SEPARATION OF HAEM INCORPORATION FROM PROTEIN SYNTHESIS IN LIVER MICROSOMES

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Summary
The incorporation of C^{14} δ amino laevulinic acid (δALA) into rat liver microsomal haem was studied. We found that haem is incorporated into microsomes in a form that turns over rapidly; that haem is non-specifically bound to microsomes; and that incorporation is not dependent on protein synthesis. These results show that turnover of microsomal haem cannot be taken as an indicator of turnover of microsomal haemoproteins.

Microsomal haemoproteins, especially cytochrome P-450, play a central role in the drug metabolizing enzyme system of the liver. Interest in these proteins has been stimulated by the finding that drugs and environmental contaminants, such as phenobarbitone and DDT, cause a massive increase in enzyme activity and haem content.[1]

Since no practical isolation technique for cytochrome P-450 has been devised, we and a number of other groups,[2,3,4] have followed the synthesis and turnover of the haem prosthetic groups of microsomes by labelling with radioactive δ ALA, a specific haem precursor.[5] We hoped in this way to measure synthesis and turnover rates of the microsomal P-450 cytochrome, in order to understand the mechanism by which phenobarbitone exerts its inducing effect. Phenobarbitone does not alter the half-life of the microsomal haem,[2] but our further observations make it more difficult to interpret these previous results, and those of other workers, in terms of turnover of the protein moiety of the cytochrome.

Materials and Methods

2 μC/100 g of 4-C-^{14} δALA (Radiochemicals, Amersham, Bucks.) (Specific activity 19 mC/mMole) was injected intraperitoneally into 150-200 g Sprague-Dawley rats (Carworth Europe, Huntingdon). These were killed at fixed time intervals after injection, their livers removed and 5 g pieces homogenized in 15 ml of 150 mM KCl. Microsomes were prepared and microsomal haem activity found by extracting the microsomes with 100 mM HCl-acetone. Cytochrome b_5 was obtained by trypsin digestion.[6] Experiments were carried out to determine a suitable dose of cycloheximide to inhibit protein synthesis. Cycloheximide (Sigma Chemical Co) dissolved in 0.15N saline was given intraperitoneally to stock rats; control rats received equivalent doses of 0.15N saline. 90 min later 1μC/100g of 1-C^{14}-L-leucine (Specific activity 10mC/mmole) was injected intraperitoneally into both groups and the rats killed 30 min later. Their livers were removed, 2g pieces were taken and homogenized in 18 ml of 150 mM KCl. An aliquot of homogenate was taken, precipitated with trichloroacetic acid and washed as described elsewhere.[8] The precipitate was dissolved in 1N naOH and counted in a liquid scintillation counter.

In experiments using C^{14} δALA, 5mg/kg of cycloheximide was given intraperitoneally 90 min before injection of the labelled compound and the animals killed 1 hour later. The livers were removed and treated as described above.

Results and Discussion

In figure 1, the peak labelling in the microsomal haem fraction occurs after 1 h and falls rapidly until 8 h when it begins to level off. There appear to be at least two radioactive components, one with a short half-life of about 4 h, and the other with a longer half-life of about 42 h.

Table 1 shows the dose-effect response to increasing dosages of cycloheximide. Other workers have shown that maximum inhibition of protein synthesis by cycloheximide lasts from four to six hours after administration[9],

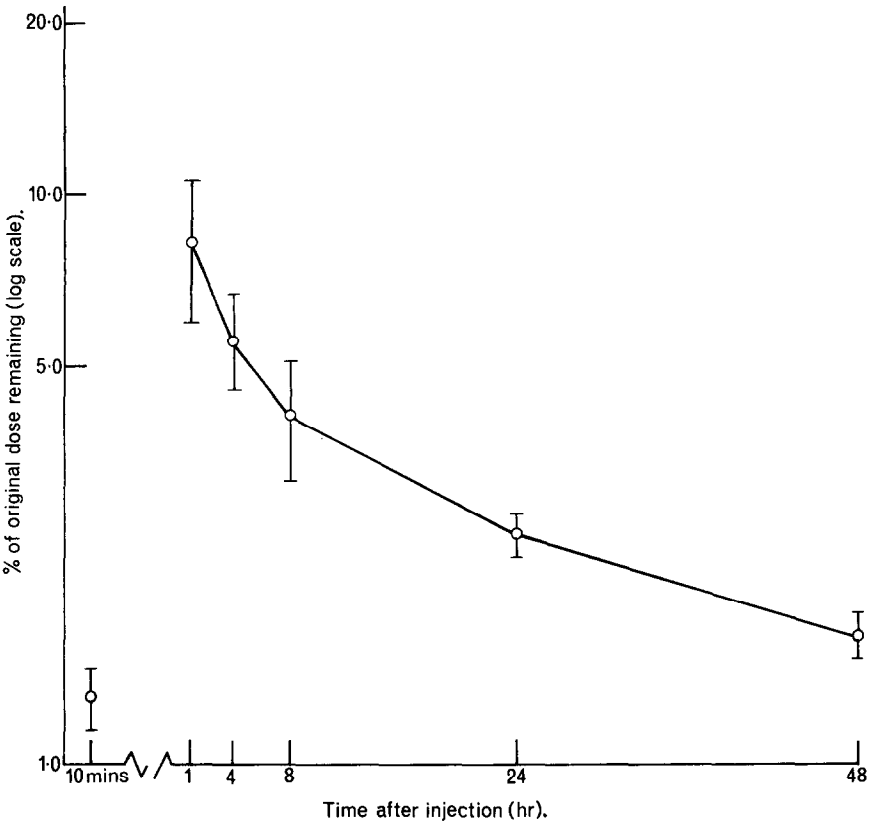

Fig. 1

Incorporation of ^{14}C - δALA into microsomal haem against time as a percentage of the dose given. Each point is the mean result ± S.D. from 3 animals.

so that our δALA experiments, when the animals were killed 2½ h after cycloheximide administration, protein synthesis would still be inhibited by over 90%.

Table 2 shows that the activity in the various liver fractions is the same in both control and cycloheximide treated rats after 1 h of labelling. Isolation of cytochrome b_5 and determination of its specific activity, which was about one fifth of the total microsomal haem specific activity, also revealed equal labelling in the two groups. In vitro experiments using a tracer dose of labelled haem added to a liver homogenate and isolation of

TABLE 1

The Effect of Cycloheximide on Amino Acid Incorporation into Protein in Rat Liver

Dose of Cycloheximide (mg/kg)	Percentage inhibition of protein synthesis	
	Stock Rats	Range
0.1	52	43-59
0.5	72	68-74
1.0	75	73-77
5.0	98	97-100

Each value is the mean percentage inhibition for 4 rats, the mean values for controls being set at 0%. Control values for stock rats were 70,300 ± 12,200 DPM/g liver.

TABLE 2

Distribution of Radioactivity in the Liver after a dose of δALA.

	Amount incorporated as % of Dose given	
	Control	Cycloheximide treated
Liver	18.0 ± 1.5 (4)	18.5 ± 0.9 (5)
Liver microsomes	10.4 ± 0.5 (4)	11.0 ± 0.6 (4)
Microsomal haem	10.0 ± 1.9 (4)	11.1 ± 2.2 (4)

Results are expressed as mean ± S.D. Figures in parenthesis indicate number of animals used.

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the cell fractions showed that microsomes had bound approximately 45% of the added haem. Increasing the amount of label added, 50 fold, did not alter the distribution.

These findings show that haem is rapidly taken up by microsomes in vivo,

in vitro and independently of protein synthesis.

In the view of the avidity with which microsomes bind haem, one cannot be sure that the radioactivity found in isolated microsomes was located there before homogenisation. The labelling of cytochrome b_5 in the presence of cycloheximide shows that haem exchange may be taking place or that preformed apoprotein may be present.

From the known time course of induction of cytochrome P-450, it seems likely to us that the haem component with the longer half life represents turnover of cytochrome P-450, while the shorter half life component is a precursor, perhaps free haem bound to microsomes. Some of this could be incorporated into microsomal cytochromes and the rest excreted as "early labelled bilirubin"[7].

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REFERENCES

1. Conney, A.H., Pharmacol. Rev. 19 ; 317 (1967).
2. Garner, R.C. and McLean, A.E.M., Biochem. J. 114, 7P (1969).
3. Levin, W. and Kuntzman, R., Life Sci. 8 ; 305 (1969).
4. Greim, H and Remmer, H., FEBS abstract (Madrid meeting) No. 332, p.117 (1969).
5. De Matteis, D.F. Pharmacol. Rev., 19 ; 523 (1967).
6. Sargent, J.R. and Vadlamudi, B.P., Biochem. J. 107 ; 839 (1968).
7. Schmid, R., Marver, H.S. and Hammaker, L., Biochem. Biophys. Res. Commun., 24 ; 319 (1966).
8. Garner, R.C., Biochem. Pharmac. In press.
9. Yeh, S.D.J., and Shils, M.E., Biochem. Pharmacol., 18 ; 1919 (1969).