found in eviscerated rabbits. It appears, therefore, that the tendency towards higher specific activity in eviscerated groups is related to decrease in total phosphate pool and a subsequent increase in specific activity of phospholipide precursors.

Summary. In the rabbit, plasma phospholipide synthesis continued at a reduced rate in absence of liver, intestine and spleen. Removal of kidney further reduced plasma phospholipide synthesis. The rate of synthesis of kidney, lung or muscle phospholipides from phosphate precursors does not appear to be altered by evisceration.

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Metabolic Conversion of Primidone (Mysoline) to Phenobarbital.* (22813)

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5-Ethyldihydro - 5 - phenyl - 4.6-(1*H*,5*H*)-pyrimidinedione (primidone, Mysoline), a drug used in the treatment of epilepsy, differs structurally from phenobarbital only in that the former has a methylene group in the 2-position and the latter a carbonyl group. The only metabolic reaction that primidone has heretofore been reported to undergo is conversion to 2-ethyl-2-phenyl malonamide(1). Another reaction that suggested itself as a possibility is oxidation of the methylene group in primidone to yield phenobarbital as a product. The present investigation has shown that this reaction does indeed occur, and this report concerns a study of this metabolic conversion and its significance in therapeutics.

Methods. Administration of primidone. Crystalline primidone supplied through the kindness of Dr. A. Stanley Cook, Ayerst Laboratories, N. Y. City, was used in experiments on dogs. It was administered in gelatin capsules by mouth. The patients received commercial tablets. *Lack of contamination of primidone with phenobarbital.* A sample of crystalline primidone was partitioned between ether and water in a countercurrent design calculated to effect 99% separation of primidone and phenobarbital. Spectrophotometric examination of the fractions that would have contained phenobarbital demonstrated that the sample of primidone could have contained no more than 0.01% of phenobarbital. Information obtained from the manufacturer indicates that the method of synthesis excludes the possibility of any initial contamination with phenobarbital. *Spectrophotometric measurements* were made with a Beckman DU ultraviolet spectrophotometer. *Isolation of phenobarbital and p-hydroxyphenobarbital from urine.* The procedure described by Butler(2) was used. All samples of urine were subjected to acid hydrolysis. *Determination of phenobarbital in plasma.* For dog plasma the method of Butler *et al.*(3) was used. Unchanged primidone does not interfere to a significant extent with this method. A con-

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centration of 500 mg/l of primidone is measured as an apparent phenobarbital concentration of 2 mg/l. Plasma from patients receiving primidone and diphenylhydantoin was subjected to a countercurrent distribution procedure for the purposes of separating phenobarbital from diphenylhydantoin and of furnishing evidence of the identity of the phenobarbital in plasma. Samples of 10 to 15 ml of plasma were extracted with ether, the ether evaporated, and residues put through a 30 cell extraction train of the type described by Craig *et al.*(4). The light solvent was a mixture of 30% (v/v) ethyl ether and 70% 2,2,4-trimethylpentane and the heavy solvent a buffer consisting of 0.75 mole K_2HPO_4 + 0.25 mole KH_2PO_4 per 1 (pH 7.2). Each effluent fraction of light solvent was extracted with 4 ml of a buffer of pH 11 (0.10 mole $NaHCO_3$ + 0.09 mole NaOH per 1). Concentration of phenobarbital was calculated from the difference between absorbancy of the buffer extract at 240 mμ and that at 260 mμ. With this system of solvents phenobarbital appears in maximal concentration in the nineteenth effluent fraction of light solvent. From the amount of phenobarbital in the peak fraction the concentration in original sample of plasma was calculated.

Results. Phenobarbital and p-hydroxyphenobarbital in urine. Primidone was administered to a dog in a daily dose of 200 mg/kg for 6 days. Urine was collected for 7 days beginning the day after first dose. From this urine there were isolated in crystalline form 77 mg of phenobarbital and 16 mg of *p*-hydroxyphenobarbital. These products were identified by melting points and mixed melting points. From a 21 hour specimen of urine from a patient receiving a daily dose of 1 g of primidone there was isolated in crystalline form 2.5 mg of *p*-hydroxyphenobarbital, which was identified by its absorption spectra in media of different values of pH. Phenobarbital was not isolated in crystalline form, but in the fraction in which it would be expected there was a substance with absorption spectra characteristic of phenobarbital. The amount of phenobarbital as calculated from absorption measurements was 0.9 mg.

Phenobarbital in plasma. By spectrophotometric procedures the presence of phenobarbital has been demonstrated and its concentration measured in samples of plasma from 2 dogs and 2 patients receiving primidone. The dogs were given, respectively, 200 and 250 mg/kg of primidone daily for 6 days. The patients were a 79 kg man receiving 1 g of primidone plus 0.4 g of diphenylhydantoin daily and an 81 kg man receiving 2 g of primidone plus 0.3 g of diphenylhydantoin daily. The former patient had been receiving primidone for 3 weeks and the latter for 8 weeks. Both denied taking any drugs other than those prescribed, *viz.* primidone and diphenylhydantoin.

The methods used for measuring phenobarbital both in dog plasma and in human plasma are not subject to interference from unchanged primidone. In samples of plasma from both species it was shown that the substance determined as phenobarbital has spectra characteristic of that compound both in the univalent anionic form and the bivalent anionic form. When extracts of human plasma were passed through the Craig countercurrent distribution train, the material determined as phenobarbital appeared in maximal amount in the nineteenth effluent fraction, the same fraction in which phenobarbital is in maximal amount. From the tenth to the thirtieth fraction, the amount of the material determined as phenobarbital conformed closely to that expected for pure phenobarbital. Thus in the fractions in this range the material determined as phenobarbital behaves as a homogeneous substance with a partition coefficient between the organic solvent and the buffer identical to that of phenobarbital. The evidence seems conclusive that phenobarbital is present in plasma after the administration of primidone and that the analytical methods used have not measured a significant amount of any substance other than phenobarbital.

In the dogs receiving primidone for 6 days there was progressive accumulation of phenobarbital in the plasma, the highest concentration being reached on the day after dosage

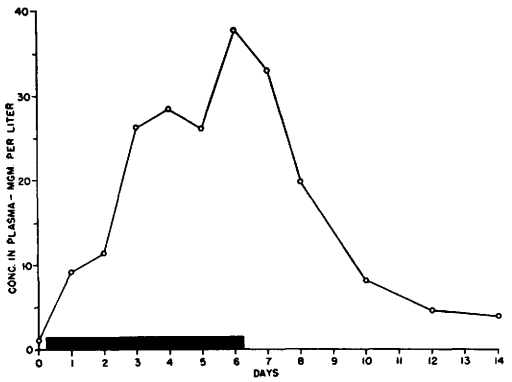

FIG. 1. Plasma phenobarbital concentrations resulting from oral administration of 200 mg/kg of primidone daily for 6 days to a 9.7 kg female dog. Period of drug administration is indicated by the bar.

was discontinued. The peak concentration was 27 mg/l in the dog on a daily dose of 250 mg/kg and 38 mg/l in the dog on a daily dose of 200 mg/kg. Fig. 1 shows the course of accumulation and elimination of phenobarbital in the latter dog.

In the patient receiving 1.0 g of primidone daily the plasma concentration of phenobarbital was 16 mg/l. In the patient receiving a daily dose of 2.0 g the plasma concentration of phenobarbital was 39 mg/l.

Discussion. The isolation of phenobarbital and its hydroxy derivative from urine and the demonstration of a substance in plasma with distribution coefficient and absorption spectra characteristic of phenobarbital give unequivocal evidence that primidone is in part converted to phenobarbital. During the course of this investigation it came to our attention that G. L. Plaa, J. M. Fujimoto, and C. H. Hine at the University of California, San Francisco, had independently found evidence of the presence of a substance with the spectral characteristics of phenobarbital in the blood of patients and in the urine of rats receiving primidone.

Comparison of the plasma concentrations of phenobarbital resulting from the administration of primidone and of phenobarbital itself permits a rough estimate of the proportion of primidone converted to phenobarbital. Calculations based on the phenobarbital concentrations found in other dogs and other patients receiving phenobarbital in chronic schedules lead to the conclusion that the dogs of the present study converted of the order of 5% and the patients of the order of 15% of the administered dose of primidone to phenobarbital. Thus the extent of the conversion is only small; but owing to the high dosage in which primidone is used and the slow rate at which phenobarbital is eliminated, that product may accumulate to reach levels sufficient to exert a significant effect. The concentrations of phenobarbital found in the plasma of the two patients of this study are in the range of those found in patients treated with antiepileptic doses of phenobarbital itself. There can be no doubt that concentrations of phenobarbital as high as those present in these two patients can be effective in suppressing seizures even in the absence of other drugs. While it seems clear that phenobarbital must, in some patients at least, make a significant contribution to the therapeutic effects of primidone, the role of the unchanged drug is not clear. If, as has been reported, seizures can be controlled in some patients by primidone and not by phenobarbital alone, the therapeutic results in such patients cannot be attributed entirely to the phenobarbital produced by the oxidation of primidone.

Summary. Primidone (Mysoline) is in part converted to phenobarbital by dog and by man. Concentrations of phenobarbital found in plasma of patients receiving therapeutic doses of primidone are high enough to exert a significant antiepileptic effect.

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