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Effect of 3,5-diethoxycarbonyl-1,4-dihydro collidine on the metabolism of iron in mouse liver

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MANY chemically unrelated drugs stimulate the hepatic formation of porphyrins in the experimental animals and in liver cells cultured *in vitro*.[1,2] These drugs enhance the activity of the hepatic 5-amino-laevulinate (ALA) synthetase,[3] the rate limiting enzyme in the biosynthetic pathway of porphyrin and haem, but the exact mechanism underlying this effect is not yet known. Granick[3] has suggested that the chemicals that induce porphyria act by interfering with the feed-back control exercised by haem at the level of ALA synthetase.

There are three main mechanisms by which the porphyrinogenic drugs may interfere with the feed-back control exercised by haem: (1) the drugs might inhibit the synthesis of haem; (2) they might compete with haem for some regulatory site where haem has to become bound in order to control ALA synthetase or (3) they might increase the rate of haem utilization or breakdown.

Evidence has been presented in favour of each of these three mechanisms.[3–5] For example, Onisawa and Labbe have described an inhibition of haem synthesis in mice made porphyric by 3,5-diethoxy-carbonyl-1,4-dihydro collidine (DDC).[4] Since their experiments were carried out on animals treated for several days with relatively high doses of DDC, a decreased iron-protoporphyrin chelation due to an aspecific toxic effect of the drug cannot be excluded. Moreover in a recent study we found that 5-ALA synthetase activity reached a maximum within a few hours after DDC administration[6] whereas the inhibition of the ferrochelatase had been observed by Onisawa and Labbe after 7–10 days of treatment.[4]

For these reasons a further study of the effect of DDC on the incorporation of ^{59}Fe by the liver has now been studied *in vivo* and *in vitro* within a few hours of DDC administration.

Male Swiss S.M. mice weighing 30 ± 4 (S.D.) g were used. The DDC was prepared according to the method of Eisner[7] and dissolved in corn oil at the concentration of 20 mg/ml. Ferric chloride (^{59}Fe) solution (0·2 mc/ml) was supplied by Sorin (Saluggia, Italy). Animals were fasted overnight before dosing and their fasting continued until they were killed. They were injected intraperitoneally with DDC solution (133 mg/kg body weight) and killed 4 hr later; control mice received the same volume of corn oil alone. The *in vivo* uptake of ^{59}Fe into liver tissue and its incorporation into liver haem were determined at several intervals varying from 15 to 120 min after i.p. injection of 3 μc of ^{59}Fe/mouse. The incorporation of ^{59}Fe into liver haem was also studied *in vitro* using both liver homogenates[8] and isolated mitochondria.[9] Liver haem was crystallized by the method of Labbe and Nishida,[10] using red blood cells to supply carrier haem. The specific activity of the crystallized haem[11] and the radioactivity of total liver homogenate were determined in a well type scintillation counter. All determinations were done in duplicate.

The effect of a single i.p. injection of DDC on the liver uptake of ^{59}Fe and on its incorporation into haem is shown in Table 1.

TABLE 1. ^{59}Fe *in vivo* UPTAKE BY LIVER AND ITS INCORPORATION INTO LIVER HAEM

Time after ^{59}Fe inj (min)	Control			DDC		
	counts/min/ total/liver (a)	counts/min/ mg haem (b)	ratio a/b	counts/min/ total liver (a)	counts/min/ mg haem (b)	ratio a/b
15	960 ± 119	25 ± 5	38·4	1149 ± 192	16 ± 3	71·8
30	1032 ± 161	29 ± 5	34·9	1881 ± 365	25 ± 5	75·2
60	1976 ± 184	67 ± 8	29·4	2255 ± 289	48 ± 10	46·9
120	3219 ± 290	190 ± 13	16·7	2431 ± 284	73 ± 6	34·0

Values given are the means ± S.E.M. of six observations for each group.

DDC treatment inhibited the incorporation of radioactive iron into liver haem at all the time intervals studied: the greatest inhibition was observed 2 hr after injection of the isotope. This effect of DDC was even more evident when the ^{59}Fe recovered in the liver haem was related to the total amount of isotope present in the liver.

The results of *in vitro* incorporation of ^{59}Fe into haem are shown in Fig. 1. Liver preparations determined from DDC treated mice showed a much greater ability to incorporate iron into haem than did their respective controls

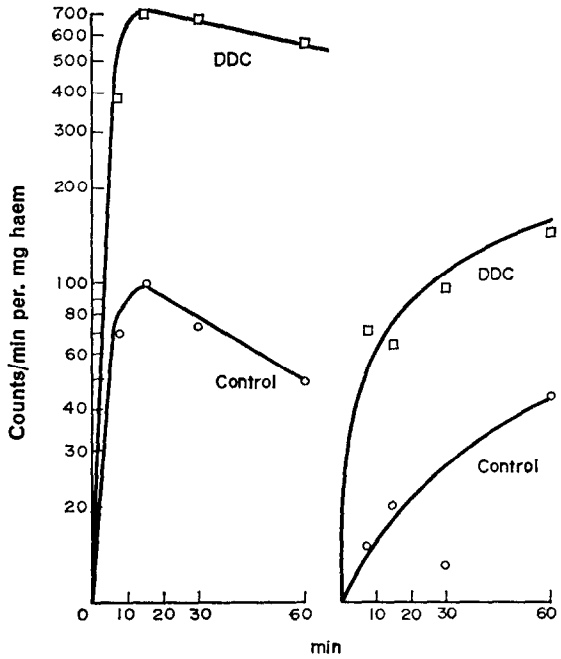

FIG. 1. (a) *In vitro* ^{59}Fe incorporation into haem by liver homogenate. (b) *In vitro* ^{59}Fe incorporation into haem by liver mitochondria.

Our *in vivo* incorporation results indicate that inhibition of haem synthesis by DDC can already be observed in the early stages of treatment and it is unlikely to be due to an aspecific toxic effect of the drug. In contrast with the results observed *in vivo*, a stimulation of ^{59}Fe incorporation into haem was noted *in vitro* after treatment with DDC. The reason for this unexpected finding is not yet clear but recent data* suggest that an increased supply of protoporphyrin substrate is involved in this *in vitro* effect of DDC rather than a stimulation of the chelatase enzyme.

* F. DE MATTEIS, personal communication.

There are several lines of evidence which suggest the existence of a causal connection between lower levels of liver haem, as judged from the amount of cytochrome P-450, and porphyria.* A decreased level of cytochrome P-450 has been observed in mice and rats treated with DDC[12] and AIA.[5]† In the case of AIA an increased destruction of liver haem is responsible for the decrease in the level of cytochrome P-450;[5,13] it is possible that DDC may cause the same effect by impairing the synthesis of haem.

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Plasma levels and urinary excretion of [^{14}C]cyclophosphamide and its radioactive metabolites in rats pretreated with prednisolone*

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THE CYTOTOXIC action of cyclophosphamide (CP) is believed to be related to its metabolic activation by hepatic microsomal enzymes.[1,2] Hayakawa *et al.*[3] reported that simultaneous administration of prednisolone (P) with CP resulted in lower plasma levels of nitrobenzylpyridine-alkylating metabolites of CP in the rat. Since glucocorticoids are often used in conjunction with CP to treat neoplastic diseases, this finding has important therapeutic implications. The availability of [^{14}C]labeled CP permitted the potential drug interaction between P and CP to be investigated in more detail. In this study we have measured the plasma levels and urinary excretion of CP and its radioactive metabolites in rats pretreated with single and repetitive doses of P.

Methods

Male Sprague–Dawley rats (Simonsen Laboratories) weighing 220–300 g were subjected to single or repetitive oral doses of 6·6 or 66 mg/kg of P (CalBiochem) suspended in a 1% carboxymethyl cellulose vehicle. Control rats received the vehicle only. Repetitive P-treatment consisted of 10 daily oral

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