# SHORT REPORT

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## COLCHICINE DOES NOT INHIBIT RABBIT RETICULOCYTE GLOBIN SYNTHESIS

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**Summary**

*Colchicine at concentrations of up to 5 mM does not inhibit protein synthesis in intact rabbit reticulocyte cells.*

Colchicine, an alkaloid isolated from the plant *Colchicum autumnale*, has been used for centuries in the treatment of gout[1]. The drug is also a potent inhibitor of cell division, due to its ability to bind to tubulin and hence disrupt microtubules[2]. Taylor has demonstrated, using cultures of KB cells, that colchicine is able to enter cells, rapidly equilibrating with external colchicine, and does not affect the rates of DNA, RNA, or protein synthesis[3]. However, the low endogenous protein synthesizing activity of such cells does not provide a critical test for the noninhibitory action of colchicine.

Intact rabbit reticulocyte cells can sustain a linear rate of protein synthesis for periods greater than one hour at 37 °C[4]. Being an enucleated precursor of the erythrocyte, globin synthesis in reticulocytes is not complicated by nucleic acid metabolism. Using such a system, we report that colchicine, at concentrations of up to 5 mM, has no inhibitory effect on protein synthesis.

Reticulocytes were obtained by cardiac puncture of rabbits which have been made anemic by daily subcutaneous injections of a solution of phenylhydrazine hydrochloride[4]. The cells were sedimented, washed with normal saline and made 25% (v/v) with a citrate-bicarbonate buffer solution[5] at pH 7.5, containing 0.01 mM ferrous ammonium sulfate and a complement of twenty naturally occurring L-amino acids in the range of 0.1–1.0 mM, including $^3$H-L-leucine (specific activity = 5 mCi/mmole). Incubation was conducted at 37 °C and 50 $\mu$l aliquots were removed at varying times for

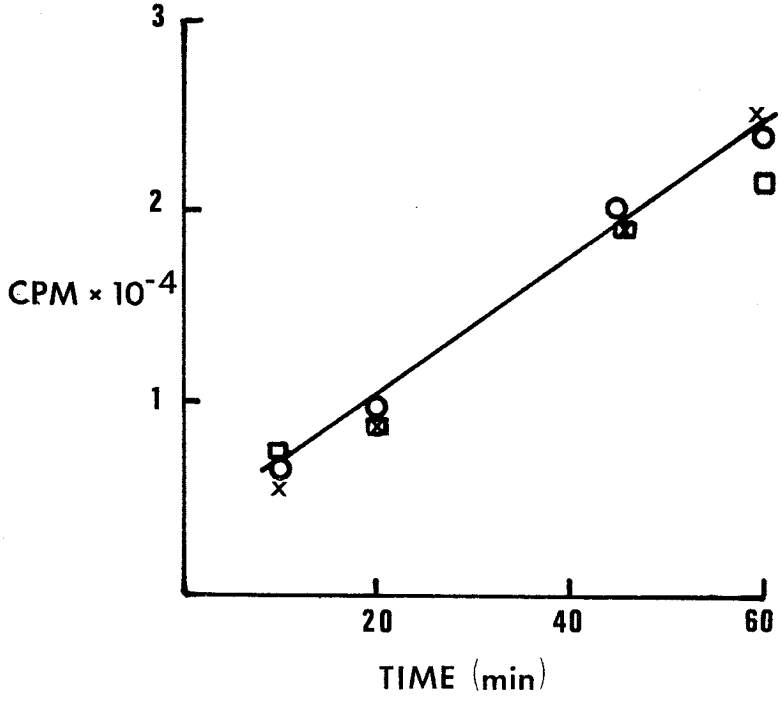


**Fig. 1.** Incorporation of $^{3}$H-L-leucine into trichloracetic acid-precipitable material in intact rabbit reticulocytes, in the absence (O) (control) and in the presence of 2 mM (×) and 5 mM (□) colchicine. 50 $\mu$l aliquots were assayed for radioactivity. The reaction mixtures were incubated at 37°. For clarity only the linear curve through control values has been drawn.

radioactivity determination[4] (counting efficiency=12%). Incorporation of $^3$H-leucine into trichloracetic acid-precipitable material in the absence of colchicine was linear for one hour, and the presence of 2 mM or 5 mM colchicine did not perturb the rate of incorporation (Fig. 1). In one hour, 14 chains of globin were synthesized per ribosome (using the information given in Table II, ref. 6); this is comparable with the values of 26–31 chains of globin synthesized per ribosome as reported by Woodward *et al.*[4]. A value of greater than one globin chain synthesized per ribosome indicates *de novo* globin synthesis and multiple chain turnover, a necessary property of any active protein synthesizing system. A value of less than unity, on the other hand, reflects only chain completion by ribosomes attached to mRNA and an inability to reinitiate protein synthesis.

It is of interest to note that treatment of red cells with 2 mM colchicine doubles the rate of sodium influx and efflux[7]. This is in contrast to the inhibition of globin synthesis by valinomycin, an effect not due to changes in intracellular potassium concentration *per se*, but due, purportedly, to changes in potassium gradient across the cell membrane[8].

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**References and Notes**

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5. The incubation mixture contained : NaCl, 40 mM ; $MgCl_2$, 3.7 mM ; KCl, 0.07 mM ; glucose, 0.15% (w/v) ; Tris-HCl, 24 mM ; trisodium citrate, 1.2 mM ; sodium bicarbonate, 1.8 mM.
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## บทคัดย่อ

ได้พบว่า colchicine ซึ่งมีความเข้มข้นต่ำกว่า 5 mM ไม่มีผลต่อการสังเคราะห์โปรตีนในเม็ดเลือดแดงชนิด reticulocyte ของกระต่าย