mimetic amines which release NE, dopamine is not associated with depletion of NE stores. Presumably, this reflects rapid resynthesis of NE from dopamine.

It is noteworthy that the same dose of MK-485 that prevented NE release in these experiments has been reported to prevent the increase in blood pressure associated with the administration of L-dopa.[1,2] Norepinephrine release from the peripheral sympathetic nerves may thus be an important factor in the pharmacology of L-dopa. Although it does not provide an explanation for the occurrence of orthostatic hypotension which commonly accompanies the clinical use of L-dopa, NE release may be related to the cardiac arrhythmias[1,3] or hypertensive reactions[1] occasionally seen in patients receiving this drug.

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Liver *N*-demethylating activity—temperature effect and phenobarbital induction in different species

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DIFFERENCES in the activities of drug-metabolizing enzymes in the livers of various species have been reported.[1,2] The view that the drug-metabolizing microsomal system has developed as one feature of the adaptation from life in an aqueous medium to that of terrestrial life has been suggested by Brodie *et al.*[2] Induction of microsomal drug-metabolizing enzymes has been widely reported by many authors, mainly in the rat.[3,4]

It seemed of interest therefore, to study whether the species differences in liver drug-metabolizing enzymes are also reflected in the inducibility of these enzymes. This seemed of particular interest in view of the induction by phenobarbital of this system in the new-born, who has a low activity of liver microsomal drug-metabolizing enzymes.[5–8]

Liver *N*-demethylating activity was studied in a fish (*Carpus carpus*), frog (*Rana ridibunda*), lizard (*Agama lizard*), hen and rat. The liver was homogenized in 1·15% KCl (3 vol.) and centrifuged at 10,000 *g* for 10 min. The supernatant, containing cell sap and microsomes, was used for the assay. *p*-Chloro-*N*-methylaniline was used as substrate.[9] The final composition for the incubation was: 0·4 ml enzyme (10,000 *g* supernatant), NADP—1·6 μmoles; magnesium chloride—30 μmoles; nicotinamide—20 μmoles; glucose 6 phosphate—16 μmoles; phosphate buffer pH 7·4 (100 μmoles) and substrate (*p*-chloro-*N*-methyl-aniline)—3 μmoles. The final volume was 2·0 ml. Blanks—lacking either enzyme or substrate—were incubated simultaneously. Incubation was carried out with continuous shaking at 37° or 7° for 10 min. The product of *N*-demethylation, *p*-chloro-aniline was estimated (after adding *p*-dimethylamino-benzaldehyde) at 445 mμ in a spectrophotometer.[9] For induction of drug metabolizing enzymes phenobarbital-sodium was injected i.p. at a dose of 75 mg/kg for 3 days and the liver enzyme activity was assayed on the fourth day. In frogs, lizards and fish where no appreciable induction was observed in preliminary experiments (see below) the induction period was prolonged to 7 days.

Table 1 shows the basic activity of liver *N*-demethylation and the effect of phenobarbital treatment in the various species. It is evident that although there is a gradual increase of the *N*-demethylase activity from the carp (fish) to the rat (mammalian), the induction by phenobarbital shows an even more striking difference, being practically negligible in the carp, frog and lizard.

TABLE 1. *N*-DEMETHYLATION BY LIVER MICROSOMES AT 37°

	Control	Phenobarbital	Difference phenobarbital–control
Rat	135 ± 8 (N = 5)	230 ± 9 (N = 5)	95 (P < 0·001)
Hen	96 ± 9 (N = 5)	140 ± 14 (N = 5)	44 (P < 0·05)
Lizard	74 ± 5 (N = 10)	91 ± 8 (N = 11)	17 (N.S.)
Frog	79 ± 7 (N = 7)	89 ± 4 (N = 8)	10 (N.S.)
Fish	56 ± 5 (N = 6)	59 ± 5 (N = 9)	3 (N.S.)

N-demethylation activity expressed in μmoles *p*-chloro-aniline formed/g liver × 60 min. min.

Results given as mean ± S.E. N—number of animals.

Phenobarbital-animals treated with phenobarbital-sodium; control—animals injected with saline. N.S.—difference between control and treated animals—not statistically significant.

TABLE 2. *N*-DEMETHYLATION BY LIVER MICROSOMES AT 7°

	Control	Phenobarbital	Difference phenobarbital–control
Rat	60 ± 3 (N = 5)	44 ± 4 (N = 5)	−16 (P < 0·02)
Hen	46 ± 3 (N = 5)	50 ± 4 (N = 5)	4 (N.S.)
Lizard	52 ± 4 (N = 10)	64 ± 4 (N = 11)	12 (P < 0·05)
Frog	54 ± 7 (N = 3)	42 ± 5 (N = 5)	−12 (N.S.)
Fish	31 ± 3 (N = 6)	46 ± 3 (N = 8)	15 (P < 0·02)

N-demethylation activity expressed in μmoles *p*-chloro-aniline formed/g liver × 60 min.

Results given as mean ± S.E. N—number of animals.

Phenobarbital—animals treated with phenobarbital-sodium; control–animals injected with saline. N.S.—difference between control and treated animals—not statistically significant.

TABLE 3. DIFFERENCE BETWEEN *N*-DEMETHYLATING ACTIVITY AT 37° AND 7°

	Control	Phenobarbital
Rat	75	186
Hen	50	90
Lizard	22	27
Frog	25	47
Fish	25	13

Figures denote differences between *N*-demethylating activity at 37° and 7° for the same group.

When the *N*-demethylating activity was tested at 7° the differences between the five species (control groups) were much smaller or negligible (Table 2). Furthermore, no increase in activity in the phenobarbital treated rats or hens was observed. On the other hand, the carp (and to a lesser degree also the lizard) showed a significant increase of the enzyme activity following phenobarbital treatment. It seems, therefore, that phenobarbital can induce liver microsomal *N*-demethylase in the carp; however, the induction is significant at 7° but not at 37°. An interesting aspect of this phenomenon becomes evident when the difference in activity between incubation at 37° and at 7° [Δ(37–7)] is tabulated for the control and for the phenobarbital-treated animals (Table 3). It is evident that the difference for the rat and for the hen is higher in the phenobarbital treated than in the respective control groups. But in the carp it is larger in the control than in the treated fish. Induction of different components of the *N*-demethylating system to a different extent in the rat and carp may be one possible reason for these observations. This hypothesis is also supported by the finding of an increase of 30 per cent in liver microsomal protein in the rat following phenobarbital treatment while no increase in microsomal protein was found in the treated carp.

Another interesting feature is the similarity of the Δ(37–7) in the control animals in carp, frog and lizard and the larger coefficient in the hen and rat. The physiological meaning of this observation could be that in the poikilothermic species a substantial proportion of the drug-metabolizing system is active even at relatively low environmental temperatures.

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