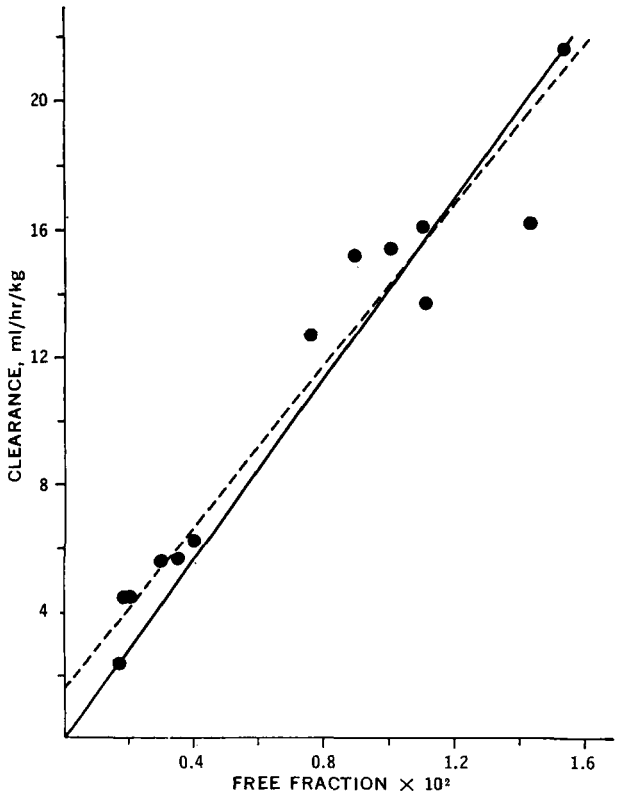

Figure 1—*Relationship between total plasma clearance of warfarin and fraction of free (not protein bound) drug in the serum of individual rats. These rats received intravenous doses of ^{14}C-warfarin, 0.6 mg/kg. Least-squares fit of the data yielded the stippled regression line; the continuous regression line was forced through the origin. The correlation coefficient is 0.97, $p < 0.001$.*

was essentially independent of total concentration for any one animal under the experimental conditions (total concentration, 0.3–4 μg/ml). Total plasma clearance[4] of warfarin ranged from about 2 to 22 ml hr^{-1} kg^{-1}. A plot of total clearance *versus* the fraction of free drug in the serum is linear, and the least-squares regression line intersects close to the origin, as predicted by Eq. 4 (Fig. 1).

In summary, our studies showed that individual differences in serum protein binding of warfarin (from 98.5 to 99.8%, equivalent to a range in the free fraction of from 0.002 to 0.015) can cause pronounced variations in the elimination rate constant (from 0.017 to 0.117 hr^{-1}) and apparent volume of distribution (from 137 to 206 ml/kg) of warfarin and that the effect of plasma protein binding on warfarin elimination can be quantified and predicted on the basis of a linear relationship between the total plasma clearance of warfarin and the free fraction of this drug in the serum. These observations may have clinical relevance in that *in vitro* protein binding studies with serum samples obtained from patients *prior to drug administration* may be useful for predicting the possibility of quantitatively unusual distribution, elimination, and pharmacological effect (4) characteristics of certain highly plasma protein bound drugs such as warfarin in individual patients.

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Received December 12, 1973.
Accepted for publication February 27, 1974.
Supported in part by Grant GM 20852-01 from the National Institute of General Medical Sciences, National Institutes of Health, Bethesda, MD 20014
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GLC Microdetermination of Plasma Anticonvulsant Levels

Keyphrases □ Anticonvulsants—plasma levels, GLC microdetermination □ GLC—microdetermination, anticonvulsant plasma levels

To the Editor:

The treatment of infants from birth for convulsive disorders is hindered by the lack of a rapid method for monitoring anticonvulsant blood levels using a small volume of plasma. The method of Berlin *et al.* (1) for diphenylhydantoin was modified by Solow[1] for the simultaneous determination of multiple drug levels. However, this method has a fairly low extraction efficiency for primidone, a primary anticonvulsant. Kållberg *et al.* (2) reported a rapid method for phenobarbital which we found also yielded almost total recovery of diphenylhydantoin. However, the recovery of primidone again was extremely low (16%), and the aqueous solution of trimethylphenylanilinium hydroxide, which was used for the final extraction,

[4] Total plasma clearance = $V_{area}\beta$, where the terminal slope of a plot of the logarithm of the drug concentration in plasma *versus* time equals $-\beta/2.3$.

[1] E. B. Solow, Indiana University, Indianapolis, IN 46202, personal communication.

degraded phenobarbital to phenylethylmalonamide even at low injector temperatures (230°).

A method developed in this laboratory (3) has proven applicable to the analysis of small plasma samples. For the analysis of a 100-μl sample of plasma, the internal standard for phenobarbital, primidone, and diphenylhydantoin is 0.50 ml of a solution of 3 mg 5-(*p*-methylphenyl)-5-phenylhydantoin/500 ml 0.1 *N* NaOH. If ethosuximide is to be determined in the sample, 0.50 ml of a solution of 1 mg of 2,2,3-trimethylsuccinimide in 100 ml of distilled water is also added to the plasma. After the addition of 0.5 ml of a 1.0 *M* phosphate buffer (pH 2.7), the aqueous phase is extracted with 10 ml of ether by shaking for 10 min. The sample is centrifuged and the ether is transferred to another tube[2].

If ethosuximide is present, amyl acetate (50 μl) is added to the ether before evaporation (4). After the ether has been evaporated at 55° under nitrogen, 1 μl of the amyl acetate residue is injected into the gas chromatograph.

To determine phenobarbital, primidone, and diphenylhydantoin in this sample, the amyl acetate or ether residue is dissolved in 0.5 ml of toluene. The drugs are then extracted from the toluene, as previously reported (3), with 12 μl of 2 *M* tetramethylammonium hydroxide[3], and the sample is withdrawn from the lower phase for chromatography.

The column, a 91.5-cm × 2-mm (i.d.) glass U-tube, is packed with 7% OV-17 on 80–100-mesh Chromosorb W-HP. The amyl acetate fraction is run isothermally at 125° for ethosuximide. The tetramethylammonium hydroxide fraction is programmed from 150 to 240° at 10°/min. The carrier gas flow rate is adjusted to around 10 ml/min to give a retention time of 4.0 ± 0.2 min for diphenylhydantoin at 240°.

The limits of detection for phenobarbital, primidone, diphenylhydantoin, and ethosuximide are well below therapeutic levels. Reproducibility is comparable to that of the macrodetermination, and samples analyzed by both methods give good agreement.

Preliminary findings indicate that the anticonvulsants trimethadione, its metabolite dimethadione, and paramethadione can also be quantitated in the amyl acetate fraction with ethosuximide. With this extraction, it is not necessary to use the salting-out technique of Booker and Darcey (5) to get significant recovery of dimethadione. While trimethadione can be seen if chromatographed at 125°, it is better resolved from the solvent front if the temperature is programmed from 100 to 125° at 4°/min. The other succinimide, hydantoin, and barbiturate anticonvulsants can be determined in the tetramethylammonium hydroxide fraction as previously reported (3).

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Received September 4, 1973.

Accepted for publication February 26, 1974.

Supported by a Veterans Administration Medical Research Grant (MRIS-9335-01) and the Florida Epilepsy Foundation.

The authors thank Mrs. E. B. Solow for her helpful suggestions and Mr. Mitchell Thomas and Miss Dora Mitchell for their technical assistance.

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Enhancement of Optical Rotation of Levodopa by Cyclization

Keyphrases □ Levodopa—enhanced optical rotation using cyclization, purity determination □ Optical rotation, levodopa—enhanced using cyclization, purity determination

To the Editor:

The optical rotation of levodopa is an important purity criterion, since it provides the only means to control the presence of the allegedly toxic and therapeutically inactive D-isomer. The specific rotation, $[\alpha]_D^{25}$, of levodopa is only about −12°, which is insufficient to control optical purity. Barooshian *et al.* (1) proposed a TLC limit test for D-dopa in levodopa, involving conversion of the enantiomers to diastereomeric dipeptides. Jouin and Saias (2) and Coppi *et al.* (3) described methods based on stereospecific enzymatic destruction of levodopa. The BP 1973 (4) provides requirements of $[\alpha]_{365}^{20}$ −119 to −125° for levodopa drug substance and $[\alpha]_D^{20}$ −38.5 to −41.5° for its dosage forms, the rotation being increased by addition of aluminum sulfate and sodium acetate.

Pesez and Fabre (5) reported that addition of 2.5 g of aluminum chloride and 2 g of methenamine to a solution of 200 mg of levodopa in 5 ml 1 *N* hydrochloric acid, dissolution, and addition of acid to 10 ml afforded a specific rotation at the sodium D-line of −142° after the solution was allowed to stand in the dark at room temperature for 3 hr. They attributed this large increase in optical rotation to an effect of methenamine on the aluminum complex of levodopa. The chemistry of their procedure was investigated; it was found that the use of aluminum chloride is supererogatory, that the enhancement of optical rotation obtained is most probably due to pheno-