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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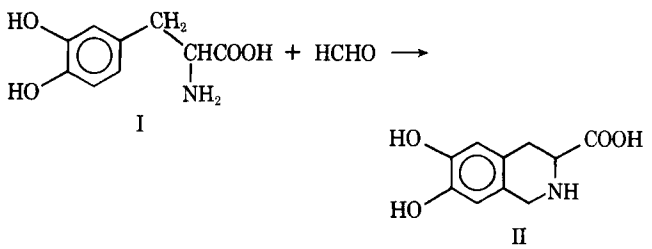

Scheme I

[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.

be free of endotoxin, *e.g.*, by treating this carefully cleaned equipment at 180° for several hours.) Ten microliters of the sample is added and mixed with the aid of the capillary tube (a special pipet holder with a punched rubber cap is recommended[3]). A negative control and a positive control are placed in the same manner on two separate test fields. Air bubbles should be prevented or eliminated using a needle, which has been previously heated to red heat and then allowed to cool. All of the small droplets placed on a slide must show some slight movement when the plate is gently vibrated. The slide is then placed in a "moist chamber" consisting, for example, of a flat plastic box, the bottom of which has been covered with a piece of moistened cellulose. Two glass rods serve as support of the slide.

The whole assembly is incubated at 37° for 30 min. After 30 min, the slide is observed against a bright background. The slide is always held in a horizontal position and may be gently tilted or vibrated through an angle of a few degrees only. A solid gel or an increase in viscosity is easily detected. The incubation may be prolonged, but usually the result remains unchanged. Very hygroscopic solutions tend to increase the volume of the droplets upon prolonged standing and, therefore, to reduce the sensitivity of the test. A nonsaturated moist chamber might be used in these cases.

(1) J. F. Cooper, J. Levin, and H. N. Wagner, *J. Lab. Clin. Med.*, **78**, 138(1971).
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(3) H. D. Hochstein, R. J. Elfin, J. F. Cooper, E. B. Seligman, and S. M. Wolff, *ibid.*, **27**, 139(1973).

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[3] Micropipets and holder, Hirschmann GmbH, Eberstadt, West Germany.

Rapidly Dissolving Forms of Digoxin: Hydroquinone Complex

Keyphrases □ Digoxin—rapidly dissolving digoxin-hydroquinone complex, bioavailability characteristics □ Hydroquinone-digoxin complex—dissolution, bioavailability characteristics □ Bioavailability—digoxin from digoxin-hydroquinone complex

To the Editor:

Numerous recent publications attested to the wide variance in bioavailability found among commercial digoxin tablets (1–5). The main cause of unsatisfactory performance seems to be directly related to the extremely low water solubility of the active substance. Although significant improvements in the efficiency of absorption from oral dosage forms have been obtained through formulation technology, it appears that intrinsically more rapidly dissolving forms of digoxin would provide greater assurance of more reproducible and more bioavailable digoxin products.

In this preliminary communication, we report the development of such forms of digoxin. The approach is based on an earlier concept reported by our group, which utilized free energy of dissolution of molecular complexes to elicit substantially faster dissolution of relatively insoluble solids (6, 7).

Figures 1 and 2 show phase solubility diagrams in water of hydroquinone in the presence of digoxin at

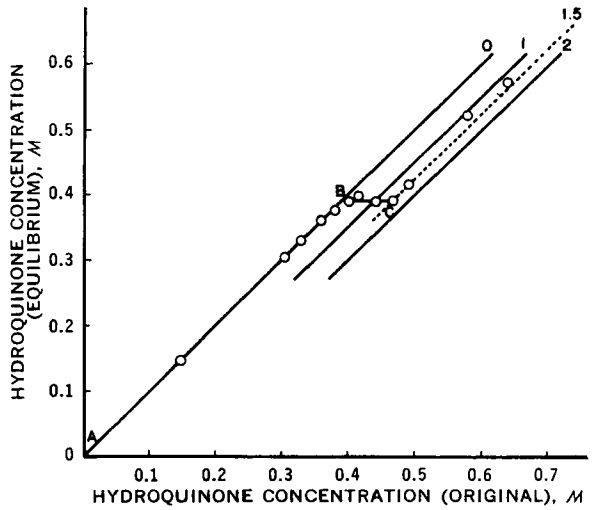

Figure 1—*Plot of hydroquinone concentrations in solution at equilibrium in the presence of 0.05 mole solid digoxin/liter against original concentrations of hydroquinone. The system was equilibrated at 25°. Point B corresponds to the triple point. Line 1 corresponds to the formation of a 1:1 complex, line 1.5 to a 2:3 complex, and line 2 to a 1:2 complex.*

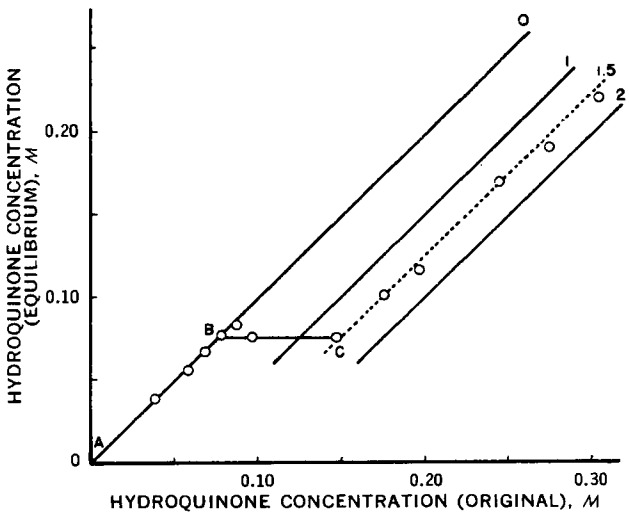

Figure 2—*Plot of hydroquinone concentrations at equilibrium* versus *original concentrations equilibrated at 1.5°. All samples initially contained 0.05* M *(39 mg/ml) of digoxin.*