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## The effect of ethchlorvynol on the drug-metabolizing enzymes of rats and dogs

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THERE HAS been much concern recently about drug interactions, especially with respect to drugs that induce the enzymes which metabolize other drugs. For example, phenobarbital and chloral hydrate have been implicated in an increased rate of metabolism of bishydroxycoumarin.[1, 2] The enzymes that are induced by such drugs as phenobarbital have been shown to metabolize (and thus control the serum concentration and pharmacological effect of) such varied drugs as barbiturates, coumarins, codeine and morphine, erythromycin, phenothiazine tranquilizers, zoxazolamine, imipramine, and several steroids.[3–5]

Since two widely used sedatives, phenobarbital and chloral hydrate, have been shown to induce these liver microsomal enzymes, it was of interest to investigate the effect of another sedative, ethchlorvynol. (Ethchlorvynol has recently been reported to perhaps show such a property in man.[6]) Experiments in which ethchlorvynol was administered to dogs and rats are reported below.

### MATERIALS AND METHODS

*Rats.* Phenobarbital, ethchlorvynol, glutethimide or methyprylon* was administered orally or i.p. to 160–200 g male Sprague–Dawley rats daily. All doses were 100 mg/kg. After the fifth dose the rats were starved for 18–24 hr. Liver microsomes from individual rats were then isolated.[7] The final suspension in 0·25 M sucrose was stored at −15° until analysis (less than a week of storage).

*Dogs.* The half-life of bishydroxycoumarin was determined after an oral dose of 40 mg/kg. Serum samples were taken periodically 1–7 days after dosing. A minimum of four determinations of measurable bishydroxycoumarin was used to determine the half-life. The dogs were then fed a 500 mg capsule of ethchlorvynol (50–100 mg/kg) daily for 24 days. The determination of the half-life of bishydroxycoumarin was then repeated. To serve as a measure of the sensitivity of the dogs to enzyme induction, they were then fed 16 mg/kg phenobarbital for 20 days, and the half-life of bishydroxycoumarin was determined again.

*Analytical determinations*

The protein content of the rat liver microsome preparations was analyzed by the biuret procedure;[8] the bishydroxycoumarin concentration in plasma was determined by extraction and u.v. analysis.[9]

*Chemicals*

NADPH, enzymatically reduced, was purchased from Sigma Chemical Company. Drugs were commercial samples. All other chemicals were reagent grade.

* Luminal, Placidyl, Doriden, and Noludar, respectively.

*Microsome assays*

The activity of the drug-metabolizing enzymes was measured by following the rate of consumption of NADPH at 340 mμ.[10] The reaction was monitored in a Gilford 2000 multiple sample absorbance recorder equipped with an auxiliary offset control, and a cell compartment thermostated at 38°. Each cell (except the blank) contained 0·24 μmole NADPH, 225 μmole sodium phosphate buffer, pH 7·4, 100 μmole aminopyrine, and water to a volume of 2·9 ml. The concentration of aminopyrine was such that maximum rates were observed. After temperature equilibration of the cells, a vial of the microsomes was thawed and 0·05–0·1 ml (corresponding to approximately 0·5 mg protein) was added to each cell. The reaction was followed for at least 10 min. The rates were calculated from the linear portion of the curve.

*Statistical analyses*

*NADPH oxidation.* The significance of the differences between sample and control mean oxidation of NADPH was calculated by using pooled variances and a two-tailed *t*-test.

*Half-life of bishydroxycoumarin.* The apparent first-order disappearance constant for each experiment was calculated from a least squares analysis of time vs. the log of the concentration of bishydroxycoumarin in plasma. The disappearance constants were compared by a two-tailed *t*-test.

## RESULTS AND DISCUSSION

The NADPH consumption method of studying the drug-metabolizing enzymes was suggested by the work of Orrenius.[11] He demonstrated that rat liver microsomes incubated in the presence of aminopyrine consume oxygen and NADPH and produce formaldehyde in a 1:1 mole ratio.

The specific activities of drug-metabolizing enzymes in microsomes from rats treated with ethchlorvynol, glutethimide, methyprylon or phenobarbital are listed in Table 1. Of the drugs tested, phenobarbital and methyprylon significantly increased the activity of the drug-metabolizing enzymes analyzed in the presence of aminopyrine. This confirms earlier reports.[1, 3–5] Although increases in

TABLE 1. LIVER DRUG-METABOLIZING ENZYME LEVELS IN RATS TREATED DAILY WITH 100 MG/KG OF VARIOUS DRUGS FOR 5 DAYS

| Treatment | Route | No. of rats | mg protein isolated/g liver (mean ± S.D.) | mμmole NADPH consumed/mg protein (mean ± S.D.) | |
|---|---|---|---|---|---|
| | | | | No aminopyrine | + Aminopyrine |
| | | | Study I | | |
| Control* | i.p. | 3 | 6·53 ± 1·32 | 14·4 ± 1·6 | 14·1 ± 1·3 |
| Ethchlorvynol* | i.p. | 4 | 6·43 ± 0·47 | 10·1 ± 2·8 | 11·0 ± 2·8 |
| Phenobarbital* | i.p. | 3 | 9·82 ± 0·49† | 15·6 ± 1·0 | 22·6 ± 1·0† |
| | | | Study II | | |
| Control* | Oral | 3 | 6·89 ± 1·57 | 9·6 ± 3·9 | 8·6 ± 3·3 |
| Ethchlorvynol* | Oral | 3 | 6·88 ± 1·37 | 7·4 ± 0·8 | 9·8 ± 1·4 |
| Glutethimide* | Oral | 3 | 6·78 ± 0·78 | 11·6 ± 3·1 | 13·0 ± 2·3 |
| Methyprylon* | Oral | 3 | 6·68 ± 0·27 | 15·8 ± 2·9 | 14·8 ± 1·4† |
| | | | Study III | | |
| Control | Oral | 4 | 8·16 ± 0·42 | 18·3 ± 4·0 | 22·1 ± 2·6 |
| Phenobarbital | Oral | 4 | 11·34 ± 0·53† | 18·4 ± 1·0 | 33·0 ± 2·1† |

* In 5% Dextran.

† Significantly different from control at P = 0·05 level.

enzyme activity were seen after oral administration of glutethimide, they were not statistically significant. This drug has been shown to be a weak inducer when administered intraperitoneally[12] or subcutaneously.[3] No significant changes were seen after the oral or i.p. administration of ethchlorvynol.

TABLE 2. CHANGE IN HALF-LIFE OF BISHYDROXYCOUMARIN PLASMA LEVELS IN DOGS TREATED (ORALLY) WITH ETHCHLORVYNOL AND PHENOBARBITAL

| Dog no. | Sex | Control | Ethchlorvynol (24 days) | | | Phenobarbital (20 days) | | |
|---|---|---|---|---|---|---|---|---|
| | | $T_{1/2}$ (days)* | Dose (mg/kg/day) | $T_{1/2}$ (days)* | P† | Dose (mg/kg/day) | $T_{1/2}$ (days)* | P† |
| 1 | Female | 1·24 ± 0·051 | 42 | 1·20 ± 0·040 | >0·10 | 16 | 0·815 ± 0·046 | <0·005 |
| 2 | Male | 0·630 ± 0·086 | 45 | 0·465 ± 0·028 | >0·10 | 16 | 0·498 ± 0·046 | >0·15 |
| 3 | Female | 3·55 ± 0·25 | 62 | 4·74 ± 0·61 | >0·05 | 16 | 2·07 ± 0·18 | <0·05 |
| 4 | Female | 4·55 ± 0·22 | 41 | 5·05 ± 1·88 | >0·5 | | | |

* Mean ± S.D.

† Probability that the $T_{1/2}$ before and after treatment are the same.

The consumption of NADPH by the microsomes was increased by the addition of aminopyrine only in the case of phenobarbital-treated rats. Presumably there are several reactions in which NADPH may be consumed, and the increase in rate when aminopyrine is added simply reflects the fact that phenobarbital treatment has so increased the capacity of the microsomes to activate NADPH that there is not enough endogenous substrate present to allow the reaction to proceed at maximum rate. The protein isolated per mg liver was significantly increased only in the case of phenobarbital treatment.

None of the four dogs treated with ethchlorvynol showed a significant shortening of the half-life of bishydroxycoumarin (Table 2). In agreement with other reports,[1] when three of these dogs were later treated with phenobarbital, two showed a highly significant decrease in bishydroxycoumarin half-life. It should be noted that dog 2, which showed no response to phenobarbital, had a control half-life of bishydroxycoumarin half as long as any of the other dogs. Perhaps prior to the experiment he had accidentally been exposed to an insecticide or another inducer. These agents have been shown to exert extremely long-lasting effects in dogs.[13]

These data suggest that ethchlorvynol neither induces nor inhibits the liver enzymes that metabolize drugs. Further studies in man at clinical doses will be necessary to confirm this point.

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# Sulphur and selenium compounds related to acetylcholine and choline—VIII. Comparative studies of succinoylcholine, succinoylthiolcholine and succinoylselenolcholine*†

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IN AN attempt to obtain information about the structure of the active sites of acetylcholinesterase and of the depolarizing membrane of conducting tissue, the synthesis and study of sulfur and selenium isologs of acetylcholine, choline and of related compounds have been undertaken.[1–7] Since replacement

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† For earlier papers in this series, see References 4-10.