. Incubation flasks contained 0.15 ml of packed cells in 3.4 ml of media consisting of 40% Eagle's medium with supplemental amino acids,[1] 40% NCI culture medium, 20% rabbit plasma, glucose 100 mg%, penicillin 84 mg%, streptomycin 84 mg%, and $NaHCO_3$ 0.01 M. L-Valine-C^{14} (uniformly labeled) was the isotopic substrate. Incubation flasks were flushed with 95% O_2 - 5% CO_2 for 5 minutes, stoppered and incubated at 37° C without shaking.

For the isolation of hemoglobin, the cell suspensions were centrifuged at 1500 x g for 15 minutes, the media were removed and the cells were washed twice with 4 volumes of isotonic sodium chloride. The cells were lysed with 1 volume of distilled water and were centrifuged at 20,000 x g for 45 minutes at 4° C. The supernatant

[1] alanine, .0007M; arginine HCl, .00016M; aspartic acid, .001M; histidine HCl, .0009M; lysine, .0008M; methionine, .00012M; phenylalanine, .00052M; proline, .00053M; serine, .00061M; threonine, .0005M; tryptophane, .00012M; tyrosine, .00026M; leucine, .001M; cysteine, .00015M

solution was applied to starch block electrophoresis in 0.05 M Veronal buffer pH 8.6. The hemoglobin spot was eluted and the optical density and radioactivity were determined.

Results: Hemoglobin formation as a function of the concentration of added hemin is shown in Figure 1. At a concen-



1. The effect of various concentrations of added hemin on the formation of globin. The cell suspension was incubated for 30 minutes at 37° C with added hemin; the control was incubated without hemin. At 30 minutes, 1-Valine-C^{14}, 12 mumoles, 8.4 x 10^7 cpm/umole was added and the incubation was continued for 2 hours. Results are expressed as the radioactivity of the globin of the experimental samples relative to the control which is assigned a value of 100.

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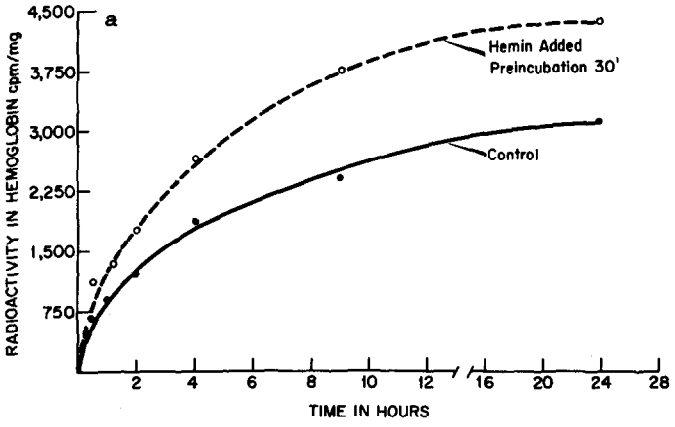
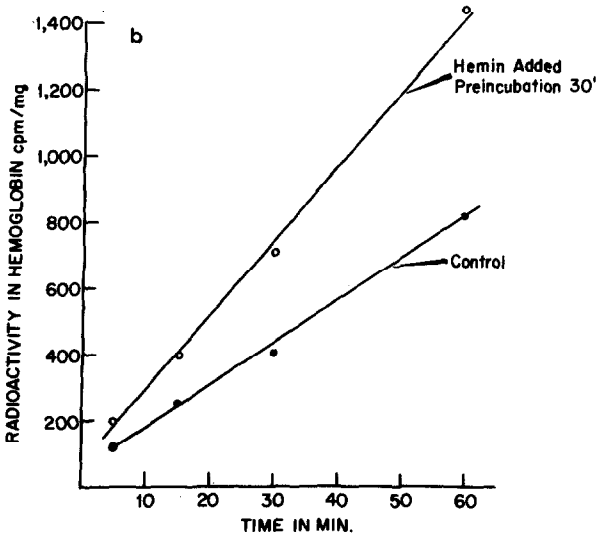
tration as low as 5 x 10^{-5} M there is a considerable increase in C^{14}-valine incorporation. The increased formation of labeled hemoglobin in the presence of added hemin is demonstrable throughout a 24-hour period of incubation (Fig. 2a). The effect occurs very rapidly and is readily observed during the first hour of incubation (Fig. 2b). In these experiments, the reticulocytes were incubated with hemin for 30 minutes prior to the addition of C^{14}-valine; in other experiments in which hemin and C^{14}-valine





2. The effect of added hemin on globin formation. After incubation for 30 minutes at 37° C with added hemin (1×10^{-4} M) or without it (control) 1-Valine-C^{14}, 12 mumoles, 8.4×10^{7} cpm/umole was added and the incubation was continued for various periods of time, (a) hours, (b) minutes.

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were added simultaneously, enhanced formation of protein was observed in 15 minutes.

Previous studies by Kruh and Borsook (1956) and from this laboratory (Kassenaar et al, 1957) have demonstrated enhanced forma-

tion of hemoglobin on the addition of iron. At concentrations of iron and of hemin which singly produced their maximal stimulatory effects on the synthesis of globin, little or no further increase was observed when both hemin and iron were added together. The question of whether iron exerts its stimulatory effect on the synthesis of globin independently or by virtue of its enhancing effect on the synthesis of heme is under study.

It should be noted that the most marked effects of hemin or of iron in increasing the formation of hemoglobin are observed in cells derived from animals that have been rendered iron-deficient by diet and extensive phlebotomy. The stimulatory effects of hemin or of iron may be minimal or absent in cells with normal or elevated concentrations of iron.

Discussion: The principal possible mechanisms by which added hemin increases the formation of globin are: (1) hemin may have a primary accelerating effect on the biosynthesis of the polypeptide chains; (2) the addition of hemin may promote the conversion of the newly formed polypeptide chains to tetramers of hemoglobin; (a) if the subunits of hemoglobin are less stable than the tetramer, a stabilizing effect of heme would be reflected in increased formatio of hemoglobin; (b) if the subunits of hemoglobin should exert a feedback inhibition of polypeptide synthesis, the conversion of subunits to the tetramer would diminish the concentration of the subunits and would lead to a secondary increase in the rate of polypeptide chain synthesis; (c) there may be a combination of the effects of 2(a) and 2(b).

A major question is concerned with the specificity of the enhancing effect of hemin on the synthesis of globin. Preliminary studies indicate that the addition of hemin results in increased formation of globin while the synthesis of the bulk of other proteins in the reticulocyte is not stimulated.

Hammel and Bessman (1964) have observed independently an increase in protein synthesis in avian erythrocyte nuclei incubated in the presence of added hemin.

The inhibitory effect of heme on its own synthesis coupled with its effect in increasing the formation of globin provides a partial basis for a schema of regulation of the synthesis of hemoglobin. This schema is presented as a working hypothesis which is currently being subjected to experimental test. It includes: (1) the inhibition by heme of its own synthesis and (2) the stimulatory effect of heme on the formation of globin. In addition, it raises the speculative possibilities of (3) the stimulation of heme synthesis by globin and (4) the inhibition by globin or its subunits of its own synthesis. The first two processes have been described and considered in these papers. If the third and fourth processes are found to be operative, they could contribute to a dual system of regulation, by heme and by globin, which might be more sensitive than regulation by either mechanism alone.

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