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from the toluene, as previously reported (3), with 12 μl of 2 *M* tetramethylammonium hydroxide[3], and the sample is withdrawn from the lower phase for chromatography.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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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-

Table I—Relationship of Optical Rotation and Concentration for Levodopa with Methenamine and Hydrochloric Acid

Levodopa, %	0.212	0.499	1.013	1.521	2.000
α	−0.349°	−0.822°	−1.672°	−2.527°	−3.312°
$[\alpha]_D^{25}$	−164.5°	−164.7°	−165.0°	−166.1°	−165.6°

lic cyclization of levodopa (I) with formaldehyde, liberated from methenamine, to form L-3-carboxy-6,7-dihydroxy-1,2,3,4-tetrahydroisoquinoline (II) (Scheme I). Evidence supporting this hypothesis and a modified procedure are presented.

Methenamine hydrolyzes in acid solution to formaldehyde and ammonium ion. The 2 g used in the Pesez and Fabre procedure is 14.3 mmoles, which on complete hydrolysis would form about 86 mmoles of formaldehyde and 57 mmoles of ammonium ion, far in excess of the 1 mmole of levodopa and less than 10 mEq of acid present in the reaction mixture. Repeating their procedure[1] gave $[\alpha]_D^{25}$ −146°, and the pH of the solution was 3.2. When the aluminum chloride was omitted, the $[\alpha]_D^{25}$ was −165.6° and the pH was 5.6. Compound II was synthesized from I and formaldehyde according to the directions given by Bell *et al.* (6). It gave a melting range of 287–290° [lit. (6) mp 286–288°] and UV maxima at 282 and 210 nm as reported. Its specific rotation (c 0.14) in 20% hydrochloric acid was 122.2°, about 10% higher than the literature value of −110.5° for a 1.67% solution in this medium. However, it was found that acid concentration had a significant effect on rotation, the values for $[\alpha]_D^{25}$ (c 0.14) being −127.8° in 1 *N* hydrochloric acid and −131.3° at pH 1.04. At pH greater than 7, solutions turned red due to catechol moiety oxidation. The specific rotation of II, determined at pH 5.6 (c 0.2), was −151°. This is equivalent to a molar rotation $[\Phi]_D$ of −315.9°, which is about 3% lower than the $[\Phi]_D$ of −324.4° calculated for levodopa at the same concentration and in the same medium.

It was found that the reaction requires about 2.5 hr for completion, using the Pesez–Fabre conditions without aluminum chloride, making 3 hr a reasonable time specification. A plot of levodopa concentration *versus* observed rotation was rectilinear for amounts between 0.2 and 2.0% (w/v) as indicated in Table I. The data suggest that the method might be useful for quantitative estimation of the drug.

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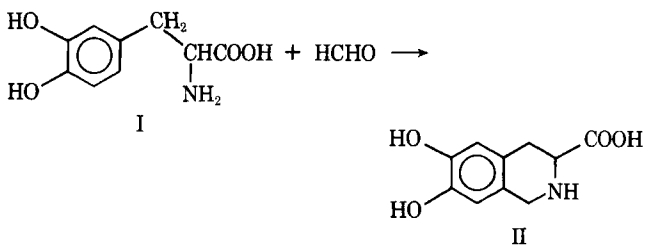

Scheme I

[1] Determined in a Perkin-Elmer model 141 polarimeter, using 1-dm cells.

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Slide Test as a Micromethod of a Modified *Limulus* Endotoxin Test

Keyphrases □ *Limulus* endotoxin test—slide test as a micromethod □ Bacterial endotoxin—slide test as a micromethod of modified *Limulus* endotoxin test □ Endotoxin test, modified *Limulus*—slide test as a micromethod

To the Editor:

A cell lysate prepared from amebocytes of *Limulus polyphemus* (the horseshoe crab) has been described as a sensitive reagent for the *in vitro* detection of bacterial endotoxin and as a comparable test to the official pyrogen bioassay using rabbits (1, 2). The test is based upon the fact that the addition of a solution containing endotoxin to a suitable *Limulus* amebocyte lysate preparation and the subsequent incubation at 37° result in a solid gel or an increase in viscosity. Unfortunately, the determination of the end-point presents some difficulties when the described test tube method (3) is used so an objective interpretation of the results is prevented.

The slide test proposed here offers two major advantages: easier determination of the end-point and significant reduction in the amount of reagent needed.

Ten microliters of *Limulus* reagent[1] is placed on the surface of a slide (*e.g.*, glass plate used for the Latex test with a black backside[2]) using a calibrated glass capillary tube. (Slide and capillary tubes must

[1] E-Toxate, Sigma Chemical Co., St. Louis, Mo., or Pyrogent, Mallinckrodt Chemical Works, St. Louis, Mo.

[2] Test plate code RFZ, Behring Werke A.G, Marburg-Lahn, West Germany.