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A RECIPROCAL RELATIONSHIP BETWEEN THE INDUCTION OF δ-AMINOLEVULINIC ACID SYNTHETASE AND DRUG METABOLISM PRODUCED BY *m*-DICHLOROBENZENE

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Abstract—The daily administration of large doses of *m*-dichlorobenzene (*m*-DCB) causes experimental hepatic porphyria in rats by induction of δ-aminolevulinic acid synthetase (ALA synthetase). However, smaller doses of *m*-DCB produce a biphasic stimulation of both urinary coproporphyrin excretion and liver ALA synthetase. The decline of ALA synthetase and urinary coproporphyrin despite continued daily dosage is associated with an increase in activity of the liver drug-metabolizing systems and decreasing serum *m*-DCB levels. The most probable explanation for the self-limiting action of *m*-DCB is stimulation by the drug of its own metabolism.

PHYSIOLOGIC control of heme synthesis appears to be exerted primarily through regulation of the initial and rate-limiting enzyme, δ-aminolevulinic acid synthetase (ALA synthetase).[1,2] A wide variety of drugs have been shown to induce this enzyme in the liver of experimental animals or cultured embryonic chick livers.[3,4] Heme, the end-product of this biosynthetic pathway, prevents the induction of ALA synthesis, possibly by feedback repression.[3,5–7]

Several investigators have noted that the rapid turnover of the microsomal cytochrome, P-450, accounts for at least 50 per cent of all the heme synthesized in the unstimulated liver of the rat[8] and mouse.[9] Cytochrome(s) P-450 plays a critical role as the terminal oxidase in the metabolism of drugs, steroids and heme.[10,11] Many drugs have been shown to induce the drug-metabolizing enzymes and concomitantly to increase microsomal cytochrome P-450.[10] Many of the compounds that stimulate drug metabolism (phenobarbital, chlorinated insecticides, griseofulvin, steroids) also induce ALA synthetase,[9] presumably to provide the heme needed for the increased synthesis of the microsomal cytochrome. Phenobarbital induction of ALA synthetase and the increase in cytochrome P-450 and microsomal protein are all abolished by the simultaneous administration of heme.[6] Hence, control of drug metabolism can be exerted by inhibiting ALA synthetase or other steps in the heme biosynthetic pathway.[12]

The following data suggest that induction of ALA synthetase can be suppressed when a drug which induces ALA synthetase also stimulates its own metabolism. Thus, induction of drug-metabolizing enzymes is suggested as an additional control mechanism influencing the induction of ALA synthetase.

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METHODS

Chemicals

m-Dichlorobenzene and 2,4-dichlorophenol (reagent grade) were purchased from Eastman Chemicals; bishydroxycoumarin (Dicumarol) from Abbott Laboratories (converted to the sodium salt by reflux with sodium hydroxide); hexobarbital from Winthrop Laboratories; and coproporphyrins I and III, and δ-aminolevulinic acid hydrochloride (ALA) from Calbiochemical Company.

Animals

Young Sherman strain female rats, 75–120 g, were fed regular laboratory chow and water *ad lib.*, except where specifically stated. Animals were dosed by gastric intubation with *m*-dichlorobenzene (*m*-DCB) or 2,4-dichlorophenol (2,4-DCP) dissolved in peanut oil. The volume of drug administered was always 0·5 ml/100 g. Control animals were dosed with peanut oil.

For urinary porphyrin determinations, 24-hr urine collections were made with animals individually housed in metabolic cages. The urine was collected in light-protected, ice-chilled bottles.

Hexobarbital sleeping times were determined by administering 150 mg/kg of sodium hexobarbital i.p. and recording the time required for the return of the righting reflex.

Livers were fixed, sectioned and stained with hematoxylin and eosin for microscopic examination.

Bishydroxycoumarin metabolism

Twenty-four hr after the last dose of *m*-DCB or peanut oil, rats were injected with 20 mg/kg bishydroxycoumarin in saline i.p. and 5 hr later blood was drawn by cardiac puncture under ether anesthesia. The half-time of bishydroxycoumarin (20 mg/kg) in the male rat is reported to be 5 hr.[13] Bishydroxycoumarin levels were determined by the method of Nagashima *et al.*[14]

ALA synthesis in vitro

The ALA synthetase activity was measured in the whole liver homogenate by the method of Marver *et al.*[15] The reaction was terminated at 30 min by the addition of 0·5 ml 10% $ZnSO_4$ and 0·5 ml NaOH to each flask. Nine flasks were pooled for ALA analyses.

Analyses

Porphyrin determinations. Urinary coproporphyrin and uroporphyrin were determined by a modification of the method of Schlenker *et al.*[16] The eluting solution was changed to acetone–ether–glacial acetic–water (5:5:1:1), which gave a better recovery in our laboratory. The recoveries for coproporphyrin and uroporphyrin were 75–100 per cent and 60–80 per cent respectively. The porphyrin absorption was read on a Cary 14 recording spectrophotometer at 380 mμ, 430 mμ, and the maximum absorption peak. The extinction coefficients ($E^{\mu g/ml}_{1\,cm}$) used were 0·747 for coproporphyrin and 0·652 for uroporphyrin as listed by Schlenker *et al.*[16]

Coproporphyrin isomeric configuration was determined by ascending chromatography against de-esterified commerical coproporphyrins I and III in a lutidine–water system.[17]

ALA and porphobilinogen measurement. ALA and porphobilinogen (PBG) were determined by the method of Marver *et al.*[18] The ion-exchange columns and wash volumes were decreased to $\frac{2}{3}$ size. After reacting the pyrroles with Erhlich's reagent, the ALA-pyrrole was read at 553 mμ with an extinction coefficient of 53, and the PBG chromagen at 555 mμ with an extinction coefficient of 68. Recovery of commercial ALA-HCl was usually 85–90 per cent.

Gas chromatography. The serum and liver concentrations of *m*-DCB and 2,4-DCP were determined by gas chromatography 6–7 hr after the last *m*-DCB administration. Blood was drawn by cardiac puncture under ether anesthesia. Slightly larger rats (200–230 g) were used because of the considerable quantity of serum required for analysis. Serum samples were acidified to pH 3, extracted with four parts of nanograde benzene, and the organic phase was dried with a small quantity of anhydrous sodium sulfate, parallel to the method of Dale *et al.*[19] Liver samples (0·7–1·8 g) were ground with a minimal amount of sodium sulfate by mortar and pestle, and then extracted with 20 ml benzene for 30 min on an automatic shaker. This sample preparation is similar to that described by Radomski and Fiserova-Bergerova.[20]

The gas chromatograph used was a Micro-Tek 2000 equipped with a tritium electron capture detector, dual channel E-2 electrometer, and 1 mV strip charter recorder.* The column used was a 6 ft $\times$ $\frac{1}{8}$ in. U-shaped glass column packed with 15% FFAP† on 40/50 Chromosorb W. The operating parameters were as follows: inlet block, 210°; column oven, 130° (*m*-DCB), 190° (2,4-DCP); detector, 210°; carrier gas, prepurified nitrogen, 45 ml per min at 40 psi; and power source, d.c. mode of operation at 15 V. A polarizing voltage of 15 V gave a standing current of $2{\cdot}56 \times 10^{-9}$ amp at an electrometer setting of $10^2 \times 64$. Under these conditions, *m*-DCB and 2,4-DCP had retention times of 1·4 and 3·3 min respectively.

The response factor, ratio of nanograms injected to resulting peak height in millimetres, of each compound was determined by injecting 1–5 μl 2,4-DCP or *m*-DCB standards and measuring the resulting peak heights at an electrometer setting of $10^2 \times 8$. This attenuation on the electrometer was used throughout the analysis, except that aliquots of samples injected were varied to align with previous standard responses.

The lower limits of detectability of the method are based on a minimum peak height of 7 mm, which corresponds to a signal-to-noise ratio of 2. With this criterion, the minimum detectable amounts of *m*-DCB and 2,4-DCP were 0·6444 and 0·918 ng respectively.

The data were analyzed by Student's *t*-test.

RESULTS

Porphyrin excretion

Rats were dosed daily with 800 mg/kg of *m*-DCB. As shown in Fig. 1, excretion of urinary coproporphyrin was significantly higher than control levels on days 2, 3, 5 and 9. However, maximal stimulation occurred on day 3, and the excretion of coproporphyrin was significantly lower on days 5 and 9, despite continued daily administration of m-DCB.

* Westronics Recorder, Westronics, Inc., Fort Worth, Tex.
† FFAP, Varian Aerograph, Walnut Creek, Calif.

Thin-layer chromatography of the urine revealed only the coproporphyrin III isomer. Uroporphyrin was not detected. Although the urinary excretion of ALA and PBG measured on days 1, 5 and 9 was significantly higher than that of controls, there was wide individual variation and no consistent trend between days 1 and 9.

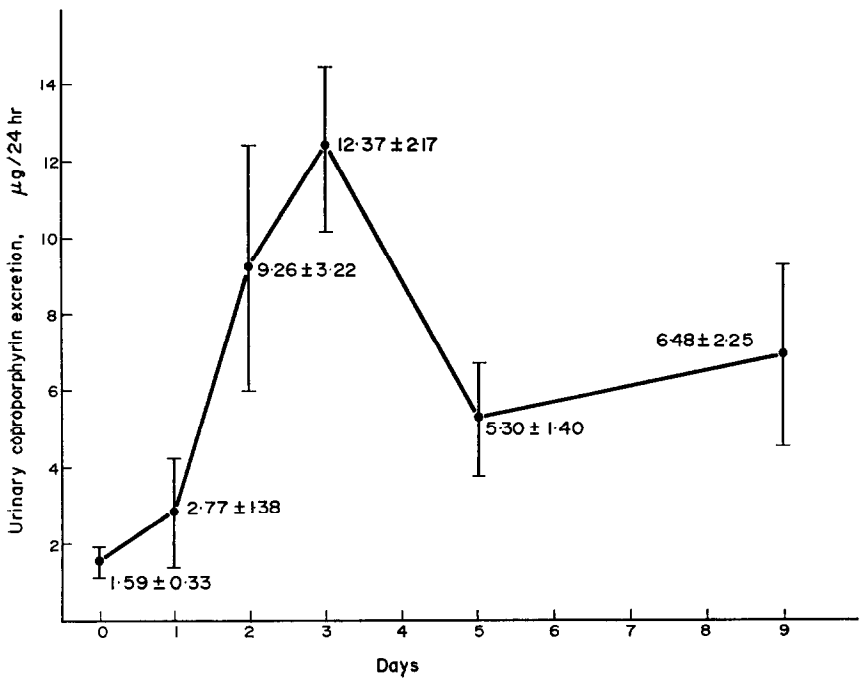

FIG. 1. Effect of *m*-DCB on urinary coproporphyrin excretion. Four female rats (90–120 g) were treated daily with 800 mg/kg *m*-DCB. The 24-hr urinary excretion of coproporphyrin (mean ± S. D.) is plotted versus time. The first dose was given on day 0, and the urinary coproporphyrin value for each day represents a urinary collection for the previous 24 hr. Urinary coproporphyrin excretion was significantly lower on days 5 and 9 than on day 3.

Livers from rats treated 1, 3 or 5 days with *m*-DCB (800 mg/kg) were essentially normal with minimal vacuolization when examined microscopically. There was no evidence of liver damage.

A few animals dosed with slightly larger amounts of *m*-DCB (900–1000 mg/kg) developed a more florid picture with gross coproporphyrinuria (>25 μg/24 hr), measurable uroporphyrinuria, and red staining of the periorbital hair and facial whiskers which fluoresced slightly under ultraviolet light.

The same biphasic pattern of urinary coproporphyrin exeretion was observed in animals dosed daily with 2,4-DCP (800–1000 mg/kg), the major metabolite of *m*-DCB,[21] and with *p*-dichlorobenzene (900 mg/kg), a compound known to produce chemical porphyria in rats.[22]

ALA synthetase

Animals were dosed daily with *m*-DCB (800 mg/kg) or peanut oil for 1, 3 or 5 days and sacrified 24 hr after the last dose. The hepatic ALA synthetase activity *in vitro* is

TABLE 1. EFFECT OF *m*-DICHLOROBENZENE ON ALA SYNTHETASE ACTIVITY*

Treatment	ALA synthetase (mμmoles/g/hr)		
	Day 1	Day 3	Day 5
Control†	13·3 ± 4·4 (7)	18·8 ± 4·6 (6)	16·1 ± 4·8 (7)
m-DCB†	52·9 ± 15·3 (7)	40·5 ± 6·8 (7)	30·5 ± 6·2 (8)

* Female rats were treated orally for 1, 3 or 5 days with *m*-DCB (800 mg/kg) or peanut oil and sacrificed 24 hr after the last dose. All values represent the means ± S. D. The number of animals in each group is given in parentheses.

† ALA synthetase activity is lower on day 5 in *m*-DCB-treated animals than on day 1 ($P < 0.01$) or day 3 ($P < 0.02$).

reported in Table 1. The enzyme activity rose 3- to 4-fold after the first dose of *m*-DCB and then declined. All *m*-DCB-treated groups were significantly higher than controls. However, the ALA synthetase activity was significantly lower at 5 days than at 3 days ($P < 0.01$) or at 1 day ($P < 0.01$). ALA synthetase activity in control rats was 16·3 ± 6·4 mμmoles/g/hr ($N = 31$). None of the control groups in Table 1 varied significantly from this value.

One possibility for the decrease in ALA synthetase activity per gram of liver is the increase in liver size. However, Tables 1 and 2 show that the greatest increase in liver weight occured between days 1 and 3, while ALA synthetase activity per gram did not decrease significantly until day 5. Nor did the increase in liver size quantitatively account for the decrease in activity.

The degree of enzyme induction after a single dose of *m*-DCB is dose related (Table 3). In these experiments, rats were starved for 24 hr, dosed with *m*-DCB, and then starved until sacrificed. *m*-DCB produces maximal enzyme induction in starved rats at 800 mg/kg, and the induction of ALA synthetase activity is much greater than that produced by the same dose in fed rats (Table 1, day 1). The maximal stimulation caused by 2,4-DCP (445 mg/kg) under identical conditions was 3-fold (77 mμmoles/g/hr).

TABLE 2. EFFECT OF *m*-DICHLOROBENZENE ON LIVER WEIGHT*

Time	Treatment	Liver wt. (g)	Body wt. (g)	Liver/body wt. × 100
Day 1	Control	4·97 ± 0·63	121 ± 13	4·10 ± 0·22
	m-DCB	4·75 ± 0·67	111 ± 10	4·28 ± 0·24
		NS	NS	NS
Day 3	Control	5·57 ± 0·68	138 ± 9	4·04 ± 0·39
	m-DCB	6·49 ± 0·96	112 ± 11	5·77 ± 0·38
		(NS)	($P < 0.001$)	($P < 0.001$)
Day 5	Control	5·96 ± 0·62	143 ± 11	4·18 ± 0·31
	m-DCB	7·31 ± 0·87	117 ± 11	6·24 ± 0·49
		($P < 0.01$)	($P < 0.01$)	($P < 0.001$)

* Data are from animals in Table 1. NS = not significant.

TABLE 3. EFFECT OF VARYING DOSES OF *m*-DICHLOROBENZENE ON ALA SYNTHETASE IN THE FASTED RAT*

Treatment (mg/kg)	No. of animals	ALA synthetase activity (mμmoles/g/hr)
Control	4	21·0 ± 3·9
m-DCB		
200	3	49·1 ± 13·1
400	4	73·8 ± 12·3
800	4	125·1 ± 27·0
1600	4	39·1 ± 18·7

* Female rats (90–120 g) were starved for 24 hr and then dosed orally with *m*-DCB or peanut oil. Starvation continued until sacrifice 24 hr later. All values represent the means ± S. D.

These experiments demonstrate that daily oral dosage with *m*-DCB results in induction of hepatic ALA synthetase and an increase in urinary coproporphyrin excretion, which peak at 1 and 3 days, respectively, while both parameters are significantly lower by 5 days. The adaptation, or tolerance, to *m*-DCB suggested that the animals might be detoxifying the drug more rapidly. This in turn would lower the effective concentration of *m*-DCB, or its metabolites, which stimulates ALA synthetase.

Metabolism of hexobarbital and bishydroxycoumarin

To test the possibility that *m*-DCB stimulates its own detoxification, presumably by induction of the drug-metabolizing enzymes in the smooth endoplasmic reticulum, we first studied the effect of *m*-DCB on the metabolism of hexobarbital and sodium bishydroxycoumarin.

Young female rats were treated for 1, 3 or 5 days with *m*-DCB or peanut oil. As seen in Fig. 2, the hexobarbital sleeping times were significantly shortened after one dose of *m*-DCB ($P < 0{\cdot}02$), and by 3 days were only 20 per cent of control ($P < 0{\cdot}001$).

To study the effect of *m*-DCB on the rate of metabolism of bishydroxycoumarin, rats were treated as described above. Five hr after bishydroxycoumarin administration, the serum concentrations of bishydroxycoumarin were significantly lower in rats treated with *m*-DCB for 5 days than in controls (Table 4, $P < 0{\cdot}02$).

Serum and liver concentrations of m-*DCB and* 2,4-*DCP*

The above experiments suggest that *m*-DCB does stimulate the hepatic degradation of some drugs. The following experiments were designed to test the hypothesis that *m*-DCB stimulates its own detoxification and thus lowers the effective drug concentration. Since we have shown that 2,4-DCP, the major metabolite of *m*-DCB in the rabbit, also stimulates ALA synthetase and coproporphyrin excretion, it was necessary to measure both 2,4-DCP and *m*-DCB simultaneously after *m*-DCB administration.

Liver and serum samples were analyzed as described in Methods. The efficacy of the extraction procedures was tested by fortifying serum and liver samples from control rats with *m*-DCB and 2,4-DCP in the concentration range detected in the dosed animals. The *m*-DCB recoveries are presented in Table 5. The 2,4-DCP recoveries were 65–89 per cent in serum and 93–100 per cent in the liver.

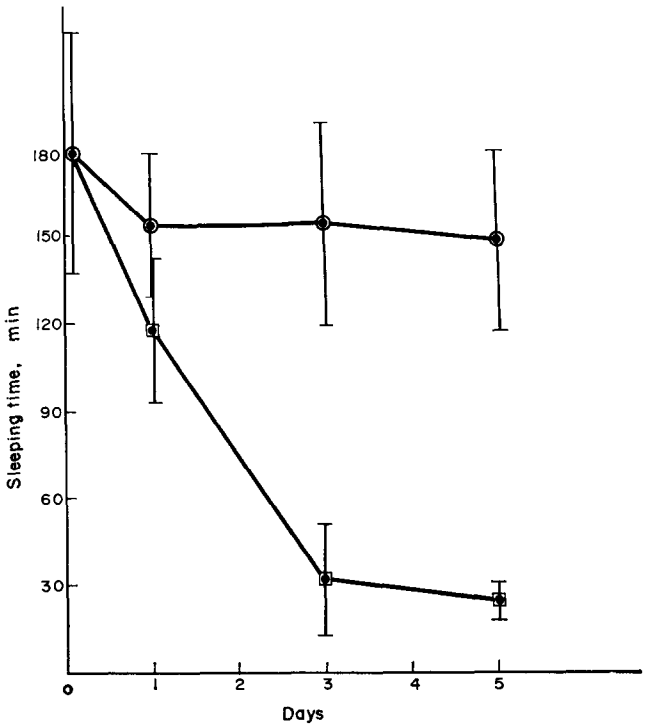

FIG. 2. Effect of *m*-DCB on hexobarbital sleeping time. Female rats (75–100 g) were treated for 0, 1, 3 or 5 days with *m*-DCB (800 mg/kg) or peanut oil. They were injected with 150 mg/kg of sodium hexobarbital i.p. 24 hr later, and sleeping times were measured. All points represent the means ± S. D. of seven or eight animals. Sleeping times are significantly reduced by *m*-DCB at days 1 ($P < 0.02$), 3 ($P < 0.001$) and 5 ($P < 0.001$). Control animals, ○—○; *m*-DCB-treated animals, □—□.

TABLE 4. EFFECT OF *m*-DICHLOROBENZENE ON BISHYDROXYCOUMARIN METABOLISM*

Treatment	Serum bishydroxycoumarin concn (μg/ml)		
	Day 1	Day 3	Day 5
Control	107 ± 16 (4)	120 ± 45 (4)	133 ± 10 (4)
m-DCB	94 ± 27 (4)	78 ± 36 (4)	82 ± 29† (3)

* Female rats were treated with *m*-DCB (800 mg/kg) or peanut oil for the times indicated. Twenty-four hr after the last dose, sodium bishydroxycoumarin was administered i.p., and 5 hr later the animals were anesthetized with ether and bled by cardiac puncture. Each value represents the mean ± S. D. The number of animals in each group is given in parentheses.

† $P < 0.025$.

TABLE 5. RECOVERY OF *m*-DICHLOROBENZENE FROM LIVER AND SERUM*

Serum *m*-DCB (μg/ml)	% Recovery	Liver *m*-DCB (μg/g)	% Recovery
1·288	94·9	6·0	110·2
3·386	110·7	12·0	106·4
5·152	104·4	30·9	94·2
7·728	86·0	41·8	110·0
9·016	83·4	63·0	81·6

* *m*-DCB was added to liver or serum samples from untreated rats in the amounts listed above. All values are the average of duplicate runs.

Rats were treated daily with 1, 3 or 5 doses of m-DCB. As seen in Table 6, the serum *m*-DCB concentration was higher on day 3 than on day 1, but was significantly lower on day 5 than on day 3 ($P < 0{\cdot}02$). The hepatic concentration of *m*-DCB showed the same pattern, but the difference between the concentration at 3 days and 5 days was not quite significant ($0{\cdot}10 > P > 0{\cdot}05$). Rats pretreated for 4 days with 40 mg/kg of phenobarbital, a known inducer of drug metabolism, before a single dose of m-DCB had slightly lower concentrations of *m*-DCB in the serum and liver than animals given only the single dose of *m*-DCB, but neither difference was significant. 2,4-DCP could not be detected in the serum or liver of any of the animals. The detectable level depended on the size of the sample taken (serum, $<0{\cdot}026$–$0{\cdot}083$ μg/ml; liver, $<0{\cdot}74$–$3{\cdot}4$ μg/g).

TABLE 6. EFFECT OF DAILY DOSAGE ON THE LEVEL OF *m*-DICHLOROBENZENE IN LIVER AND SERUM*

Treatment	Serum *m*-DCB (μg/ml)	Liver *m*-DCB (μg/g wet wt.)	Liver wt. (g)	Body wt. (g)
1 dose *m*-DCB	3·25 ± 1·31	13·01 ± 6·92	7·62 ± 0·51	230 ± 10
3 doses *m*-DCB	8·89 ± 1·66	44·17 ± 6·31	9·67 ± 0·22	202 ± 13
5 doses *m*-DCB	5·91 ± 2·02	32·10 ± 16·18	12·14 ± 1·46	193 ± 11
4 doses phenobarbital × 1 dose *m*-DCB	2·24 ± 0·49	8·49 ± 2·37	9·17 ± 0·41	235 ± 7

* Female rats (200–230 g) were treated orally with *m*-DCB (800 mg/kg) for 1, 3 or 5 days; or phenobarbital (40 mg/kg) i.p. for 4 days followed by 1 dose of *m*-DCB on the fifth day. The animals were anesthetized with ether 6–7 hr after the last dose of *m*-DCB, and blood was drawn by cardiac puncture. All values represent the means ± S. D. of six animals.

DISCUSSION

The administration of *m*-DCB daily to rats induces ALA synthetase. At large doses (900–1000 mg/kg), this drug produces experimental hepatic porphyria similar to that described by Rimington and Ziegler[22] with *p*-dichlorobenzene. However, with daily doses of 800 mg/kg of *m*-DCB, we observed a biphasic stimulation in the excretion of urinary coproporphyrin and ALA synthetase activity, which peaked by 3 days and then declined. Drug metabolism was also stimulated by *m*-DCB. The increase in ALA

synthetase and the changes in drug metabolism reported above were not parallel. In fact, the decrease in urinary excretion of porphyrins and in ALA synthetase activity at 5 days coincided with the time of maximal stimulation of drug metabolism occurring with this dosage. These data suggest that the decrease in ALA synthetase after 3 days may be a result of increased metabolism of *m*-DCB by the liver microsomal systems.

The serum concentration of *m*-DCB peaked on day 3 and was significantly lower by day 5, supporting the hypothesis that the decrease in ALA synthetase is a result of increased metabolism of *m*-DCB. It should be noted that the serum concentrations of *m*-DCB did not strictly parallel ALA synthetase activity. Although the serum concentration of *m*-DCB was decreasing by day 5, it was still higher on day 5 than on day 1. In contrast, induction of ALA synthetase was maximal by day 1. However, ALA synthetase was measured 24 hr after the last dose of *m*-DCB, while the serum levels of *m*-DCB were measured 6–7 hr after the last dose.

The porphyria seen after *m*-DCB could be caused by either *m*-DCB or a metabolite. Parke and Williams[21] demonstrated that *m*-DCB is oxidized in rabbits mainly to 2,4-DCP, which is excreted as conjugates of glucuronic and sulfuric acids. 2,4-DCP has been implicated as a causative agent of porphyria by Bleiberg *et al.*,[23] and we have found that it causes a biphasic excretion of coproporphyrin similar to that seen after *m*-DCB administration.

We found no 2,4-DCP present in the liver or serum of animals treated with *m*-DCB. If 2,4-DCP is a metabolite of *m*-DCB in the rat, the circulating level is less than 3 per cent of the *m*-DCB present. Therefore, it probably does not contribute to the observed increase in ALA synthetase seen after *m*-DCB administration.

Alternative explanations for the decrease in ALA synthetase are possible: (1) The relatively high serum levels of *m*-DCB which occur after three doses may cause liver damage and nonspecific depression of enzyme activities. This possibility is not supported by morphological examination of the tissue or by the fact that activity of the liver drug-metabolizing enzymes is higher at this time. In addition, a higher dose of *m*-DCB caused greater porphyrinuria when administered daily. (2) Administration of the drug could result in increased production of repressors or inhibitors of ALA synthetase. Heme, hemoglobin, hemin and bilirubin have been shown to inhibit induction of ALA synthetase.[5,24] Damage to the red blood cells or the liver could increase the concentration of one of these known inhibitors. We found no increase in the serum bilirubin levels or any suggestion of hemolysis after 5 days of *m*-DCB treatment. However, the production of suppressive compounds during porphyria is not ruled out.

Induction of drug-metabolizing enzymes has been reported to be accompanied by an increase in the level of the terminal oxidase, cytochrome P-450, and the changes are often parallel.[10] Drugs which induce P-450 and liver microsomal drug metabolism apparently stimulate heme synthesis in the liver, since blocking heme synthesis by administration of heme or 3-amino-1,2,4-triazole prevents phenobarbital induction of P-450.[6,12] In addition, drug-mediated increases in cytochrome P-450 are blocked by a variety of inhibitors of RNA and protein synthesis, suggesting that the increase in cytochrome P-450 is a result of *de novo* protein synthesis.[10,25] Thus, the several-fold increase in P-450 seen with induction of drug-metabolizing enzymes may be accompanied by induction of the rate-limiting enzyme, ALA synthetase.[9,10] The induction of ALA synthetase by known inducers of drug metabolism is not unexpected,

since P-450 accounts for more than 50 per cent of the heme synthesized in the normal liver.[8] However, the control of induction of drug metabolism and ALA synthetase is not completely understood, since many of the strongest inducers of drug metabolism are merely weak inducers of ALA synthetase and vice versa.[9]

The results reported in this paper suggest that *m*-DCB stimulated its own degradation, and repeated dosage resulted in lower plasma *m*-DCB levels than a single dose. This diminished the induction of ALA synthetase and lessened the porphyrogenic response. We suggest that many drugs may be potentially porphyrogenic, but porphyrogenic activity is limited by the ability of the drug to increase its own metabolism.

Addendum—Since this manuscript was submitted for publication, several reports have been published which confirm our observations. Moore *et al.*[26] have demonstrated that daily administration of phenobarbital to rats results in a maximal rise in hepatic δ-ALA synthetase activity at about 4 days and then a fall off in activity at a time when stimulation of hepatic drug metabolism reaches a maximum.[27] Kaufman *et al.*[28] have found that pretreatment of rats with phenobarbital abolished the ability of progesterone or AIA to induce δ-ALA synthetase and increased the rate of metabolism of AIA. The ability of a drug to induce chemical porphyria appears to depend on the balance of its ability to induce δ-ALA synthetase and the rate of inactivation of the inducing drug. It is interesting to note that hexachlorobenzene, which is not metabolized,[29] is one of the few drugs which produces a sustained induction of δ-ALA synthetase.[30]

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