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# Effect of Haemin on Endogenous Protein Synthesis in Oocytes of the Queensland Cane Toad *Bufo marinus*

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*Abstract*

Although general protein synthesis as measured by L-[$^3$H]leucine incorporation is unaffected by injection of haemin into oocytes of *B. marinus*, the synthesis of at least two proteins is repressed. It appears that haemin is capable of exerting inhibitory effects on eukaryote protein synthesis as well as stimulating it as previously reported by other workers.

## Introduction

Haemin has been shown to stimulate protein synthesis in a number of cell types from mammals (Beuzard *et al.* 1973; Legon *et al.* 1973; Mathews *et al.* 1973; Rhoads *et al.* 1973) as well as the translation of rabbit α-globin mRNA in oocytes of *Xenopus laevis* (Lane *et al.* 1971; Giglioni *et al.* 1973). While determining optimum conditions for translation of injected mRNA, we also examined the effect of haemin on protein synthesis in oocytes of the Queensland cane toad *Bufo marinus*. We report here that haemin has little effect on general protein synthesis in the oocytes, but causes a selective repression of at least two proteins.

## Materials and Methods

*Microinjection*

Female *B. marinus* were obtained from the Apex Club, Innisfail, Qld. Oocytes were obtained by dissection; prior injection with pituitary hormone was unnecessary. It has been shown previously (May and Glenn 1974) that oocytes of this species are capable of translating injected rabbit haemoglobin mRNA and that endogenous protein synthesis is unaffected by the microinjection procedure.

*Measurement of Protein Synthesis*

Protein synthesis was measured by incubating injected oocytes in Barth's medium (Gurdon 1967) containing either L-[$^3$H]leucine (specific activity 60 Ci/mmole), L-[$^3$H]phenylalanine (51 Ci/mmole), L-[$^{14}$C]phenylalanine (416 mCi/mmole), L-[$^3$H]valine (17·2 Ci/mmole), or L-[$^{14}$C]valine (260 mCi/mmole). Oocytes were incubated at 22°C, then homogenized in 0·052M tris–glycine buffer, pH 8·9, containing unlabelled amino acid (0·5%). Protein was precipitated with 5% trichloroacetic acid containing unlabelled amino acid (0·5%). The precipitate was dissolved in alkali, then reprecipitated and washed twice with trichloroacetic acid, ethanol, and ether and finally dissolved in NCS scintillant and counted by liquid spectrometry.

*Gel Electrophoresis*

Sodium dodecylsulphate (SDS) polyacrylamide gel electrophoresis was performed by the method of Weber and Osborn (1969), except that the concentrations of ammonium persulphate and TEMED (*N,N,N'N'*-tetramethylethylenediamine) were halved. Electrophoresis of samples was carried out

at room temperature at 6 mA per gel. After electrophoresis the gels were frozen in dry ice and sliced. Gel slices (1 mm) were incubated overnight at 37°C in 0·25 ml of 1% SDS and then 2 ml of toluene–Triton X100 scintillant added (5 parts toluene scintillant: 3 parts Triton X100). In the dual labelling experiments the spill of $^{3}H$ into the $^{14}C$ channel was 0·08% and there was a 6·8% spill of the $^{14}C$ into the $^{3}H$ channel; all values have been corrected for spill over.

*Stock Solution*

A stock haemin solution of 10 mg/ml was freshly prepared before each experiment by dissolving haemin in 1N KOH, diluting with 10 mM tris–KCl buffer, pH 7·8, and adjusting the final pH to 7·8 with HCl, as described by Burnham and Lascelles (1963).

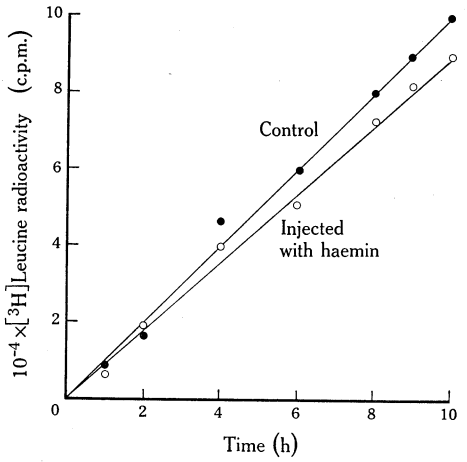


**Fig. 1.** Effect of haemin on general protein synthesis. Batches of oocytes were injected with either haemin (0·038 mM final intracellular concentration) or with buffer (0·01M tris–KCl, pH 7·8) and incubated with [$^{3}$H]leucine in Barth's medium.

## Results and Discussion

*Effect of Injected Haemin on Protein Synthesis*

Oocytes were injected with 50 nl of 10 mM tris–KCl buffer, pH 7·8, containing haemin at different concentrations. (The initial concentration of haemin used was 20 times the intracellular level, and as the volume of the oocyte is approximately 1 $\mu$l this gives a 20-fold dilution of the haemin when 50 nl were injected.) The injected oocytes were then incubated for 23 h at 22°C in Barth's medium containing 10 $\mu$Ci L-[$^{3}$H]leucine. At the end of this period the oocytes were homogenized and processed as described in Materials and Methods. It can be seen from the following tabulation that haemin, over a wide concentration range, had little effect on general protein synthesis. This is in agreement with the results of Giglioni *et al.* (1973).

| Intracellular haemin concn (mM): | Control | 0·001 | 0·01 | 0·03 | 0·05 | 0·1 | 0·2 | 0·4 |
|---|---|---|---|---|---|---|---|---|
| Endogenous protein synthesis (%) | 100[A] | 96 | 92 | 94 | 106 | 90 | 103 | 101 |

[A] Equivalent to 66 000 c.p.m.

**Fig. 2.** SDS polyacrylamide gel profiles of proteins derived from oocytes injected with buffer (10 mM tris–KCl, pH 7·8) or with haemin (0·038 mM final concn) and then incubated with variously labelled amino acids:

(*a*) ▲ Buffer, [$^{3}$H]phenylalanine; △ haemin, [$^{14}$C]phenylalanine.
(*b*) ▲ Buffer, [$^{3}$H]valine; △ haemin, [$^{14}$C]valine.
(*c*) ▲ Buffer, [$^{3}$H]valine; △ buffer, [$^{14}$C]valine.

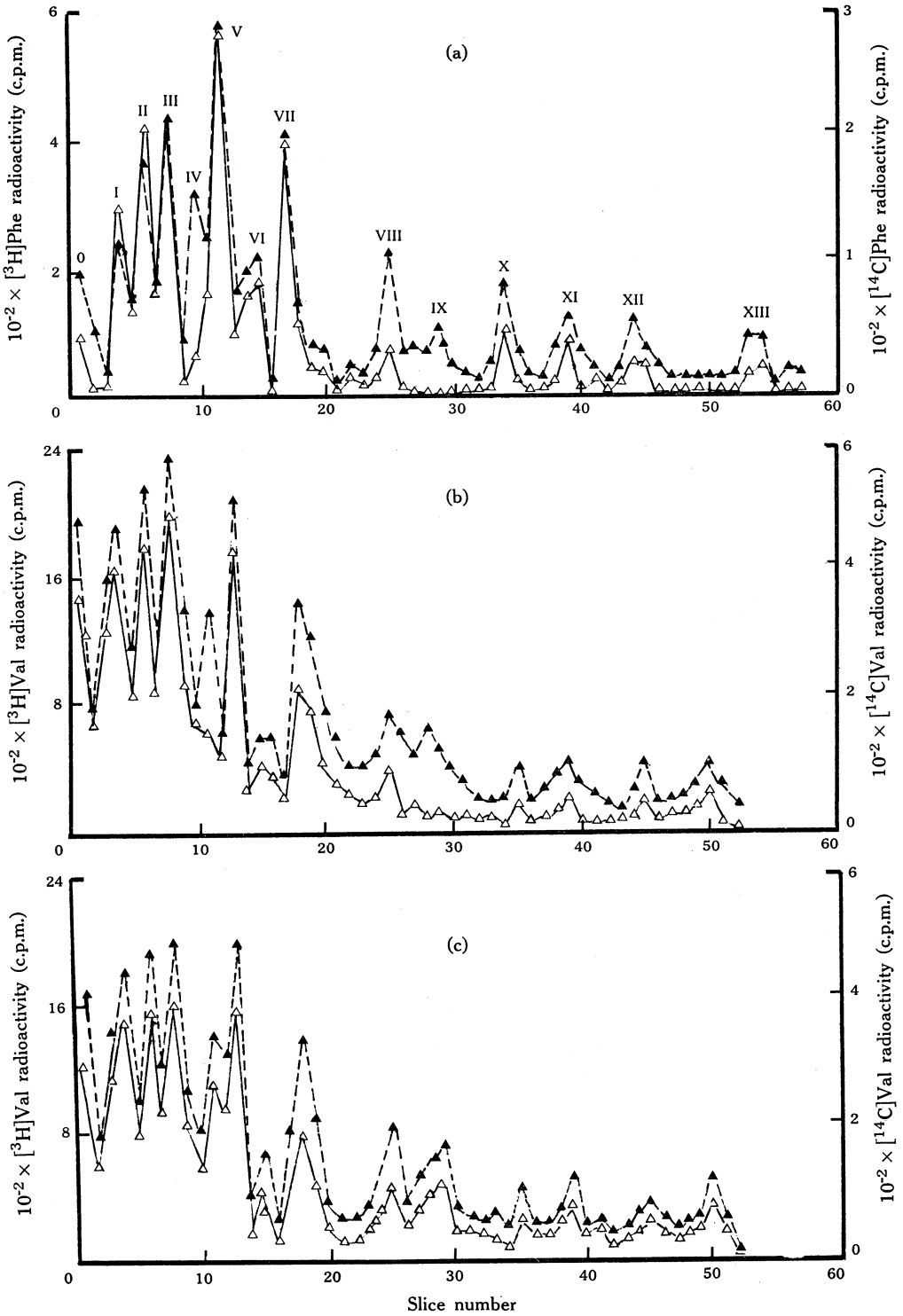
(a)
(b)
(c)
0
I
II
III
IV
V
VI
VII
VIII
IX
X
XI
XII
XIII
$10^{-2} \times [^3H]$Phe radioactivity (c.p.m.)
$10^{-2} \times [^{14}C]$Phe radioactivity (c.p.m.)
$10^{-2} \times [^3H]$Val radioactivity (c.p.m.)
$10^{-2} \times [^{14}C]$Val radioactivity (c.p.m.)
Slice number

Fig. 2

Since the haemin could have had an effect during the first few hours of incubation, samples of oocytes injected with 0·03 mM haemin (final intracellular concentration) were processed at intervals over a 10-h period. Samples of oocytes injected with 10 mM tris–KCl buffer, pH 7·8, were used as a control. At no time did the haemin appear to stimulate protein synthesis (Fig. 1).

*Selective Effect of Haemin on Protein Synthesis*

Although the overall rate of protein synthesis was unaltered by haemin there could be effects on the synthesis of individual proteins. There is some precedent for this view since it has been shown (Edwards 1973) in isolated liver cells that haemin stimulates synthesis of tyrosine aminotransferase but represses that of δ-amino-laevulinate synthase, while general protein synthesis remains unaffected. In order to examine this possibility we carried out dual labelling experiments and compared the SDS polyacrylamide gel profiles of proteins derived from control oocytes injected with buffer with those from oocytes injected with haemin.

Oocytes injected with buffer alone were incubated with [$^3$H]phenylalanine and those injected with haemin (0·03 mM final intracellular concentration) with [$^{14}$C]-phenylalanine. After incubation the oocytes were homogenized in 0·052M tris–glycine buffer, pH 8·9, containing L-phenylalanine and freeze-dried. The lyophilized material was taken up in SDS (1%)–β-mercaptoethanol (1%) and incubated at 37°C for 2 h. Samples were clarified by centrifugation at 100 000 *g* for 60 min and the soluble fractions mixed, giving an excess of $^3$H over $^{14}$C, and then examined by SDS poly-acrylamide gel electrophoresis.

Fig. 2*a* shows that most of the proteins found in the control oocytes have their counterparts in the oocytes injected with haemin. Proteins IV and IX, however, appear to be missing, or much reduced in oocytes injected with haemin. This experiment was repeated using valine as the radioactive label instead of phenylalanine. Fig. 2*b* shows that proteins IV and IX are again absent, or much reduced, in the haemin-treated samples. In a control experiment two batches of oocytes were injected with 10 mM tris–KCl buffer, pH 7·8, one batch was incubated with [$^3$H]valine and the other with [$^{14}$C]valine. Extracts were prepared in the usual manner and examined by SDS gel electrophoresis. Fig. 2*c* shows that in these gels proteins IV and IX were present in both the $^3$H and the $^{14}$C samples.

These results show that most proteins synthesized in oocytes of *B. marinus* are totally unaffected by the injection of haemin but at least two appear to be repressed. It would thus appear that haemin is capable of stimulating protein synthesis in eukaryotes as in reticulocytes and Krebs ascites cells, or inhibiting it as in liver cells or oocytes. At present there is no obvious explanation for the apparently variable effects of haemin on the translation of different mRNA species.

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