

THE EFFECT OF COBALT ON THE SYNTHESIS OF GLOBIN BY RABBIT RETICULOCYTES IN THE PRESENCE AND ABSENCE OF IRON

Herbert M. SCHULMAN

*Lady Davis Institute for Medical Research, Jewish General Hospital, 3755 Cote St. Catherine Road,
Montreal 249, Quebec, Canada*

Received 29 October 1970

1. Introduction

There are conflicting reports concerning the effect of cobaltous ions on the synthesis of hemoglobin by reticulocytes. This results, not from conflicting hypotheses about the mechanism of action of cobalt, but rather from conflicting experimental observations. Various authors have reported that cobalt stimulates globin synthesis by reticulocytes [1–4], while we previously reported that cobalt inhibits globin synthesis [5]. These discrepancies are apparently resolved by the findings reported in this communication which indicate that cobalt inhibits globin synthesis when transferrin and iron are present in the incubation medium, while it stimulates globin synthesis in the absence of transferrin and iron.

2. Methods

Reticulocytes were obtained from phlebotomized rabbits and purified on dextran gradients as previously described [6]. The incubation medium was identical to the one previously described [7]. The medium containing iron had 1.0% (w/v) rabbit transferrin and 60 $\mu\text{g}/\text{ml}$ $\text{Fe}(\text{NH}_4)_2\text{SO}_4$. When present cobaltous ion was added to the incubation medium at a final concentration of 10^{-3} M CoCl_2 . Cells were incubated as 10% (v/v) suspensions at 37° . The ^{14}C -amino acids were from a reconstituted algal hydrolysate. For radioactive protein determinations, whole washed cells were lysed with two volumes of ice cold distilled water and the lysates were precipitated with ice-cold acidified acetone (3% conc. HCl in acetone v/v). The precipitates were collected on Whatman no. 42 filter papers,

washed exhaustively with acidified acetone, glued to aluminum planchettes, dried and counted in a Nuclear Chicago Low Background counter. It had previously been demonstrated that this procedure is an accurate measure of globin synthesis, since glycine accounted for only 5% of the radioactivity in the ^{14}C -amino acid mixture [see 5].

Rabbit transferrin was purchased from Pentex, Incorporated. It is contaminated with hemopexin, but behaves identically to pure transferrin in supporting hemoglobin synthesis in rabbit reticulocytes [8].

3. Results

Table 1 shows that when reticulocytes are incubated in the presence of iron and cobalt they synthesize about 50% as much globin as when they are incubated in the presence of iron alone. However when the cells are incubated in a medium lacking iron and the amount of globin synthesized is compared to a medium lacking iron but supplemented with cobalt a relative stimulation is apparent. This relative stimulation increases with the increasing amount of time that the cells have been incubated in the absence of iron. Nevertheless this relative cobalt stimulated globin synthesis does not reach the level of synthesis observed with the medium containing only iron.

4. Discussion

We have shown that cobalt stimulates globin synthesis in rabbit reticulocytes relative to the amount

Table 1
Incorporation of ^{14}C -amino acids into globin

Time of incubation prior to the addition of ^{14}C -amino acids (min)	+ iron -cobalt (a)	+ iron + cobalt (b)	-iron -cobalt (c)	-iron +cobalt (d)	b/a $\times 100$	d/c $\times 100$
0	6045	3167	2837	2760	52	97
30	6258	3258	1730	2791	52	161
60	6617	3297	919	2721	50	296
90	6407	3518	703	2531	55	360

Cells were incubated in the complete medium with or without iron and cobalt as indicated. After the preincubation time ^{14}C -amino acids were added and the cells were incubated for an additional 30 minutes after which globin was isolated and assayed for radioactivity as described in Methods. The results are expressed as counts per minute per sample. All samples contained the same number of cells. In experiments not reported here, but performed under identical conditions, more than 90% of the incorporated radioactivity could be accounted for in purified alpha and beta chains of rabbit globin.

of synthesis observed in iron-depleted cells. However, when the cells are incubated in the presence of both iron and cobalt, cobalt inhibits globin synthesis relative to the amount synthesized by the cells incubated only with iron.

Although cobalt protoporphyrin stimulates globin synthesis in the reticulocyte cell free system [9], there is no direct evidence supporting the hypothesis [3] that cobalt protoporphyrin is incorporated into newly synthesized globin.

Acknowledgements

The author is grateful to Miss Rose Sidloi and Mr. Charles Tu for technical assistance and the Canadian Medical Research Council for financial support of this work.

References

- [1] H. Morell, J.C. Savoie and I.M. London, *J. Biol. Chem.* 233 (1958) 932.
- [2] H.S. Waxman and M. Rabinovitz, *Biochim. Biophys. Acta* 129 (1966) 369.
- [3] R. Jackson and T. Hunter, *FEBS Letters* 9 (1970) 61.
- [4] H.S. Waxman, *J. Clin. Invest.* 49 (1970) 701.
- [5] H.M. Schulman and A. Jobe, *Biochim. Biophys. Acta* 169 (1968) 241.
- [6] H.M. Schulman, *Biochim. Biophys. Acta* 148 (1967) 251.
- [7] H.M. Schulman, *Biochim. Biophys. Acta* 155 (1968) 253.
- [8] J. Martinez-Medellin and H.M. Schulman, unpublished.
- [9] S.D. Adamson, E. Herbert, S.F. Kemp, *J. Mol. Biol.* 42 (1969) 247.